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TESI DI DOTTORATO

Characterization of new technological and nutritional properties of milk from cows of 6 breeds reared in multi-breed herds

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1 Summary

Improved animal production, largely as a result of genetic selection, was one of the greatest achievements of the last century. The dominant dairy cattle breed, at a global level, is the Holstein-Friesian. The breed has undergone an extreme genetic selection for several decades, towards high milk yield, and incorporated by high-nutrient and milk output systems. However, the high genetic pressure on only one trait, i.e. milk yield, resulted in unfavorable impacts on the welfare of the cows (i.e., metabolic stress, lameness, mastitis, reduced fertility and longevity). Moreover, as animals tend to adapt to the environment they are selected in, it is likely that selection for increased yield may also lead to environmental sensitivity. For instance, the negative correlation between production and fitness traits in less favorable environments is indicative of a decline in adaptability in the modern dairy cows. An increased importance exists, nowadays, for farm animal welfare that is recognized by all stakeholders in the farm animal production chain.

These considerations, together with the unchanged primary goal of the dairy industry for high milk quality for the consumer's market, has lead, in some cases, in the use of crossbreeding between Holstein-Friesian and other dairy and dual-purpose breeds. In some countries, dual-purpose breeds such as the Simmental, Montbéliarde, Normande, and specialized breeds such as the Brown Swiss and Jersey are considered the breeds of choice for crossbreeding. These breeds, including the local Italian (North-east Italy) breeds of Rendena and Alpine Grey, tend to offer superior milk quality, complemented by high beefing merits. This combination can result in increased revenue from male calves and cull cow sales.

Nevertheless, comparison of milk quality of these different breeds is lacking in the literature, especially due to practical difficulties in the recording system of lots of animals, that are reared in different mixed-breed farms. To alleviate this problem, the Cowplus project has been developed at the Department of Agronomy, Food, Natural resources, Animals and Environment at University of Padova. The project permitted the sampling of 1,508 cows reared in 41 multi-breed herds, located in

Trentino region in the north-eastern Italian Alps. Farms were selected from a pool of 610 herds enrolled in the Italian milk recording system. Cows were recorded for body characteristics, daily milk yield and composition, renneting aptitude, and cheese-yield. In total, 6 dairy and dual-purpose breeds were used. As part of the Cowplus project, this study aimed in: 1) the quantification and characterization of the effects of high or low herd productivity (defined according to the milk net energy yielded daily by the cows); 2) quantifying the variability of the herds within herd productivity class; 3) performing a within-herd comparison between the 3 dairy and the 3 dual-purpose breeds; 4) analyzing the effects of the days in milk (DIM) and the parity of the cows, on traditional milk quality and milk renneting aptitude (*Chapter 1*), cheese-making ability (*Chapter 2*), milk mineral elements (*Chapter 3*), and productivity and efficiency indicators of lactating cows (*Chapter 4*).

More precisely, the goal of the first chapter was to test the afore mentioned effects on coagulation properties, and assess the repeatability and reproducibility of traditional milk coagulation properties (**MCP**) and curd-firming over time (**CF$_t$**) modeled and derived traits. Milk samples were collected from all the 1,508 cows and analyzed in duplicates (3,016 tests) using two lactodynamographs (Formagraph, FOSS) to obtain 240 curd-firmness (**CF**) measurements in 60 min (one every 15 sec) for each duplicate. Results showed that the effect of herd-date on the traditional and modeled MCP was modest while individual animal variance showed the highest incidence. The repeatability of MCP was high (> 80%) for all traits excluding those depending on the last part of the lactodynamographic curve (57 to 71%). The reproducibility, taking also into account the effect of instrument, was equal or slightly lower than repeatability. Milk samples collected in farms characterized by high level of productivity exhibited delayed coagulation but greater potential curd firmness compared to milk samples collected from low productivity herds. Large differences in all MCP traits were observed among breeds, both between specialized and dual-purpose breeds, and within the two groups, even after adjusting for milk quality and yield. Milk samples from Jersey cows, both for milk quality and MCP, and also from Rendena cows (but only for coagulation time) were superior respect to milk from Holstein-Friesian cows, while intermediate results were found for the other breeds of Alpine origin.

The second chapter aimed at evaluating the same effects on 508 model cheeses derived from 508 cows of 6 different breeds. For each cow 6 milk composition traits, 4 recovery traits (**REC**) of milk nutrients (fat, protein, solids and energy) in the curd, and 3 actual % cheese yield traits (**%CY**), expressing the fresh cheese, cheese solids and cheese water as percentages of the processed milk were analyzed (these traits were obtained during the experimental cheese-making process). In addition, 2 theoretical %CYs (fresh cheese and cheese solids) were calculated from the milk composition, and 2 overall cheese-making efficiencies (fresh cheese and cheese solids) were calculated as the % ratio between actual and theoretical %CYs. Daily milk yield (**dMY**) was also measured and estimates were made of 3 actual daily cheese yield production traits (**dCY**) per cow (fresh cheese, cheese solids and water retained in the cheese). Results showed that cows reared in high productivity herds yielded more milk with greater nutrient contents and more cheese per day, and had greater theoretical %CY, although to a lesser extent, actual %CY. However, they did not differ from low productivity herds in terms of REC traits (except solids), while they had a lower solid cheese-making efficiency. Individual herds within productivity classes were an intermediate source of total variation with respect to REC traits (11.3% to 17.1%), and to actual and theoretical %CY and estimates of efficiency (10.0% to 17.2%), and a major source for milk yield and dCY traits (43.1% to 46.3%). Breed within herd greatly affected all traits. Compared with the dual-purpose breeds, the 3 specialized dairy breeds (Holstein, Brown Swiss and Jersey) had, on average, a similar dMY, better milk composition, greater actual and theoretical %CY, similar fat and protein REC, and slightly lower cheese-making efficiency. Of the specialized dairy cow breeds, Holsteins produced more milk, but Brown Swiss cows produced milk with a greater nutrient content, greater nutrient REC, higher actual and theoretical %CY and a higher cheese-making efficiency, so the two large breeds had the same dCY. Small Jersey cows produced much less milk, with much more fat and protein and greater REC traits than the two large-framed breeds resulting in greater actual and theoretical %CY but similar efficiencies. Although the Jersey breed had lower dMY and dCY, the difference was much smaller for the latter. The differences among Simmental and the local Rendena and Alpine Grey were not very large. Compared with medium-framed cows of the local breeds, Simmentals had greater dMY, tended to have better milk composition, REC and %CY traits (but similar efficiencies), and also had much greater dCY. Among the local breeds, the higher dMY of Rendena was offset by the greater nutrient content of milk from the Alpine Greys, so their dCY was similar.

The objective of the third chapter was to test the same previous effects on 240 milk samples from 240 cows of 6 different breeds. Fifteen minerals were determined by Inductively Coupled Plasma - Optical Emission Spectrometry (**ICP-OES**). Results revealed that the effect of herd-date was large especially on environmental minerals (from 47 to 91% of the total variance), while it ranged from 11% to 61% considering both macro- and micro-minerals. Milk samples collected in farms characterized by high level of productivity exhibited richer mineral profile compared to milk samples collected from low productivity herds. Parity influenced exclusively macro-minerals, with the exception of Ca and S, while DIM influenced almost all minerals, with few exception related to the environmental elements. Large differences were observed among breeds, both between specialized and dual-purpose breeds, even after adjusting for milk quality and yield. Milk samples from Jersey and Brown Swiss cows were superior respect to milk from Holstein-Friesian cows, both for milk quality and mineral profile, while intermediate results were found for the other breeds of Alpine origin. Moreover, the variance of individual animals was much greater than variance of individual herds within herd productivity class.

The fourth chapter focused on the concepts of production, productivity and efficiency. As breed of cows and herd characteristics are the most important factors affecting milk productivity and efficiency, the aim of this chapter was to obtain independent evaluation of these factors on the data (body size and production) and milk characteristics from the 41 multi-breed herds on all 1,508 lactating cows from the 6 breeds. Nine productivity indicators and two simplified indicators of cow

efficiency for cheese production, one energetic and one economic, were calculated. Results showed that breed within herd greatly affected all traits. On average the 3 dairy breeds were not much different from the 3 dual-purpose breeds, but large differences characterized both groups of cows. Jersey cows were the less productive, but, after correcting for herds effect and scaling for body size, they showed the highest efficiency among the dairy breeds. Holstein was the most productive dairy breed, but Brown Swiss cows had better milk quality and more efficient cheese-making aptitude and thus produced more cheese per day than Holsteins. Dual-purpose breeds were less variable than dairy ones, with Simmental with larger body size and production, but not productivity and efficiency respect to local Rendena and Alpine Grey breeds. If on one hand within herd comparison and correctly scaling of production traits reduced strongly herd differences in productivity, on the other hand they did not reduce very much the differences in terms of milk composition, technological properties and efficiency of cheese-making (recovery of milk nutrients in cheese), so that the differences among breeds remained strong and their importance on the overall efficiency evaluation of the breeds increased.

2 Riassunto

L’aumento della produzione animale, soprattutto a causa della selezione genetica, è stato uno dei più grandi successi del secolo scorso. La razza bovina da latte dominante a livello internazionale è la Frisona. Questa razza per molti decenni ha subito una forte selezione genetica verso l'alta produzione di latte, anche attraverso sistemi di produzione intensivi. Tuttavia, l'alta pressione genetica sulla produzione di latte ha determinato impatti negativi sul benessere degli animali (es., stress metabolico, zoppia, mastite, ridotta fertilità e longevità). Inoltre, dal momento che gli animali tendono ad adattarsi all'ambiente in cui sono selezionati, è probabile che la selezione per un maggiore rendimento abbia portato anche a sensibilità ambientale. Per esempio, la correlazione negativa tra le caratteristiche di produzione e di fitness in ambienti meno favorevoli è indicativo della diminuzione della capacità di adattamento delle moderne vacche da latte. Esiste una crescente importanza, oggi, per il benessere degli animali d'allevamento che viene anche riconosciuto da tutti gli attori della catena di produzione degli animali da reddito.

Queste considerazioni, insieme all'obiettivo primario del settore lattiero-caseario di alta qualità del latte, ha portato, in alcuni casi, all'utilizzo di programmi di incrocio fra Frisona e altre razze specializzate da latte e a duplice attitudine. In alcuni paesi, razze a duplice attitudine come la Pezzata Rossa, la Montbéliarde, la Normanna, e razze specializzate come la Bruna e la Jersey, sono preferite a scopo di incrocio. Queste razze, tra cui le italiane razze locali Rendena e Grigio Alpina (Nord-Est Italia), tendono ad offrire una superiore qualità del latte, accompagnata da una maggior produzione di carne, e quindi più alto valore sia dei vitelli che delle vacche a fine carriera.

Tuttavia, un serio confronto di queste diverse razze è carente in letteratura, in particolare a causa di difficoltà pratiche nel campionamento di numerosi animali, che vengono allevati in diversi allevamenti a razza mista. Per ovviare a questo problema, è stato sviluppato il progetto Cowplus presso il Dipartimento di Agronomia, di Alimentazione, delle Risorse naturali, Animali e Ambiente presso l'Università di Padova. Il progetto ha permesso il campionamento di 1508 bovine allevate in

41 aziende multi-razza, situate in provincia di Trento, nelle Alpi italiane nord-orientali. Le aziende sono state selezionate da un pool di 610 allevamenti iscritti al sistema di controlli funzionali del latte. Gli animali sono stati campionati per la morfologia, la produzione giornaliera e la composizione del latte, l'attitudine alla coagulazione e alla caseificazione. In totale, sono state utilizzate 6 razze: 3 specializzate da latte e 3 a duplice attitudine. Nell'ambito del progetto Cowplus, gli obiettivi di questa tesi di dottorato sono stati: 1) la quantificazione e la caratterizzazione degli effetti di alta o bassa produttività dell'azienda (definite in base all'energia netta di lattazione prodotta giornalmente dalle vacche); 2) quantificare la variabilità delle aziende entro classe di produttività aziendale; 3) confrontare, a parità di azienda, le 3 razze specializzate con le 3 razze a duplice attitudine; 4) analizzare gli effetti dei giorni di lattazione (DIM) e dell'ordine di parto delle bovine, sulla qualità e l'attitudine alla coagulazione (*Capitolo 1*), l'efficienza di caseificazione (*Capitolo 2*), e il profilo minerale del latte (*Capitolo 3*), e sugli indicatori di produttività di efficienza degli animali (*Capitolo 4*).

Più precisamente, l'obiettivo del primo capitolo è stato quello di verificare gli effetti sopracitati sulle proprietà di coagulazione del latte tradizionali e modellizzate, e di valutarne la ripetibilità e la riproducibilità. I 1,508 campioni di latte sono stati analizzati in doppio (3,016 analisi) utilizzando due lattodinamografi (Formagraph, FOSS) per ottenere 240 misurazioni di consistenza del coagulo in 60 minuti (una ogni 15 secondi) e per ogni ripetuta. I risultati hanno mostrato un contenuto effetto dell'azienda sui parametri tradizionali e modellizzati, mentre la varianza del singolo animale ha mostrato una più alta incidenza. La ripetibilità delle MCP tradizionali è risultata elevata (> 80%) per tutti i caratteri, esclusi quelli legati alla fase finale della curva lattodinamografica (dal 57 al 71%). La riproducibilità, anche tenendo conto dell'effetto dello strumento, è risultata uguale o leggermente inferiore alla ripetibilità. I campioni di latte raccolti nelle aziende caratterizzate da un elevato livello produttivo hanno presentato una coagulazione più ritardata, ma un potenziale maggiore di consistenza del coagulo rispetto ai campioni di latte provenienti da allevamenti a bassa produttività. Grandi differenze sono state osservate tra le razze in merito all'attitudine alla coagulazione del latte, sia tra le specializzate da latte e a duplice attitudine, sia entro i due gruppi, anche dopo aver corretto per la qualità e la produzione giornaliera di latte. I campioni di latte di Jersey, sia per la composizione che per l'attitudine alla coagulazione del latte, e anche di Rendena (ma solo per il tempo di coagulazione) sono stati superiori rispetto al latte di Frisona, mentre risultati intermedi sono stati trovati per le altre razze di origine alpina.

Il secondo capitolo è stato diretto a valutare gli stessi effetti su 508 caseificazioni individuali delle 6 razze. Per ogni bovina sono stati ottenuti: 6 parametri di composizione del latte, 4 caratteri di recupero dei nutrienti dal latte (REC - grasso, proteina, solidi ed energia) nella cagliata, e 3 caratteri di resa reale in % (%CY), che esprime il formaggio fresco, la sostanza secca e l'acqua ritenuta nel formaggio, come percentuali del latte trasformato (ottenuti tramite una procedura individuale di micro-caseificazione). Inoltre sono state calcolate 2 rese teoriche (*%Th*-CY) (resa in sostanza secca e a fresco) dalla composizione del latte, e 2 efficienze (*%Ef*-CY) di caseificazione calcolate come rapporto in % tra resa reale e teorica. Inoltre, è stata misurata la produzione giornaliera di latte (dMY) oltre alle stime individuali di resa giornaliera in formaggio (dCY), sostanza secca e acqua del formaggio. I risultati hanno mostrato che gli animali allevati in aziende ad alta produttività hanno prodotto un latte più ricco in nutrienti e reso più formaggio al giorno (%CY e dCY). Tuttavia, nessuna differenza è stata rilevata fra aziende ad alto e basso livello produttivo in termini di recupero di nutrienti nella cagliata (ad eccezione della sostanza secca), mentre l'efficienza in sostanza secca della cagliata è stata inferiore. La singola azienda, a parità di livello produttivo, è risultata una fonte di variazione intermedia sui recuperi (dal 11.3% al 17.1%), sulle rese reali e teoriche e sulle stime di efficienza (dal 10.0% al 17.2%), e una delle principali fonti per la produzione giornaliera di latte (dMY), così come per le dCYs (dal 43.1% al 46.3%). La razza, a parità di ambiente, ha fortemente influenzato tutti caratteri analizzati. Rispetto alle razze a duplice attitudine, le 3 razze da latte (Frisona, Bruna e Jersey) hanno avuto, in media, una migliore composizione del latte, una maggiore resa reale e teorica, simile recupero di grasso e proteina nella cagliata, e una leggermente inferiore efficienza casearia. Delle razze specializzate, la Frisona ha prodotto più latte, ma la Bruna ha prodotto il latte con un maggior contenuto di nutrienti, un maggiore recupero di questi nella cagliata, una più alta resa reale e teorica e una migliore efficienza casearia, così che la produzione giornaliera in formaggio è stata simile. Le più piccole Jersey hanno prodotto molto meno latte però con molto più grasso e proteina, e % più alta del recupero di nutrienti rispetto alle due razze grandi, presentando così una maggiore resa reale e teorica, anche se simile efficienza casearia. Anche se la razza Jersey ha mostrato inferiore produzione giornaliera di latte e formaggio, la differenza è stata molto più lieve per la seconda. Le differenze tra Pezzata Rossa e le due locali Rendena e Grigio Alpina non state molto grandi. Rispetto alle due, la Pezzata Rossa ha avuto una maggiore produzione di latte con una migliore composizione, oltre ad avere maggior REC e caratteri legati alla resa (ma efficienze simili). Entro le razze locali, la più alta produzione giornaliera di latte della Rendena è stata compensata dal maggior contenuto di nutrienti del latte di Grigio Alpina, quindi la loro produzione giornaliera di formaggio è stata simile.

L'obiettivo del terzo capitolo è stato quello di testare gli effetti sopracitati su 240 campioni di latte da 240 vacche appartenenti alle diverse 6 razze. Quindici minerali sono stati determinati utilizzando lo spettrometro di emissione al plasma (ICP-OES). I risultati hanno rivelato che l'effetto dell'azienda ha avuto un'influenza maggiore specialmente su minerali ambientali (dal 47 al 91% della varianza totale), mentre ha variato dall'11% al 61% sui macro e micro-minerali. I campioni di latte raccolti nelle aziende caratterizzate da un elevato livello di produttività hanno presentato un più ricco profilo minerale rispetto ai campioni di latte provenienti dagli allevamenti a bassa produttività. L'ordine di parto ha influenzato esclusivamente i macro-minerali, con l'eccezione di Ca e S, mentre i DIM hanno influenzato tutti i minerali, con poche eccezioni relative ai micro-ambientali. Sono state osservate notevoli differenze tra le razze, sia tra le specializzate che a duplice attitudine, anche correggendo il modello statistico per la qualità e la produzione di latte. Le razze Jersey e Bruna hanno presentato una migliore qualità del latte, sia in termini di composizione chimica che in profilo minerale, rispetto alle vacche di razza Frisona. Risultati intermedi sono stati trovati per le altre razze di origine alpina. Sulla base di questo studio gli effetti della razza sui macro-minerali e alcuni degli essenziali micro-minerali sono molto più importanti rispetto agli effetti della produttività aziendale, dell'ordine di parto e giorni di lattazione.

Il quarto capitolo si è focalizzato sui concetti di produzione, produttività ed efficienza. Dal momento che la razza e l'azienda sono i fattori che più influenzano la produttività e l'efficienza del latte, lo scopo di questo capitolo è stato quello di ottenere una valutazione indipendente di questi due fattori sui dati raccolti sugli animali (dimensioni del corpo e produzione) e le caratteristiche del latte, dalle 41 aziende miste e su tutte le 1,508 vacche in lattazione appartenenti alle 6 diverse razze. Sono stati calcolati a questo scopo nove indicatori di produttività e due indicatori semplificati di efficienza della vacca per la produzione di formaggio, uno energetico e uno economico. I risultati hanno mostrato che la razza, a parità di ambiente, ha fortemente influenzato tutti gli indicatori. In media, le 3 razze da latte non sono state molto diverse dalle 3 razze a duplice attitudine, ma grandi differenze hanno caratterizzato entrambi i gruppi di animali. Le Jersey sono state le meno produttive, ma, dopo la correzione per l'effetto azienda e rapportate per le dimensioni del corpo, hanno mostrato la più alta efficienza tra le razze da latte. La Frisona è stata la razza da latte più produttiva, ma la Bruna ha avuto una migliore qualità del latte e un'attitudine casearia più efficiente così come più formaggio prodotto al giorno, rispetto alla Frisona. Le razze a duplice attitudine sono state meno variabili rispetto a quelle da latte, con la Pezzata Rossa con maggiori dimensioni del corpo e maggior produzione, ma simile produttività ed efficienza per le razze Rendena e Grigio Alpina. Mentre il confronto a parità aziendale e il corretto rapporto sui caratteri di produzione hanno ridotto fortemente le differenze in produttività aziendale, non le hanno ridotte a livello di composizione del latte, abilità coagulativa, ed efficienza alla trasformazione casearia (in termini di recupero di sostanze nutritive dal latte nella cagliata), quindi le differenze tra le razze sono rimaste forti e la loro importanza sulla valutazione complessiva dell'efficienza è rimasta elevata.

3 General introduction

Since the beginning of animal domestication and for thousands of years, humans were selecting for their needs. However, intense genetic selection, in the form that is known nowadays, is an achievement of the last century. At first, selection was probably limited to submissiveness and manageability, but then breeding programs have focused on the genetic improvement of production traits, such as milk yield. The high levels of milk production of Holstein-Friesian cows formed a practical basis for the widespread popularity of this breed (Prescott, 1960). However, the heavy genetic selection for increased production worsened not only the quality and the technological properties of milk, but also the fitness and the longevity of these animals, reducing farm profitability, as well as the cows' ability to adapt to the environment in which they find themselves.

Since both farm animal welfare and milk qualitative aspects have become increasingly important from the societal point of view, and because of the increased cheese production at global level, other dairy and dual-purpose cow breeds have been reconsidered. However, one missing aspect is a fair comparison among different breeds regarding the quality and the technological aptitude of milk, or milk and cheese production. Previous studies in literature often regarded a small number of cows of two-three different breeds reared in one farm (Auldist et al., 2002; Mistry et al., 2002), or a large number of cows from many single-breed farms (Poulsen et al., 2013), so the effect of breed was confounded with that of herd and feeding strategies.

In the milk industry crossbreeding programs in dairy cattle are a feasible strategy, as they alleviate the fertility and longevity problems that can occur as a result of selection programs in dairy pure breeds (Heins and Hansen, 2012). However, they are also used to improve the milk fat and protein contents, and the technological aptitude of milk. In fact, when compared with pure Holstein-Friesian, crossbred cows are generally characterized for producing lower quantities of milk, fat, and protein (kg), with higher concentrations of milk fat and protein (%). However, the extent of these differences may vary upon the crossbreed type.

Hence, the comparison of different breeds in the same environment, and the study of the interactions between breeds and herd productivity level is necessary. Furthermore, the assessment of diverse aspects of milk production of different breeds in common environments could be useful not only for a reasonable choice as a function of the environment, but also for the use of more efficient crossbreeding programs. This evaluation could be used mainly for economic reasons, according to the productive vocation of the area, especially if the milk is destined to cheese production, and to better define the selection objectives within breed.

Besides Holstein-Friesian, that is the dominant dairy breed in the world, another extremely specialized dairy breed is Jersey. Jersey is the smallest of the dairy cattle breeds and produces, on average, less milk than Holstein-Friesian cows. However, this breed is well-known for the high quality of its milk (especially for %fat and %protein). Thus, although being less productive, Jersey cows are probably as much efficient as the Holstein. Other larger dairy breeds are addressed principally to the quality and the technological properties of milk, or to the fitness and fertility aspects, rather than milk yield. In the case of milk quality, Brown Swiss breed is recognized to have high casein index and very good milk fat to protein ratio for production of most cheeses, while Scandinavian breeds are preferred especially for longevity and fertility traits. The dual-purpose breeds (i.e., Simmental at international level, and local breeds such as the Rendena and Alpine Grey in North-east Italy), are known for compensating the lower milk yield by a better milk quality and renneting properties, and by a higher meat production (higher price of calves and dairy cull cows), compared to the Holstein-Friesian breed.

Hence, more focus should be given to those breeds. To make possible a fair comparison among such breeds we investigated different aspects of milk production, productivity and efficiency. The first aspect, besides the traditional quality and quantity of milk, regarded the study of milk renneting properties. As one of the main problems caused by the worldwide diffusion of the Holstein-Friesian breed is a general worsening of milk coagulation properties (**MCP**) (i.e., delayed milk coagulation and slower curd firming process), with a consequent decrease on percentage of cheese wheels labelled as first quality for some Italian dairy products (Bittante et al., 2011). Breed variation in terms of curd firmness over time (**CF$_t$**) pattern is known. Milk from Holstein-Friesian and some Scandinavian cattle breeds yield higher proportions of noncoagulating (**NC**) samples. As a result, samples with longer RCT and lower curd firmness (**a$_{30}$**), and samples for which curd-firming time (**k$_{20}$**) are not available. The problem is much less obvious in Brown Swiss, Simmental, and other local Alpine breeds. Bittante et al. (2012) provided with a review on the topic, but the results from the reported studies have been obtained from very different experimental conditions. Because the experimental conditions were so variable, direct comparisons among breeds could be made only by using data obtained within a single trial. Besides, these works were based on a small number of cows of two-three different breeds reared in a unique farm (Auldist et al., 2002; Jõudu et al., 2008), or on many cows from single-breed farms (Poulsen et al., 2013), so that the effect of breed was confounded with the effects of farm, feeding strategy, and sampling date, or the study was based on bulk milk samples from different single-breed farms (Mariani et al., 1984; De Marchi et al., 2007). So there is still considerable ambiguity with regard to the direct effect of breed and herd on MCP. The major limitations of traditional MCP have been in part overcome by prolonging the observation period and by using an innovative CF$_t$ modeling, which uses all available information provided by lactodynamograph. The method allows the estimation of RCT, the potential asymptotic curd firmness, the curd-firming rate, and the syneresis rate (Bittante et al., 2013). However for such traits, there are no studies available comparing the performance of different breeds. Another important aspect for the comparison among breeds concerns the cheese-making traits, efficiency and daily cheese productions, since the measurement of cheese yield (**CY)** is of economic importance at global level, and daily yield of cheese, expressed in kilograms of cheese produced daily per cow (**dCY**), is the final production target of many dairy farmers. However, the main problem in this case is that most of the studies in literature that involve cheese-making procedures have used bulk milk, principally because it is very time-consuming and labor intensive to produce a high number of small model cheeses from milk of individual cows. Also, due to practical difficulties and cost of experimental trials on %CY and its related traits, very few published studies have measured the individual %CY traits of bovine milk (Cipolat-Gotet et al., 2013), and studied their genetic and environmental relationships (Cecchinato and Bittante, 2016). Nevertheless, individual cow information is needed for the genetic analysis of these traits (Othmane et al., 2002).

One more aspect regards a more nutritional side of milk, the study of mineral elements. Macro-minerals, especially Ca, P and Mg, are also fundamental for milk coagulation (Malacarne et al., 2014), and Na, Cl and K are involved in diagnosis of mastitis in dairy cows (Hamann and Krömker, 1997). The mineral content of milk is not constant but varies according to several different factors, such as breed (Mariani et al., 2002), the stage of lactation (Gaucheron, 2005) and feed management (Van Hulzen e al., 2009). However, the effects of the most important sources of variation, herd and breed, are often confounded because of the use of data from single-breed herds and the frequent relation between specific breeds and specific environmental and management features. Much less focus has been given in the study of essential micro-minerals, especially for comparing different breeds.

Further aspects are linked to the concepts of production, productivity and efficiency of animals, since they differ to each other's, even being strictly correlated. As highlighted at the beginning, the high milk production of Holstein cows in the past decades promoted their worldwide diffusion. Productivity is often linked to the dimension or cost of the producing unit, and it is frequently expressed by a ratio between the food produced and the "size" of productive animal, where the size could be represented simply by the body weight (**BW**) of the animal, or by some predictor of its nutrients requirement for maintenance, of which the metabolic weight (**MW**) and the body protein weight (**PW**) are examples. The Jersey cows produces much less milk than Holsteins, but their size is much smaller, so if their productivity per unit BW or MW be lower or higher respect to Holsteins is questionable. Efficiency of production is even more complicated to be defined and measured. It implies a complete balance of production activity, and it could be expressed as a ratio between production output, at the nominator, and the sum of all production inputs, at the denominator. The primary genetic characteristic of a cow - its breed - has been shown to have an enormous effect on milk yield, and on its main destination: cheese yield (Banks et al., 1986; Verdier-Metz et al., 1995), but the comparisons of different breeds in scientific papers could go from few dozens of cows with a lot of precise data obtained in an experimental farm (Mistry et al., 2002; Hurtaud et al., 2009; Martin et al., 2009), to few data obtained from whole cattle populations underwent production recording systems with a large majority of single-breed herds (Malacarne et al., 2006; Bland et al., 2015). In this last case the effect of individual herds and dairy systems are confused with the effect of breed.

These aspects together allow for a complete and balanced evaluation of the different breeds, and permit to define also their productive efficiency in a panoramic view.

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5 The COWPLUS multi-breed trial

This PhD thesis was part of the general architecture of Cowplus project, that was developed with the aim to promote cattle breeding in mountain environment of Trentino region. The data set serving all the chapters is constituted of 1,508 cows of 6 different breeds (3 dairy specialized: Holstein-Friesian, Brown Swiss, Jersey, and 3 dual-purpose: Simmental, Rendena, Alpine Grey), reared in 41 multi-breed herds (selected from a starting sample of 610 dairy farms previously surveyed) in Trentino and Bolzano provinces.

The sampling of the animals lasted 9 months, from March till December 2013. Cows were checked and sampled during evening milking, for:

1) identification data:

- body size: chest girth, estimated body weight, body condition score;
- life phases: age first calving, number of lactations, calving interval, dry period, days in milk, lactation to calving;
- milk production (kg): milk yield, milk fat, milk protein.

2) Health status: rectal temperature, heart rate, respiratory profile, appetite and fecal consistency.

3) Blood serum analyses (g/L):

- total protein, albumin, globulin, BHB.

4) Milk quality:

- macro components (%): fat, protein, lactose, casein, total solids;
- acidity: pH;
- Somatic Cell Count (SCC) converted to Somatic Cell Score (SCS);
- casein fractions and whey proteins (g/L): αS1-CN, αS2-CN, β-CN, κ-CN, ALA, BLG, lactalbumin, lactoglobulin, lactoferrin;

- macro- and micro-minerals (mg/kg): Calcium, Phosphorous, Sodium, Potassium, Magnesium, Sulphur, Copper, Iron, Manganese, Selenium, Zinc, Boron, Silicon, Tin, Strontium;
- fat globules (µm): $D_{2,1}$, $D_{3,2}$, $D_{4,3}$, D_{50}, D_{mode}, span, SSA (m^2/g fat);
- fatty acid profile (%): 74 single fatty acids and 17 categories and indices;
- bacteriological analyses (cfu/mL): 7 contagious and 12 environmental pathogens.

5) Milk renneting aptitude:

- traditional coagulation properties (MCP): RCT (min), k_{20} (min), a_{30} (mm), a_{45} (mm), a_{60} (mm);
- modeled curd firming over time (CF_t) parameters: RCT_{eq} (min), CF_P (mm), k_{CF} (%/min), k_{SR} (%/min), CF_{max} (mm), t_{max}(min).

6) Cheese-making ability (on individual model-cheeses):

- REC traits (%): $REC_{PROTEIN}$, REC_{FAT}, REC_{SOLIDS}, REC_{ENERGY};
- CY traits (%): CY_{CURD}, CY_{SOLIDS}, CY_{WATER};
- theoretical CY traits (%): *Th*-CY_{CURD}, *Th*-CY_{SOLIDS};
- cheese-making efficiency (%): *Ef*-CY_{CURD}, *Ef*-CY_{SOLIDS};
- daily cheese productions (kg/d): dCY_{CURD}, dCY_{SOLIDS}, dCY_{WATER}.

7) Model-cheese quality:

- macro components (%): moisture, fat, protein, salt, total solids;
- acidity: pH;
- texture indices: hardness (N), shear force (J 10^{-3});
- colorimetric indices: L*, a*, b*, C, h°;
- macro- and micro-minerals (mg/kg): Calcium, Phosphorous, Sodium, Potassium, Magnesium, Sulphur, Copper, Iron, Manganese, Selenium, Zinc, Boron, Silicon, Tin, Strontium;
- fatty acid profile (%): 74 single fatty acids and 17 categories and indices;

- Near-infrared (NIR) spectra collection.

In addition, information on farm management, diet, production performance, agronomic management of the surfaces, the management of waste, and the energy consumption were also collected.

6 General aims

The general aims of this research were to:

1) quantify and characterize the effects of high or low herd productivity (defined according to the milk net energy yielded daily by the cows);

2) quantify the variability of herds within herd productivity class;

3) make a within-herd comparison of 3 dairy and 3 dual-purpose breeds;

4) quantify the effects of DIM and parity,

on the following traits:

a) traditional milk quality (*Chapter 1, 2, 3, 4*):

- macro components (%): fat, protein, lactose, casein, total solids;
- acidity: pH;
- Somatic Cell Count (SCC) converted to Somatic Cell Score (SCS);

b) milk renneting aptitude (*Chapter 1*):

- traditional MCP: RCT, k_{20}, a_{30}, a_{45}, a_{60};
- modeled CF_t parameters: RCT_{eq}, CF_P, k_{CF}, k_{SR}, CF_{max}, t_{max};

c) cheese-making ability (*Chapter 2*):

- CY traits (%): CY_{CURD}, CY_{SOLIDS}, CY_{WATER};
- REC traits (%): $REC_{PROTEIN}$, REC_{FAT}, REC_{SOLIDS}, REC_{ENERGY};
- theoretical CY traits (%): *Th*-CY_{CURD}, *Th*-CY_{SOLIDS};
- cheese-making efficiency (%): *Ef*-CY_{CURD}, *Ef*-CY_{SOLIDS};
- daily cheese productions (kg/d): dCY_{CURD}, dCY_{SOLIDS}, dCY_{WATER}.

d) milk mineral elements (mg/kg) (*Chapter 3*):

- macro-minerals: Calcium, Phosphorous, Sodium, Potassium, Magnesium, Sulphur;
- micro-minerals: Copper, Iron, Manganese, Selenium, Zinc, Boron, Silicon, Tin, Strontium.

e) productivity and efficiency indicators (*Chapter 4*):

- body size, condition and estimated composition of lactating cows: body weight (BW), metabolic weight (MW), body condition score (BCS), hearth girth; empty body composition (%): protein, fat and water; body composition (kg): protein, fat and water on body weight; body energy;
- milk yield, composition and estimated energy content and cheese yield: protein, fat, lactose, energy;
- cheese yield: theoretical, actual, and relative index;
- daily yield per cow: milk, cheese, protein, fat, energy.
- milk productivity ratios: milk yield (g/kg) on BW, MW and PW; energy yield (kJ/kg) on BW, MW and PW; cheese yield (g/kg) on BW, MW and PW;
- energy requirements (MJ/d): maintenance, activity, lactation, pregnancy;
- income over feed costs (IOFC, €/d);
- efficiency (%); energy and costs.

Chapter 1

Breed of cow and herd productivity affect milk composition and modeling of coagulation, curd firming and syneresis

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ABSTRACT

Milk coagulation properties (**MCP**) have been widely investigated in the past using milk collected from different cattle breeds and herds. However, to our knowledge, no previous studies have assessed MCP in individual milk samples from several multi-breed herds characterized by either high or low milk productivity, thereby allowing the effects of herd and cow breed to be independently evaluated. Multi-breed herds (n = 41) were classified into two categories based on milk productivity (high vs low), defined according to the average milk net energy daily yielded by lactating cows. Milk samples were taken from 1,508 cows of 6 different breeds - 3 specialized dairy (Holstein-Friesian, Brown Swiss, Jersey), and 3 dual-purpose (Simmental, Rendena, Alpine-Grey) breeds - and analyzed in duplicate (3,016 tests) using two lactodynamographs (Formagraph, FOSS) to obtain 240 curd firming (**CF**) measurements over 60 min (one every 15 sec) for each duplicate. The 5 traditional single-point MCPs (RCT, k_{20}, a_{30}, a_{45}, and a_{60}) were yielded directly by the instrument from the available CF measures. All 240 CF measures of each replicate were also used to estimate the 4 individual equation parameters (RCT_{eq}, CF_P, k_{CF}, and k_{SR}) and the derived traits (CF_{max}, and t_{max}) by curvilinear regression using a nonlinear procedure (PROC NLIN). Results showed that the effect of herd-date on the traditional and modeled MCPs was modest, ranging from 6.1% of total variance for k_{20} to 10.7% for RCT, while individual animal variance was the highest, ranging from 32.0% for t_{max} to 82.5% for RCT_{eq}. The repeatability of MCP was high (>80%) for all traits except those associated with the last part of the lactodynamographic curve (i.e., a_{60}, k_{SR}, k_{CF} and t_{max}: 57 to 71%). Reproducibility, taking into account also the effect of instrument, was equal or slightly lower than repeatability. Milk samples collected in farms characterized by high productivity exhibited delayed coagulation (RCT_{eq}: 18.6 vs 16.3 min) but greater potential curd firmness (CF_P: 76.8 vs 71.9 mm) compared with milk samples collected from low productivity herds. Parity and DIM influenced almost all the MCPs. Large differences in all MCP traits were observed among breeds, both between specialized and dual-purpose breeds, and within these two groups of breeds, even after adjusting for milk quality and yield. Milk quality and MCP of samples from Jersey cows, and coagulation time of samples from Rendena cows were better than in milk from Holstein-Friesian cows, while intermediate results were found with the other breeds of Alpine origin. The results of this study, also taking into account the intrinsic limitation of this technic, show that the effects of breed on traditional and modeled MCPs are much greater than the effects of herd productivity class and of parity and DIM. Moreover, the variance in individual animals is much greater than the variance in individual herds within herd productivity class. It seems that improvement in MCP depends more on genetics (breed, selection, etc.) than on environmental and management factors.

Key words: milk coagulation, curd firming, syneresis, breed, herd productivity.

INTRODUCTION

Milk coagulation properties (MCP) have consequences for cheese-making, cheese yields and cheese quality. The major cheese-making problems are fast coagulation of milk (acid, fermented milk), late or absence of coagulation of milk (especially with some milk protein genetic variants), weak curd firmness at cutting and slow syneresis of curd. Recently, Cecchinato and Bittante (2016) found strong relationships between cheese yield and curd firming patterns. Milk renneting properties also affect cheese quality (Horne and Banks, 2004), and are therefore particularly important for Protected Designation of Origin (PDO) cheeses (Mariani and Battistotti, 1999; Bertoni et al., 2005; Bittante et al., 2011).

Several techniques can be used to assess MCP (Klandar et al., 2007), but the most common approach used in both the laboratory and industry is lactodynamography. Traditionally, three single-point traits are recorded: rennet coagulation time (RCT, min); time to a curd firmness (CF) of 20 mm (k_{20}, min); and CF 30 min after enzyme addition (a_{30}, mm).

The major limitations of the traditional MCPs are: the incidence of samples not coagulating (**NC**) within 30 minutes (no RCT, k_{20} and a_{30} available); the much greater incidence of late-coagulating (**LC**) samples that fail to reach 20 mm CF (no k_{20} available); the high correlation between RCT and a_{30} (so that the latter trait has limited informative value).

Recently, a more informative method to overcome, at least in part, the above-mentioned limitations and acquire detailed information is to model the curd-firming process over time (CF_t) using the hundreds of single-point pieces of information automatically available for each milk sample analyzed (Bittante, 2011), and extend the lactodynamographic test period beyond 30 minutes (Bittante et al., 2013).

Traditional MCPs obtained from lactodynamographs have been used in several studies to compare milk from cows of different breeds, as reviewed by Bittante et al. (2012). Comparisons are difficult, however, because they are often based on a small number of cows of two-three different breeds reared in one (experimental) farm (Auldist et al., 2002; Jõudu et al., 2008), or on a large number of cows from many single-breed farms (Poulsen et al., 2013), so that the effect of breed is confounded with the effects of farm, feeding strategy, and sampling date, or they are based on bulk milk samples from different single-breed farms (Mariani et al., 1984; De Marchi et al., 2007). In addition, the effect of feeding strategy is not well known as experimental trials focus on some specific diet ingredient (Kreuzer et al., 1996; Malossini et al., 1996), and very few studies have been carried out at the population level (Tyrisevä et al., 2003).

Over time, dairy farms have moved towards larger and more industrialized setups in which cows are fed high-energy diets, while dairy herds have changed in terms of the proportions and productivity of breeds, and dairy breeds have been assiduously selected to improve productivity and milk quality.

We have, therefore, carried out a large study involving several multi-breed herds characterized by variable levels of productivity, which allows for independent evaluation of the effects of farm and of different cattle breeds. The specific aims of this study were: 1) to quantify and characterize the effects on MCP of high or low herd productivity (defined according to the milk net energy yielded daily by the cows); 2) to quantify the variability of herds within herd productivity class; 3) to make a within-herd comparison of 3 dairy and 3 dual-purpose breeds for their milk quality, traditional MCPs, and modeled CF_t; 4) to quantify the effects of DIM and parity, and assess the repeatability and reproducibility of traditional MCPs and CF_t modeled and derived traits.

MATERIALS AND METHODS

Multi-breed herds

The present study is part of the Cowplus project. A total of 1,508 cows from 41 multi-breed herds (2 to 5 breeds, with an average of 3.0) located in the Trentino Alto Adige region, north-eastern Italian Alps, were controlled once for daily milk production and sampled during the evening milking for milk quality analyses. A total of six breeds were sampled, 3 specialized dairy breeds:

Holstein Friesian (HF = 31 herds and 471 cows), Brown Swiss (BS = 36 herds, 663 cows), and Jersey (Je = 7 herds, 40 cows); and 3 dual-purpose breeds: Simmental (Si = 20 herds, 158 cows), and two autochthonous breeds, Alpine Grey (AG = 13 herds, 73 cows) and Rendena (Re = 8 herds, 103 cows). The herds comprised fifteen combinations of breeds: HF + BS + Si (n = 8 herds), HF + BS (n = 7 herds), BS + Si + AG (n = 6 herds), HF + BS + Re (n = 3 herds), HF + BS + Je (n = 3 herds), BS + AG (n = 3 herds), HF + BS + Si + AG (n = 2 herds), HF + AG (n = 2 herds), BS + Je (n = 1 herds), HF + BS + Si + AG + Re (n = 1 herd), BS + Si + AG + Re (n = 1 herd), HF + Si + Re (n = 1 herd), BS + AG + Re (n = 1 herd), HF + Si (n = 1 herd), HF + Re (n = 1 herd).

Dairy and dual-purpose breeds

The 41 mixed-breed dairy farms selected for the study had only cows enrolled in the Italian Herd Books of the 6 breeds studied and were practicing almost exclusively artificial insemination using national or imported semen from proven bulls or progeny testing young bulls.

The dairy large-framed Holstein Friesian cows in the province of Trento were obtained from semen mainly from Italian, German, American and Dutch bulls (Cecchinato et al., 2015a). In this study, the cows were characterized by a body-weight of 654±45 kg, a parity of 2.4±1.6, and DIM of 197±140.

The dairy large-framed Brown Swiss cows were obtained from semen from Italian, Austrian, German, American and Swiss bulls. Body size was very close to Holstein Friesians (656±46 kg), as was parity (2.6 ± 1.6) and DIM (188 ± 139).

The dairy small-framed Jersey breed has been recently introduced, and the cows came from semen imported mainly from the USA and Denmark. Body size was very small (413 ± 37 kg), while the other characteristics were similar to those of the other two specialized dairy breeds (parity 2.9 ± 2.1, DIM 214 ± 116).

The large-framed Simmental cows in the area belong to the dual-purpose strains of this breed reared mainly in the Alpine regions, and came from inseminations using semen from Italian, German, and Austrian bulls, as well as from French Montbeliarde bulls. Sires are often pre-selected for growth rate and muscularity through station performance testing, and the body size of the cows was only slightly greater than that of the two large-framed dairy breeds (662 ± 56 kg), as was parity (2.7 ± 1.9), while DIM was lower (177 ± 118).

The medium-framed local breeds, Rendena and Alpine Grey, are both dual-purpose breeds of Alpine origin. Bulls are selected in two steps, with pre-selection based on station performance testing (Bech Andersen et al., 1981). The Rendena breed has a dark chestnut coat, while the other has a grey coat, and both are of medium size (565 ± 48 and 527 ± 45 kg, respectively). They are similar to the Simmental breed in parity (2.8 ± 1.8 and 2.5 ± 1.7) and DIM (189 ± 94 and 158 ± 75).

Herd productivity classification

The herds were classified into two categories of productivity (**HP**), defined according to the average daily milk energy output (**dMEO**) of the lactating cows. The net energy content (NE_L) of milk was estimated with the following equation, proposed by the NRC (2001):

$$NE_L \text{ (Mcal/kg)} = 0.0929 \times \text{fat,\%} + 0.0547 \times \text{protein,\%} + 0.0395 \times \text{lactose,\%},$$

where NEL is the energy of one kg milk. The NE_L values obtained were converted to MJ/kg and multiplied by the daily milk yield of each cow (MJ/d) to obtain the individual dMEO of each cow. Individual dMEO data were subjected to an ANOVA using the GLM procedure in SAS (SAS Institute Inc., Cary, NC) in order to estimate the least square means (LSMs) of the dMEO for the selected herds after correcting for breed, DIM, and parity of the cows. After ranking the dMEO LSMs of the 41 farms, we divided the herds into high producing (**High-HP**: n = 20, average dMEO= 90.86 MJ/d) and low producing (**Low-HP**: n = 21, average dMEO= 56.35 MJ/d) on the basis of the median value.

Large-framed breeds (Holstein Friesian, Brown Swiss and Simmental) were found in herds of both high and low productivity, Jerseys only in High-HP herds, and local breeds (Rendena and Alpine grey) only in Low-HP herds.

Analysis of milk samples

Milk samples (without preservative) were adjusted to 4°C immediately after collection, and processed within 24 hours of sampling at the Milk Quality Laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) of the University of Padua.

All samples were analyzed for pH (portable pHmeter Crison Basic 25; Crison Instruments SA, Barcelona, Spain), and SCC (Fossomatic Minor, Electric A/S, Hillerød, Denmark). Milk SCC was log-transformed to SCS (Ali and Shook, 1980). Protein, fat and lactose contents were measured by a Milkoscan FT2 infrared analyzer (Foss Electric A/S) calibrated according to reference methods [ISO 8968–2/IDF 20–2 for protein (ISO-IDF, 2014); ISO 1211/IDF for fat (ISO-IDF, 2010); ISO 26462/IDF 214 (ISO-IDF, 2010) for lactose].

Milk coagulation properties were measured in duplicate using mechanical lactodynamographs (2 instruments; Formagraph, Foss, electric A/S Hillerød, Denmark) with pendula calibration carried out before each session of the trial. For each animal replicate, 10 mL of milk, heated to 35°C, was 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. Traditional single-point measurements of each milk sample [rennet coagulation time (RCT; min), time interval between gelation and attainment of curd firmness of 20 mm (k_{20}; min), and curd firmness at 30, 45 and 60 min after rennet addition (a_{30}, a_{45}, and a_{60}, mm)] were obtained directly from the instruments. A data file of the 240 curd firmness (CF) observations (1 every 15 s for the 60 min of the test) for each milk replicate was also extracted.

Modeling curd firmness and syneresis

The 4-parameter model (Bittante et al., 2013) was chosen for this study because a preliminary view of the CF_t data showed an appreciable decrease in CF in the final part of the curve of many milk samples. It uses all the information available for estimating the 4 parameters, which, unlike traditional MCPs, are not single-point measurements. The model tested was:

$$CF_t = CF_P \times [1 - e^{-k_{CF} \times (t - RCTeq)}] \times e^{-k_{SR} \times (t - RCTeq)},$$

where CF_t is curd firmness at time t (mm); CF_P is the asymptotical potential value of CF at an infinite time (mm); k_{CF} is the curd-firming instant rate constant (% × min^{-1}) k_{SR} is the syneresis instant rate constant (% × min^{-1}); and RCT_{eq} is RCT estimated by CF_t equation on the basis of all data points (min). The CF_P is conceptually independent of test duration and is not intrinsically dependent on RCT (unlike a_{30}). The parameter k_{CF} describes the shape of the curve from the time of milk gelation to infinity and is conceptually different from k_{20} as it uses all available information. The parameter k_{SR} is the curd syneresis instant rate constant (% × min^{-1}). The parameter k_{CF} is assumed to increase CF toward the asymptotic value of CF_P, whereas k_{SR} is assumed to decrease CF toward a null asymptotic value. In the initial phase of the test, the first rate constant prevails over the second, such that CF_t increases to a point in time (t_{max}) at which the effects of the 2 parameters are equal but opposite in sign; this is when CF_t attains its maximum level (CF_{max}). Thereafter, CF_t decreases, tending toward a null value due to the effect of curd syneresis and the corresponding expulsion of whey. The RCT_{eq} parameter has the same meaning as the traditional RCT measure but was estimated using all available data.

Statistical Analysis

To avoid convergence and estimation problems, the procedure (Bittante et al., 2013) was modified to include curd firmness measurements up to 45 min from the addition of rennet (180 records for each individual milk sample, one every 15 sec), while CF_P, was calculated multiplying CF_{max} by 1.34, that is the coefficient resulting from the linear regression between CF_P and CF_{max} values obtained in a preliminary analysis. The other three CF_t model parameters (RCT_{eq}, k_{CF}, and k_{SR}) were estimated by curvilinear regression using the nonlinear procedure (PROC NLIN) in the SAS software (SAS, 2001). The parameters of each individual equation were estimated using the

Marquardt iterative method (350 iterations and a 10^{-5} level of convergence) according to Bittante (2011).

Experimental data from traditional and modeled MCPs (two replicates per cow) were analyzed using the MIXED procedure (SAS Institute Inc., Cary, NC) according to the following base model:

$$y_{ijklmnopq} = \mu + HP_m + Herd_n(HP)_m + Breed_k + Parity_j + Breed_k \times Parity_j + HP_m \times Parity_j + DIM_i + HP_m \times DIM_i + Animal_l + Instrument_o + Pendulum_p(Instrument)_o + e_{ijklmnopq}$$

where $y_{ijklmnopq}$ is the observed trait (RCT, k_{20}, a_{30}, a_{45}, a_{60}, RCT_{eq}, CF_P, k_{CF}, k_{SR}, CF_{max}, t_{max}); μ is the overall intercept of the model; HP_m is the fixed effect of the m^{th} herd productivity (m = 2 levels); $Herd_n$ is the random effect of the n^{th} herd (n = 1 to 41) within the m^{th} class of herd productivity; $Breed_k$ is the fixed effect of the k^{th} breed (k = HF, BS, Je, Si, AG and Re); $Parity_j$ is the fixed effect of the j^{th} parity (j = 1 to ≥ 4); DIM_i is the fixed effect of the i^{th} class of days in milk (i = 1 to 11; class 1, 5-35 days (324 samples); class 2, 36-65 d (254 samples); class 3, 66-95 d (256 samples); class 4, 96-125 d (274 samples); class 5, 126-155 d (250 samples); class 6, 156-185 d (238 samples); class 7, 186-215 d (244 samples); class 8, 216-245 d (262 samples); class 9, 246-275 d (246 samples); class 10, 276-305 d (184 samples); class 11, > 305 d (482 samples); $Animal_l$ is the random effect of the l^{th} animal (l = 1 to 1,508); $Instrument_o$ is the random effect of the o^{th} instrument (o = 2 instruments); $Pendulum_p$ is the random effect of the p^{th} pendulum (p = 1 to 10) within the o^{th} instrument; $e_{ijklmnopq}$ is the random residual ~ N (0, σ_e^2).

The MY and chemical components of milk (one observation per cow) were analyzed using the same model without Animal, Instrument, and Pendulum (Instrument) as random factors (reduced model).

A model that also included the breed × herd productivity interaction (interaction model) was fitted to test the data from the three HF, BS and Si breeds present in both classes of herds. As this interaction was almost never significant, or, when significant, was not relevant, the results of this model analysis are not shown nor discussed. The breed × DIM interaction was not included in the model because of the low number of observations in some cells of the less represented breeds.

A further model (extended model) was used to analyze the direct effects of breed on MCP and CF_t traits corrected for the milk yield and quality traits and was obtained from the base model with inclusion of linear covariate of milk yield, fat%, protein%, lactose%, pH and SCS. Moreover, the breed effect was considered random to obtain a correct quantification of breed variance. The indirect effect of breed on MCP and CF_t traits due to breed differences in terms of milk yield and quality was obtained subtracting the breed variance yielded by the extended model from the breed variance obtained from the base model (with breed as random effect). Both direct and indirect breed variance were represented as percentage of total breed variance.

Orthogonal contrasts were estimated between the LSMs of traits for the effect of breed:

a) dairy specialized (HF, BS and Je) *vs* dual purpose breeds (Si, AG and Re);

b) within specialized, large-framed *vs* small-framed breeds (HF + BS *vs* Je), and

c) comparison of the two large-framed dairy breeds (HF *vs* BS);

d) within dual-purpose, large-framed breed *vs* medium-framed local breeds (Si *vs* Re + AG), and

e) comparison of the two medium-framed local dual-purpose breeds (Re *vs* AG) .

Orthogonal contrasts were also estimated between the LSMs of traits for the effect of parity: a) 1st *vs* ≥2nd, b) 2nd vs ≥3rd, c) 3rd *vs* ≥4th.

RESULTS

Milk quality and coagulative ability

Descriptive statistics of milk yield, chemical composition, traditional coagulation properties and curd firming modeling (CF_t) equation parameters of the milk samples are summarized in Table 1. All traits, excluding pH and lactose, exhibited high variability due to the diversity of herd productivity and of the six sampled breeds. The coefficient of variation of traditional and modeled MCPs varied between 17% for t_{max} and 64% for k_{20}.

Effects of animal and herd-date, repeatability and reproducibility

Variances in single test-day milk yield (MY), milk quality, traditional MCP, and CF_t equation parameters are summarized in Table 2. Regarding MY and chemical composition, the proportion of variance due to herd-date is very large for pH, followed by MY, while the incidence of the effect of herd-date on the other traits was smaller (8.9% for lactose content to 24.6% for protein content). As these traits were not analyzed in duplicate, the proportion of the total variance listed as animal also includes the residual component.

In the case of traditional and modeled MCPs, among the random effects, herd-date is modest, compared with the same effect on milk production and quality, which varies from 6.1% for k_{20} to 10.7% for RCT. The individual effect had the highest variance, from 61.3 to 82.5% for the majority of the traits considered (7/11), but was lower only for the traits recorded mainly in the last part of the curve (5/11). Instrument had very little effect on variability in the renneting properties of milk (from 0.0% for the time measurements to 4.7% of the total variance for a_{60}, data not shown), while pendulum within instrument affected results more than instrument, with a range from 1.0% for the two RCT traits to 13.6% for k_{CF} (data not shown). Repeatability was very good (81.7 to 94.1%) for the majority of traits analyzed (7/11), but less so in the 5 traits recorded in the last part of the CF_t curve (57.1% for t_{max} to 70.7% for a_{60}). Reproducibility was equal to or slightly lower than repeatability, because of the low effect of instrument on these traits.

Effects of herd productivity and parity

Least square means of herd productivity (HP) and parity, and *F*-values of the same factors of variation and of the HP × parity and HP × DIM interactions are summarized in Table 3. Milk yield was obviously very different in the two herd productivity categories, and also milk protein and fat contents were higher in High-HP than in Low-HP, while lactose, pH, and SCS were almost identical in the two groups.

Parity affected both milk production and quality, except for fat content. Least square means of classes of parity showed that MY increased particularly from the first to the following lactations, as expected, while the opposite trend was noted for protein content. It is also worthwhile noting (data not shown) that the milk yield of cows reared in the High-HP farms increased from the first to third lactation by an average of 3.7 kg/d, while the milk yield of cows reared in the Low-HP farms increased by only 2.2 kg/d (HP × Parity interaction: $P<0.001$), although these increases represent almost the same proportion of primiparous daily yield (+15% and +14% for High-HP and Low-HP, respectively). Acidity and SCS of milk tended to increase almost linearly across classes of parity, while lactose showed an almost opposite trend. The HP × parity interaction affected lactose slightly ($P < 0.05$), but SCS content greatly because the cell content of milk from cows reared in High-HP farms (data not shown) increased by 57% from first to third lactation, while SCS of milk from Low-HP farms increased only by 8% (HP × Parity interaction: $P < 0.001$).

Regarding traditional MCPs, milk samples from farms characterized by High-HP presented delayed RCT, although the curd firmness was higher 60 minutes after rennet addition compared with milk samples from Low-HP farms. Parity affected all the traditional coagulation traits, because milk samples from primiparous cows had faster coagulation and higher curd firming properties than milk from multiparous cows. The only significant, but not relevant, interaction was HP × DIM for k_{20}.

Moving to the CF_t model parameters and derived traits, the predicted RCT_{eq} was very similar to the measured trait (shorter in samples from Low-HP farms and from primiparous cows), although samples from Low-HP farms also had a smaller asymptotical potential value of CF (CF_P), and a smaller CF_{max} attained at a shorter t_{max} time than the samples from High-HP farms.

Results confirmed that milk from primiparous cows coagulates earlier than milk from multiparous cows (RCT_{eq}), and attains a greater CF (CF_P and CF_{max}), but has similar curd-firming and syneresis instant rate constants (k_{CF} and k_{SR}) and in the same t_{max}. Modest HP × parity and HP × DIM interactions were observed for k_{SR} .

Effect of days in milk

The variation during lactation was highly significant for all the traits analyzed. In the case of daily milk yield, an interaction between DIM and HP was also noted. In fact, it can be seen from Figure 1 that cows reared in farms characterized by High-HP displayed (as an average of the 6 breeds studied) the typical pattern observed in dairy cows: an increase in production until lactation peak (65 to 95 DIM class) and an almost linear decrease thereafter. The pattern of milk yield was different in cows reared on farms characterized by Low-HP, because the lactation peak coincided with the first DIM class (5 to 35 d) followed by an almost linear decrease.

The average values of the fat and protein contents of milk were lowest in samples collected at 35 to 65 DIM (3.79% and 3.39%, respectively), and then increased almost linearly until the end of lactation (4.78% and 4.08%, respectively). The HP × DIM interaction also affected these traits (table 3). The milk protein content of cows from High-HP was +0.25 percentage points greater than from Low-HP at the beginning of lactation, and decreased progressively to +0.06 at the end (data not shown). Milk fat content exhibited a similar, although more variable, trend, moving from +0.38 to +0.13 percentage points during lactation in favor of the cows reared in High-HP.

Lactose exhibited an opposite pattern to fat and protein, the highest value (5.12%) being in the second DIM class, the lowest in the last (4.86%). The pH increased over the first three classes of DIM (6.45 to 6.50), then to 6.52 at the end of lactation. Similarly to fat and protein, average SCS values were lowest in the second DIM class (2.15), then progressively increased until the end of lactation (3.65). Although the HP × DIM interaction was significant (Table 3), comparison of the two HP levels did not exhibit a clear trend during lactation, the values being more erratic (data not shown).

The pattern of traditional single-point MCPs during lactation is shown in Figure 2. It is evident that RCT increased rapidly during the first part of lactation, while the changes occurred more slowly during the second part. The k_{20} trait increased slightly during the first trimester of lactation, but the initial values tended to be recovered during the following two thirds of lactation.

All three curd firmness values (a_{30}, a_{45} and a_{60}) were high at the beginning of lactation, reduced during the first part of lactation, were stable during mid-lactation, and tended to increase in the last couple of months.

The patterns of the CF_t modeling parameters and derived traits are shown in Figure 3 (the RCT_{eq} pattern is not shown because it was almost identical to the traditional RCT pattern reported in Figure 2a). The pattern of the traits showing asymptotical potential and maximum curd firmness (CF_P and CF_{max}) was similar to the pattern for traditional curd firmness: a decrease at the beginning, stabilization in the middle, and improvement toward the end of lactation. The pattern of the time needed to attain CF_{max} (t_{max}) was opposite to that of CF_{max}. Moving to the two instant rate constants, k_{CF} showed a slight decrease at the beginning of lactation followed by a rapid increasing until the end of lactation, while k_{SR}, on the contrary, was the only trait not affected by stage of lactation. Anyway, the effect on CF_t modeling of different coagulation conditions (rennet concentration, pH, Ca, casein content, etc.), especially in late lactation samples, need to be further investigated.

Effect of breed

Least square means of the effects of breed and their orthogonal contrasts (*F*-value) for all the observed traits are reported in Table 4. All these least square means are obviously corrected for all the other factors of variation included in the model (herd productivity class, individual herds, and parity and DIM of the cows).

Comparing, first, the average of the three specialized dairy breeds (Holstein Friesian, Brown Swiss and Jersey) with those of the dual-purpose breeds (Simmental, Rendena and Alpine Grey), it will be noted that the difference in milk yield did not attain statistical difference. Regarding milk quality traits, specialized dairy breeds outyielded dual-purpose breeds in the three major chemical components (protein, fat and lactose) but not in average pH and SCS. The productive aptitude of cows did not *per se* affect any traditional coagulation properties, nor any CF_t parameters or derived traits, with the sole exception of the speed of the process as described by the two instant rate constants for curd firming and syneresis (on average faster with dairy than with dual-purpose breeds).

The three specialized dairy breeds differed considerably from each other for almost all the traits analyzed, while the differences were smaller among the three dual-purpose breeds. Results confirmed the small-framed dairy breed (Jersey) to have a smaller productive potential (daily milk yield: -31%) than the large-framed dairy breeds (Holstein Friesian and Brown Swiss), but higher milk protein (+11%) and fat (+35%) contents. Both the lactose content and pH of Jersey milk were slightly lower than in the heavier breeds. Milk from Jersey cows was characterized by much more favorable technological properties, whether expressed as MCP or CF_t parameters and derived traits (Table 4).

Compared with Holstein Friesians, Brown Swiss cows had a slightly lower productive potential (-12%), compensated for by greater milk fat and protein contents (+8% and +7%, respectively), and, in particular, by much more favorable milk technological properties.

Moving to dual-purpose breeds, it will be noted that the large-framed Simmental cows produce, on average, more milk (+14%) than the cows of the two medium-framed local breeds (Rendena and Alpine Grey), with a greater fat content (+10%) and a much smaller somatic cell content (-20% SCS, corresponding to -33% SCC). No differences were found between the averages of the two local breeds with respect to the coagulation, curd firming and syneresis properties of milk.

The differences recorded between the two local breeds were that Rendena cows produced more milk, with less protein, a lower pH, and more lactose and SCS than Alpine Greys. Moreover, Rendena milk samples coagulated earlier than Alpine Grey milk samples.

The breed × parity interaction affected MY, milk pH, SCS, and rate of curd firming traits (k_{20}, k_{CF}, k_{SR}, and t_{max}), but these differences were not quantitatively important.

DISCUSSION

Modeling the coagulation, curd firming and syneresis process

The major limitations of traditional MCP in cattle species, but not in sheep (Vacca et al., 2015), are the existence of NC samples (milk not coagulating within 30 min of rennet addition), from which it is impossible to estimate RCT, k_{20}, and a_{30}, as well as the high frequency of LC samples whose late coagulation make it difficult to obtain a k_{20} value (not reaching 20 mm of curd firmness within 30 min), and the high correlation between RCT and a_{30}, especially in late coagulating samples, so that the latter trait does not add much information beyond the former (Bittante et al., 2013). The use of traditional MCP with a 30 min test duration could bias comparisons among breeds, or dairy systems, characterized by different incidences of NC and LC samples. In addition, the traditional lactodynamograph setup for analyzing bovine milk was designed to explore primarily the coagulation and curd-firming process, not syneresis.

Therefore, extending the lactodynamographic analysis beyond 30 minutes and applying a 4-parameter curd firming model allowed us to model the entire pattern of coagulation, curd firming, and syneresis. The number of NC samples (without any traditional MCP measures) decreased from about 6.3% of milk samples at 30 min from rennet addition to less than 1.0% at 60 min. Also, the number of LC samples (those failing to reach a minimum curd firmness of 20 mm (no k_{20})) was 17.0% after 30 min and only 2.6% after 60 min. However, it was also possible to extract the new parameters from the majority of these very late-coagulating samples using the CF_t equation, so that milk samples without predicted CF_t parameters only accounted for 1.1% of the total. Moreover, the pattern of coagulation, curd firming, and also syneresis emerges more clearly from the model based on all 240 informative points for each sample than from 5 of those points, as with traditional traits.

The repeatability obtained was similar to that estimated by other authors on relatively small sets of data, (Caroli et al. 1990; Dal Zotto et al., 2008). The only previous study estimating the repeatability of modeled parameters (Bittante et al., 2013) used a 4-parameter model based on data from bulk milk samples from Holstein Friesian cows of one experimental herd, and not from individual milk samples from cows of 6 breeds reared in 41 mixed-breed farms, like in the present study. Moreover, repeatability was not expressed in terms of the proportion of total variance, but in the unit of measure of each trait. Comparison of the two trials could be carried out using the residual mean square error as an indication of between-replicate variability. Compared with the previous trial, in the present study the residual variability was larger for RCT_{eq} (1.6 *vs* 0.7 min, respectively), similar for CF_P (7.1 *vs* 6.9 mm, respectively) and for k_{CF} (1.5 *vs* 1.8 %/min, respectively), and smaller for k_{SR} (0.17 *vs* 0.33 %/min, respectively). In any case, repeatability was satisfactory for RCT_{eq}, CF_P and CF_{max}, but lower for k_{CF}, k_{SR} and t_{max}. It is clear that the increased interest in prolonging the duration of the test should motivate the instrument manufacturer to produce lactodynamographs with improved repeatability also in the second part of the prolonged test.

Effects of animal and herd on lactodynamographic traits

Figure 4 shows the combined effects of parity on CF_t modeling parameters and highlights the superiority of milk from primiparous over multiparous cows, confirming the results found with traditional MCP (Tyrisevä et al., 2003) as well as modeled CF_t patterns (Malchiodi et al., 2014; Bittante et al., 2015).

In addition to the recognized effects of lactation stage on milk production and quality traits, the present study confirmed the importance of DIM for both traditional MCP and CF_t equation parameters. Figure 5 clearly shows that the effect of DIM is more important for coagulation than for curd firming and syneresis traits, and that milk produced at the beginning of lactation is superior to that produced thereafter, confirming previous reports by Macheboeuf et al. (1993a), Kreuzer et al. (1996), Tyrisevä et al. (2004), Jõudu et al. (2007), and Malchiodi et al. (2014).

Some studies carried out on a large number of cows with repeated samplings made it possible to estimate the animal repeatability of lactodynamographic traits after correcting for parity and DIM. As reviewed by Bittante et al. (2012), the animal repeatability of the main traditional

MCPs was close to 60%, slightly greater than for milk yield and composition traits. The animal repeatability estimated by Caroli et al. (1990) for the same traits was slightly lower (48 to 56%). In the present study, the animal repeatability of traditional MCPs was greater, close to 80%, but it should be noted that it was calculated on replicated analyses of the same milk sample, and not on subsequent samplings.

No estimates relating to animal repeatability are available in the scientific literature for the new modeling parameters and derived traits. It should be noted that regarding the lower instrumental repeatability with the increased time interval from rennet addition, animal repeatability is 82% for RCT_{eq}, around 60% for the two instant rate constants, and much lower for the remaining traits (as for single point a_{60}).

The effect of individual herd has also been studied in several large surveys, although only a few of them included individual herd as a random factor allowing an estimation to be made of the corresponding variance component expressed as a proportion of total variance. Tyrisevä et al. (2004), Ikonen et al. (2004), and Vallas et al. (2010) sampled a large number of farms (73 to 693) and included this effect as a random effect in modeling MCP data. Herd variability in these studies represented a small proportion (lower than 10%) of total variance of traditional MCP. Slightly greater was the incidence of herd variance on total variance obtained by Cecchinato et al. (2013) on traditional MCP obtained from prolonged tests using different techniques.

The present study confirmed that the effect of herd-date within class of herd productivity represented a very low proportion (6% to 13%) of total variability for both traditional single point MCP and new CF_t modeling parameters and derived traits, especially when compared with MY and milk quality traits (Table 2). If we consider that herd clusters together several management criteria, such as housing conditions (free *vs* tie stalls), diet administration (total mixed ration, silage, summer pastures, etc.) and diet quality (percentage of starch, NDF and Crude Protein), and that herd here is combined with date of sample collection (and therefore also with season), the % variability in MCP and the modeled parameters explained by this factor is very low. This means that improvement in the MCP and CF_t parameters is basically affected mainly by variation in individual animal factors (breed, genetics, parity, stage of lactation, etc.) and much less by factors like environment, farm management, animal feeding, milking systems, etc.

Effect of herd productivity on lactodynamographic properties

To our knowledge no previous studies have investigated the effect of herd productivity (high or low) on the lactodynamographic properties of milk. Only Oloffs et al. (1992) sampled animals on about 1,400 farms and separated them into 3 classes based on average milk production levels, but the effect of these classes of herd productivity on the response variables was not shown. However, milk energy output (dMEO, MJ/d) is based on daily production of fat, protein and lactose, and this explains why, after correcting for breed, DIM and parity of cows, the herds with High-HP had not only higher daily MY but also better milk quality. Moreover, the herds with the highest average milk energy outputs are probably the best managed (i.e., in terms of diet and health). On the other hand, milk coagulation (RCT and RCT_{eq}) was faster in samples from herds characterized by Low-HP, but curd firmness tended to be reduced (Table 2). The combined effect of HP on lactodynamographic traits can be seen in Figure 6, which shows the patterns of coagulation, curd firming and syneresis obtained from the LSMs of the CF_t equation parameters for the two HP classes. It is evident that in the first phase of the test (until 30 min) milk samples from low productivity farms are better, while the opposite is true in the second phase of the test (not analyzed in a traditional 30 min test). It worth noting that the parameters relative to the second phase of the lactodynamographic test are those better correlated with cheese yield, and fat and protein recovery in the curd (Cecchinato and Bittante, 2016).

Further information can be indirectly obtained from the survey carried out by Tyrisevä et al. (2004) which classified 125 Finnish dairy farms not according to production level but to some of the cows' feeding criteria (number of daily administrations of concentrates and type of concentrate used). The RCT was not affected by feeding criteria, but a small favorable effect on a_{30} was observed including a moderate amount of oats in the diet. In particular, feeding practices leading to greater milk yield (use of compound feed and concentrates administrated 4 or more times per day) led to an improvement in MCP similar to that found in the present study's comparison of milk from farms characterized by high *vs* low average daily milk energy output (Table 3). Recently, in a survey carried out on 85 herds of Brown Swiss cows, we found that traditional farms with lower average milk yields produced milk characterized by earlier coagulation, a greater syneresis rate, and a smaller and earlier CF_{max} than milk from modern, more productive, farms (Bittante et al., 2015), a result similar to our comparison of Low-HP and High-HP.

Several experimental studies on the effects of feeding strategy on MCP have been published (Malossini et al., 1996; Coulon et al., 2004), but they often compared different forages, concentrates or supplements, and found small differences in the average milk yield of cows.

The relationships between the productivity of individual cows within herd and lactodynamographic traits have been the subject of several studies. In a review of 8 studies on phenotypic and genetic parameters at the population level, Bittante et al. (2012) found very low phenotypic correlations between individual milk yield and traditional MCP (-0.06±0.08 for RCT and -0.03±0.04 for a_{30}), and more variable genetic correlations (-0.15±0.18 for RCT and +0.04±0.22 for a_{30}). Herd productivity seems to have a greater effect than individual cow productivity within herd on both traditional and modeled MCPs.

Effect of breed within herd

In the present study, large differences among breeds were found. The comparisons of different cattle breeds in published research are almost all based on traditional MCPs obtained from lactodynamographic tests of 30 min. In these conditions, an appreciable number of milk samples do not coagulate (NC samples) and do not yield any MCP measures. As the incidence of NC samples differs in different breeds and these samples cannot yield any MCP traits, they can bias the estimation of MCP among different breeds, as discussed by Bittante et al. (2012), if the statistical models do not accommodate censoring (Cecchinato and Carnier, 2011). The bias on the k_{20} trait is even larger due to the LC samples, whose late coagulation does not allow curd firmness to reach the value of 20 mm (no k_{20} value). The entity of the bias in traditional MCP traits when comparing different breeds (in particular Holstein Friesian with Jersey and Rendena) could be predicted from the large differences across breeds in the incidence of NC and LC samples 30 min after rennet addition, as shown in Figure 7. Extending the test duration to 60 min greatly reduced the incidence of both NC and LC samples, so that the results reported in Table 4 for traditional MCP could be considered almost unbiased. The situation is even better for CF_t traits, as the incidence of samples without trait prediction is about 1%.

The specialized dairy breeds, in particular, exhibited large differences. Compared with large-framed Holstein-Friesian and Brown Swiss cows, small-framed Jersey cows are well known for their very low average milk yield but also the very high content of both fat and protein. Jersey cows also had the best renneting properties of all the 6 breeds compared in the present study. This superiority confirms previous results from the studies comparing breed reviewed by Bittante et al. (2012) and also from Poulsen et al. (2013) comparison of Jersey cows with Holstein and Swedish Red cows reared in different farms.

Of the two large-framed dairy breeds, HF and BS, BS cows yielded very favorable MCPs, and produced milk with shorter RCT and k_{20} values and higher curd firmness than milk from HF (table 4). These results agree with 9 studies reviewed by Bittante et al (2012). Within dual-purpose breeds, a large number of studies have confirmed the good average technological aptitude of milk from the large-framed Simmental breed, which was better than Holstein Friesian and close to Brown Swiss. These studies, like those on specialized dairy breeds, were mainly experimental trials carried out on research farms or, in some cases, surveys at the population level comparing milk from single-breed farms.

Medium-framed local breeds are of environmental significance, and are important from a cultural point of view, since they are related to local traditions and regional food products. Few studies have focused on measured MCPs of local breeds, such as Alpine Grey and Rendena, however, De Marchi et al. (2007) in their survey compared 5 of the 6 breeds investigated in our research, including the two local breeds (but no Jerseys). The analyses were carried out on samples of bulk milk from single-breed herds and the statistical model also included the effects of protein and fat percentages, SCS, titrable acidity, and log bacterial count, so that the effects of these five dairy cattle breeds reported also included the effects of differences in geographical area, dairy system and feeding, and management practice, but were corrected for milk quality traits. Nevertheless, the ranking of the five breeds was about the same as in the present study.

Since the differences among breeds were substantial, to distinguish the direct effect of breed on the lactodynamographic traits from the indirect effects arising from differences in milk composition and production, we included MY, protein, fat, lactose, pH, and SCS as general covariates in the basic model, and after that we calculated the differences in breed variances between the two models, with and without covariates, for each lactodynamographic trait. The results (not shown) revealed that no traits were affected by MY, favorable effects were exerted by protein (especially on curd firming traits), fat (especially on milk coagulation time), and lactose (on all traits) contents, an increase in pH was unfavorably related to lactodynamographic traits, while SCS had a minor negative effect. These effects confirmed the majority of results reported in the scientific literature (Bittante et al., 2012). The effect of breed was not totally explained by the introduction of milk quality covariates into the model, since they remained highly significant for all the traits examined. However, the proportion of total breed variance explained by MY and quality traits was very different among traits: 20% or less for RCT traits and t_{max}, and about 40 to 60% for the remaining traits (Figure 8). Even though the differences among the LSMs of the 6 breeds were smaller after taking the effect of milk yield and quality traits into account, the ranking of the 6 breeds remained almost unchanged.

No information is available in the scientific literature regarding the effect of breed of cows on the new CF_t model parameters. The only indirect information comes from a comparison of the patterns of coagulation and curd firming in milk from purebred Holstein Friesian cows with those of 2nd and 3rd generation crossbred cows (Malchiodi et al., 2014), which revealed a favorable effect of using Brown Swiss and Montbeliarde sires in the crossbreeding scheme.

The combined effects of breed on different lactodynamographic traits can be seen in Figure 9, which shows a comparison of the patterns of coagulation, curd firming and syneresis obtained from the LSMs of CF_t equation parameters for the 6 breeds. It is evident that breed exerts a strong effect, much greater than the effects of parity (Figure 4), DIM (Figure 5) and herd productivity class (Figure 6). Also immediately evident, is the superiority of the patterns characterizing the Jerseys and, in part, the Rendena breeds over the inferior Holstein-Friesian breed, while the other breeds of Alpine origin (Brown Swiss, Simmental and Grey Alpine) were intermediate. The differences among breeds are slightly less evident after correcting all the MCP traits for milk yield and quality (data not shown), but the ranking of breeds remains about the same. So the differences among breeds arise mainly from different genetic factors from those controlling milk, fat, protein and lactose secretions.

As reviewed by Bittante et al. (2012), within breed traditional MCPs have been found to exhibit heritability coefficients similar to those characterizing other milk traits, and significant effects of milk protein genetic variants (in particular, those related to κ-casein). But it has been shown that the effect of milk protein genetic variants can also explain, but only partially, the differences in the lactodynamographic profiles of different breeds (Ikonen et al., 1999; Auldist et al., 2002). Candidate gene (Tyrisevä et al., 2008; Glantz et al., 2011; Cecchinato et al., 2012 and 2015b) and genome-wide (Glantz et al., 2012; Gregersen et al., 2015; Dadousis et al., 2016) approaches have recently revealed that many other genes are involved in the control of these traits within breed, whether expressed as traditional MCPs or CF_t modeling. A comparison of different breeds with respect to the cheese-making ability of their milk based on molecular information is still wanting, and could provide new insights into the reasons for the differences in breed not mediated by milk yield and composition.

In any case, prediction of traditional MCPs at the population level by Fourier-Transform Infrared (FTIR) spectroscopy, and its use in indirect selection for cheese-making aptitude has proven to be effective (Cecchinato et al., 2009; Ferragina et al., 2015), while there is still little information on modeled CF_t traits.

CONCLUSIONS

In conclusion, this study on multi-breed herds allowed the effects of farm and of breed of cow to be independently evaluated. The use of CF_t modeling based on all the information available after rennet addition and on extension of curd firmness recording allowed for better representation of the effects of the different factors examined on coagulation, curd firming and syneresis processes. In particular, there was a relatively low incidence of the effect of herd and season on the coagulative ability of milk. Comparison of farms with high or low average daily milk energy output, revealed some contradictory effects on coagulation and curd firmness phenomena. It may be speculated that the factors differentiating herds (environment, facilities, feeding, management, health) are not very important for traditional or modeled MCP traits, and that improvements in these traits at the herd level should be based mainly on modifying individual cow factors.

Breed remained the predominant effect, showing strong differences between specialized and dual-purpose breeds, and especially within the two groups, even after correcting for milk yield and quality. In particular, results confirmed the very good milk quality and coagulative aptitude of Jersey cows, and, in part, also the dual-purpose Rendena cows, against the clear inferiority of Holstein-Friesian cows and the intermediate results of the other breeds of Alpine origin. Moreover, it was noted that, after correcting for effects of parity and DIM, there was still a very high animal effect within breed supporting that there is a strong genetic base to these traits and the possibility of genetic improvement.

Further research is needed to investigate genetic differences among breeds and within breeds among individuals at the level of some candidate genes (especially milk protein genetic variants) and of a genome-wide approach. It is also important to study the effects of herd productivity and of breed of cow directly on cheese-yield and milk nutrient recovery in cheese, which would also pave the way for studies on the relationships between direct cheese-making traits and MCP and CF_t model parameters.

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TABLES AND FIGURES

Table 1. Descriptive statistics of milk yield, chemical composition, traditional coagulation properties and curd firming time (CF_t) equation parameters and derived traits

Trait	N	Mean	SD	P1[4]	P99
Milk yield, kg/d	1,489	24.3	9.1	6.0	49.0
Chemical composition of milk:					
Protein, %	1,490	3.70	0.44	2.89	4.71
Fat, %	1,480	4.27	1.10	1.88	6.94
Lactose, %	1,502	4.98	0.29	4.10	5.52
pH	1,488	6.51	0.10	6.28	6.73
SCS[1]	1,495	2.85	1.86	-0.47	7.28
Traditional coagulation properties:[2]					
RCT, min	2,888	18.6	6.9	8.15	43.15
k_{20}, min	2,851	4.2	2.7	1.30	14.30
a_{30}, mm	2,889	40.1	19.0	0.00	72.40
a_{45}, mm	2,902	51.8	15.2	2.32	77.88
a_{60}, mm	2,910	54.2	13.7	17.54	79.54
CF_t equation 4 parameters:[3]					
RCT_{eq}, min	2,886	18.9	7.0	7.94	43.60
k_{CF}, $\%\times min^{-1}$	2,888	8.1	2.5	4.64	14.90
k_{SR}, $\%\times min^{-1}$	2,884	0.63	0.23	0.00	1.31
CF_P, mm	2,914	74.5	17.4	23.35	106.2
CF_{max}, mm	2,914	55.6	13.0	17.42	79.25
t_{max}, min	2,930	51.9	8.9	28.00	60.0

[1] SCS= 3 + $\log_2$ (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; CF_P = asymptotic potential curd firmness; k_{SR} = syneresis instant rate constant; CF_{max} = maximum curd firmness achieved within 45 min; t_{max} = time at achievement of CF_{max}. [4] P1 = 1st percentile; P99 = 99th percentile.

Table 2. Variance of the random effects, repeatability and reproducibility of traditional milk coagulation properties and of curd firming modeling (CF_t) equation parameters

	Random effects (% of total variance):		RMSE	Repeatability %^{4}	Reproducibility %
	Herd-Date	Animal			
Milk yield, kg/d	32.5	67.5	5.0	-	-
Chemical composition of milk:					
Protein, %	24.6	75.4	0.25	-	-
Fat, %	17.5	82.5	0.71	-	-
Lactose, %	8.9	91.1	0.26	-	-
pH	59.3	40.7	0.06	-	-
SCS^1	14.8	85.2	1.58	-	-
Traditional coagulation properties:^{2}					
RCT, min	10.7	82.4	1.6	94.1	94.1
k_{20}, min	6.1	80.2	0.9	88.0	88.0
a_{30}, mm	10.6	78.6	5.4	91.4	91.0
a_{45}, mm	8.5	68.2	5.9	84.0	81.9
a_{60}, mm	9.3	46.2	7.3	70.7	66.0
CF_t equation parameters:^{3}					
RCT_{eq}, min	10.5	82.5	1.6	94.1	94.1
k_{CF}, %×min^{-1}	9.6	34.8	1.5	59.1	57.9
k_{SR}, %×min^{-1}	8.4	40.1	0.17	59.8	58.2
CF_P, mm	8.4	61.3	7.1	81.7	78.2
CF_{max}, mm	8.4	61.3	5.7	81.7	78.2
t_{max}, min	13.5	32.0	5.3	57.1	57.1

^{1}SCS= 3 + $\log_2$ (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; $^3RCT_{eq}$ = 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 achieved within 45 min; t_{max} = time at achievement of CF_{max}.

$$^4RT\% = \frac{\sigma^2_{Herd(HP)} + \sigma^2_{Animal} + \sigma^2_{Instrument} + \sigma^2_{Pendulum(Instrument)}}{\sigma^2_{Herd(HP)} + \sigma^2_{Animal} + \sigma^2_{Instrument} + \sigma^2_{Pendulum(Instrument)} + \sigma^2_e} \times 100;$$

$$RD\% = \frac{\sigma^2_{Herd(HP)} + \sigma^2_{Animal} + \sigma^2_{Pendulum(Instrument)}}{\sigma^2_{Herd(HP)} + \sigma^2_{Animal} + \sigma^2_{Instrument} + \sigma^2_{Pendulum(Instrument)} + \sigma^2_e} \times 100,$$ where $\sigma^2_{Herd(HP)} + \sigma^2_{Animal} + \sigma^2_{Instrument} + \sigma^2_{Pendulum(Instrument)} + \sigma^2_e$ are variance components for herd within herd productivity level, animal, instrument, pendulum within instrument, and residual effects, respectively.

Table 3. Effect of herd productivity level and of parity

	Herd productivity (HP)			Parity (LSM)				Parity Contrasts (*F*-value):			HP × parity	HP × DIM
	High-HP (LSM)	Low-HP (LSM)	*F*-value	1st	2nd	3rd	≥4th	1st *vs* ≥2nd	2nd *vs* ≥3rd	3rd *vs* ≥4th		
Milk yield, kg/d	26.6	17.3	70.2^{***}	20.2	22.0	23.2	22.5	55.6^{***}	3.6	2.4	5.7^{***}	2.0^{*}
Chemical composition of milk:												
Protein, %	3.80	3.59	20.6^{***}	3.72	3.75	3.68	3.65	2.7	16.1^{***}	1.7	0.8	4.0^{***}
Fat, %	4.44	4.20	5.0^{*}	4.33	4.36	4.33	4.27	0.0	1.1	1.0	1.1	2.9^{**}
Lactose, %	4.96	4.98	0.2	5.06	4.98	4.92	4.92	58.6^{***}	7.7^{*}	0.0	3.0^{*}	0.6
pH	6.50	6.50	0.0	6.49	6.50	6.50	6.52	12.6^{***}	1.3	11.9^{**}	0.5	1.4
SCS[1]	2.76	2.71	0.1	2.28	2.50	2.97	3.21	38.2^{***}	19.9^{***}	2.9	8.0^{***}	3.4^{***}
Traditional coagulation properties[2]*:*												
RCT, min	18.3	16.2	7.0^{*}	16.2	18.1	17.6	17.1	7.1^{*}	1.2	0.5	0.6	0.7
k_{20}, min	3.9	4.2	0.5	3.6	4.2	4.2	4.2	6.7^{*}	0.0	0.0	2.3	1.9^{*}
a_{30}, mm	41.8	43.0	0.3	45.9	41.0	41.1	41.6	10.4^{*}	0.0	0.0	0.7	1.3
a_{45}, mm	52.9	50.6	2.1	54.4	51.3	50.5	50.7	10.0^{*}	0.2	0.0	1.3	1.4
a_{60}, mm	55.1	51.0	7.5^{**}	55.3	53.3	52.0	51.6	10.8^{*}	1.4	0.1	0.9	1.4
CF_t equation parameters[3]*:*												
RCT_{eq}, min	18.6	16.3	7.7^{**}	16.4	18.3	17.8	17.3	7.2^{*}	1.3	0.5	0.6	0.7
k_{CF}, $\% \times min^{-1}$	8.7	9.1	2.9	9.0	8.6	8.9	9.0	0.6	2.7	0.3	1.6	1.4
k_{SR}, $\% \times min^{-1}$	0.69	0.74	3.3	0.72	0.68	0.71	0.73	0.6	2.4	0.4	2.8^{*}	1.9^{*}
CF_P, mm	76.8	71.9	6.9^{*}	77.5	74.3	73.0	72.6	11.9^{***}	0.8	0.0	1.3	1.8
CF_{max}, mm	57.3	53.7	6.9^{*}	57.8	55.4	54.5	54.2	11.9^{***}	0.8	0.0	1.3	1.8
t_{max}, min	50.9	47.2	11.0^{**}	48.3	50.4	49.4	48.0	3.0	5.4^{*}	3.1	0.5	0.8

[1] SCS= 3 + $\log_2$ (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; CF_P = asymptotic potential curd firmness; k_{SR} = syneresis instant rate constant; CF_{max} = maximum curd firmness achieved within 45 min; t_{max} = time at achievement of CF_{max}. $^{*}P < 0.05$; $^{**} P < 0.01$; $^{***} P < 0.001$

Table 4. Least square means of breed effect and their orthogonal contrasts (*F*-value) of traditional coagulation properties and CF_t equation parameters and derived traits for breed and F-value of the contrasts

	Breed (LSM):						Breed Contrasts (*F*-value):					Breed × parity
	Holstein Friesian (HF)	Brown Swiss (BS)	Jersey (Je)	Simmental (Si)	Rendena (Re)	Alpine Grey (AG)	HF BS Je *vs* Si AG Re	HF BS *vs* Je	HF *vs* BS	Si *vs* Re AG	Re *vs* AG	
Milk yield, kg/d	26.5	23.4	17.1	23.6	22.0	19.3	3.2	114.4***	129.1***	33.4***	13.4***	2.2**
Chemical composition of milk:												
Protein, %	3.47	3.75	4.07	3.63	3.50	3.77	40.7***	165.7***	442.7***	0.0	54.6***	2.3**
Fat, %	4.01	4.28	5.59	4.27	3.79	3.98	116.1***	202.2***	46.6***	27.1***	3.4	1.5
Lactose, %	4.97	4.96	4.87	4.96	5.08	4.98	14.1***	6.6*	0.27	7.1*	7.9*	1.6
pH	6.51	6.51	6.49	6.50	6.49	6.52	0.4	4.4*	0.6	3.2	8.8*	2.7***
SCS[1]	3.04	2.82	2.64	2.26	2.96	2.71	2.3	1.7	6.3	12.9***	1.1	2.6***
Traditional coagulation properties[2]:												
RCT, min	19.6	18.7	13.1	18.0	15.2	18.8	0.1	26.1***	4.2*	1.6	9.4**	0.8
k_{20}, min	5.7	3.8	2.5	4.5	3.5	4.3	0.2	23.4***	100.7***	3.6	2.4	1.7*
a_{30}, mm	32.7	41.7	57.0	39.1	44.7	39.3	2.3	37.0***	52.6***	1.6	2.8	1.2
a_{45}, mm	44.3	53.6	58.6	49.7	52.7	51.4	0.4	15.1***	95.5***	1.7	0.3	0.9
a_{60}, mm	47.0	55.6	57.2	51.4	53.2	54.0	0.1	8.2**	117.0***	2.3	0.1	0.9
CF_t equation parameters:[3]												
RCT_{eq}, min	19.8	19.0	13.0	18.3	15.4	19.1	0.2	28.4***	3.5	1.6	9.8**	0.8
k_{CF}, $\%\times min^{-1}$	7.6	8.5	11.9	8.3	8.7	8.4	19.9***	124***	45.9***	1.0	0.8	2.3**
k_{SR}, $\%\times min^{-1}$	0.59	0.69	0.97	0.66	0.71	0.66	8.9**	69.5***	41.8***	0.6	1.5	2.0*
CF_P, mm	64.7	76.7	84.4	71.5	74.4	74.5	1.3	25.4***	131.2***	2.3	0.0	0.9
CF_{max}, mm	48.3	57.2	63.0	53.4	55.5	55.6	1.3	25.4***	131.2***	2.3	0.0	0.9
t_{max}, min	52.4	51.0	41.1	50.5	48.1	51.3	6.3	70.4***	8.1**	0.8	6.2	2.3*

[1]SCS= 3 + $\log_2$ (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; CF_P = asymptotic potential curd firmness; k_{SR} = syneresis instant rate constant; CF_{max} = maximum curd firmness achieved within 45 min; t_{max} = time at achievement of CF_{max}. *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Figure 1. Effect of interaction (*P* = 0.03) between herd productivity level (High-HP and Low-HP) and stage of lactation (each point represents the LSM and the bars the corresponding standard error of the interaction HP × DIM from a model including herd within HP, 6 breeds effect, parity and its interactions with HP and breed).

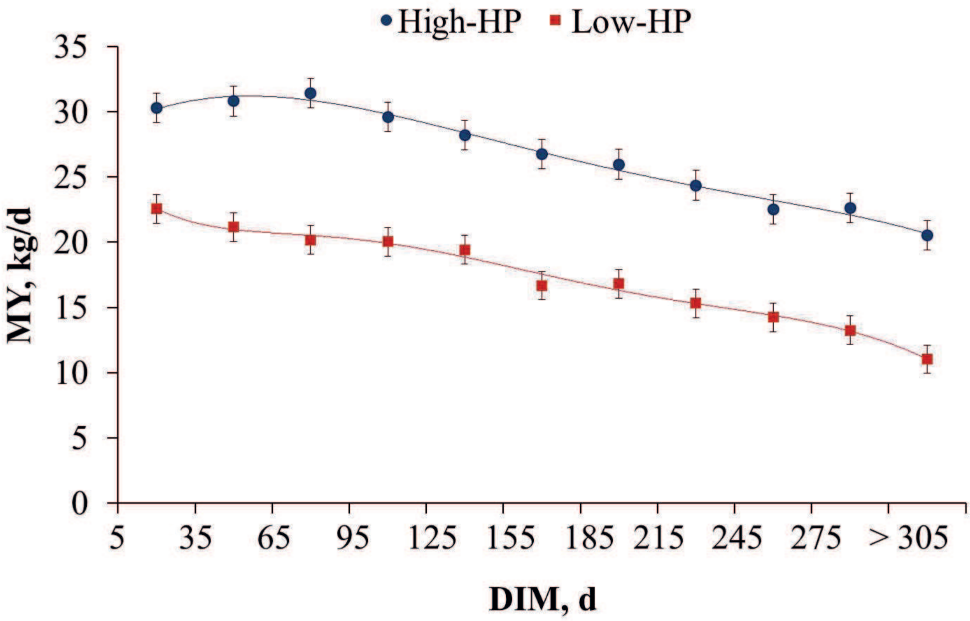
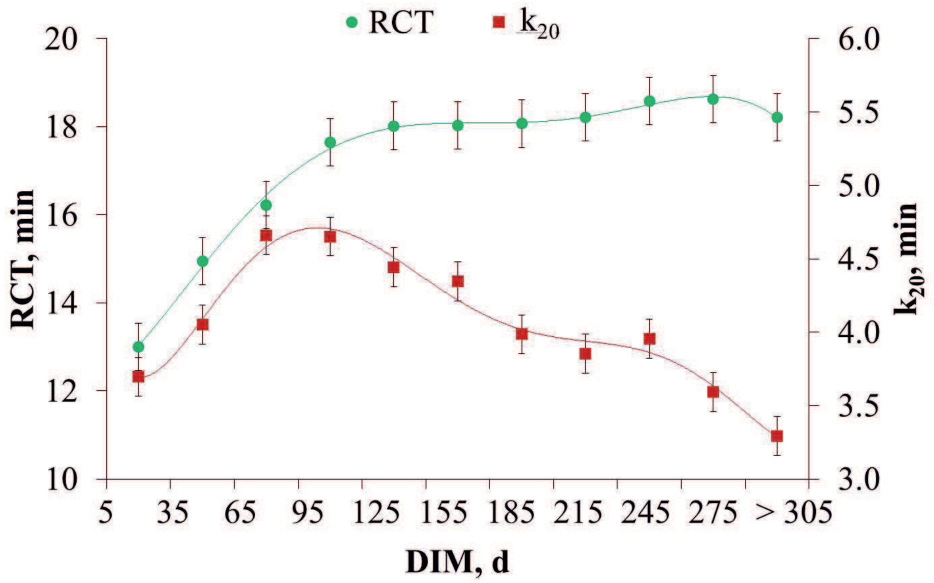


Figure 2. Effect of DIM on traditional MCP: RCT (P<0.0001) and k_{20} ($P < 0.0001$) [a] and on curd firmness at 30 (a_{30}, P<0.0001), 45 (a_{45}, P=0.002) and 60 minutes (a_{60}, p<0.0001) [b].

[a]



[b]

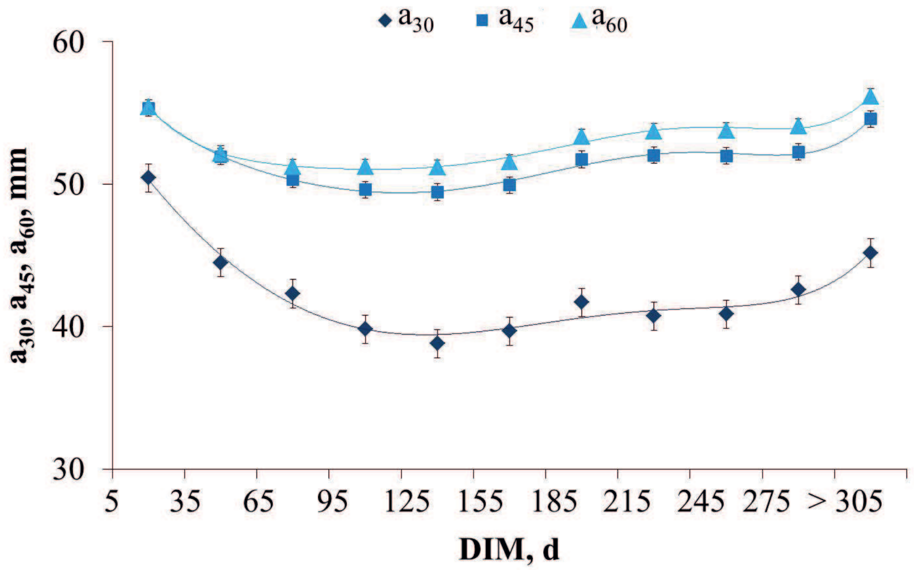
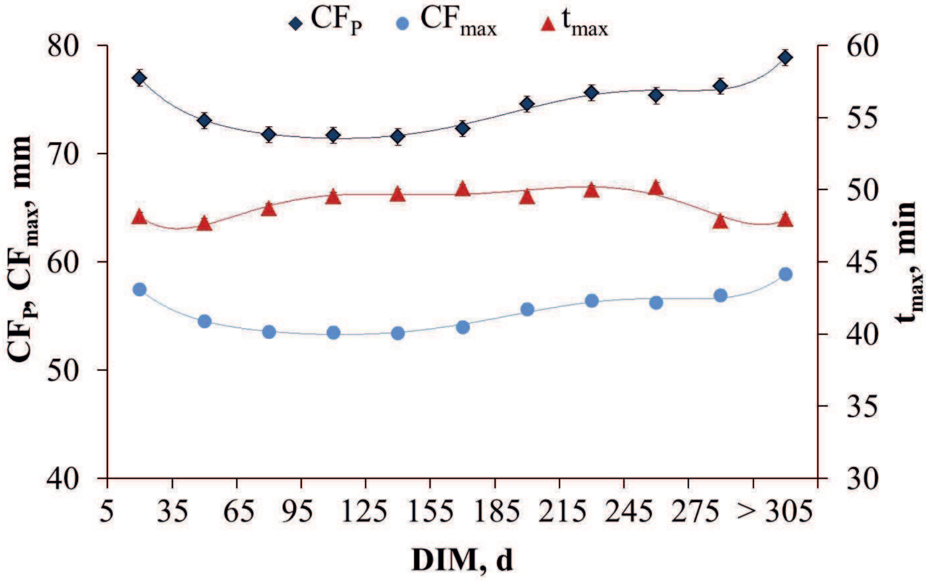


Figure 3. Effect of DIM on CF_t modeling parameters and derived traits: CF_P (P<0.001), CF_{max} (P < 0.001), t_{max} (P < 0.01) [a], and k_{CF} (P < 0.001) and k_{SR} (ns) [b] (RCTeq showed values very similar to those of RCT reported in Figure 2a).

[a]



[b]

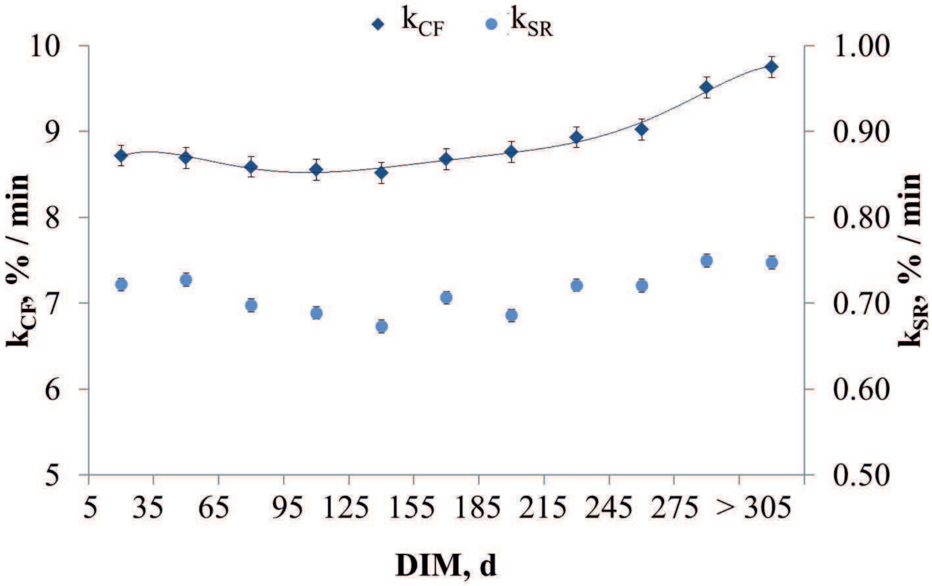


Figure 4. Pattern of curd firmness after rennet addition (CF_t modeling) of milk samples across classes of parity.

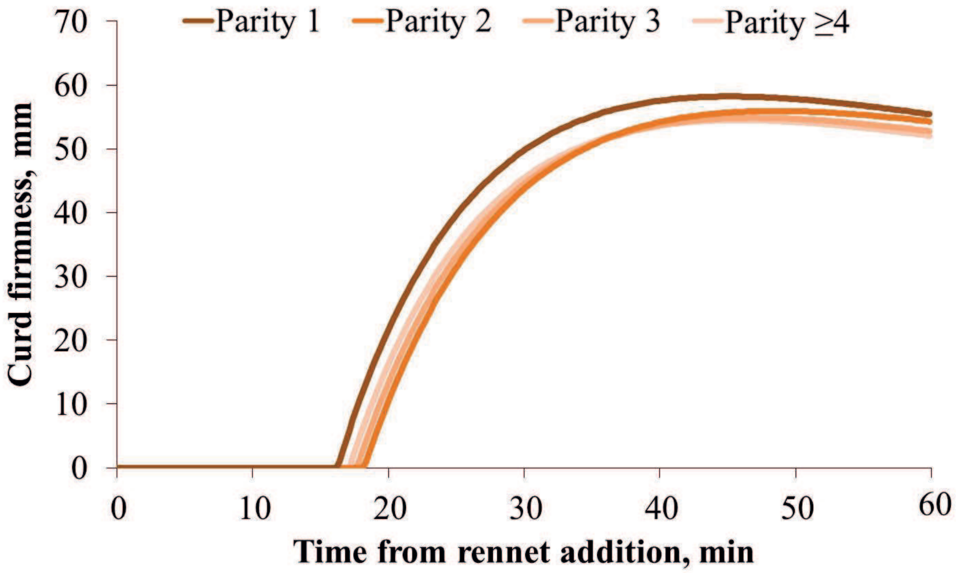


Figure 5. Pattern of curd firming after rennet addition (CF_t modeling) of milk samples according to stage of lactation.

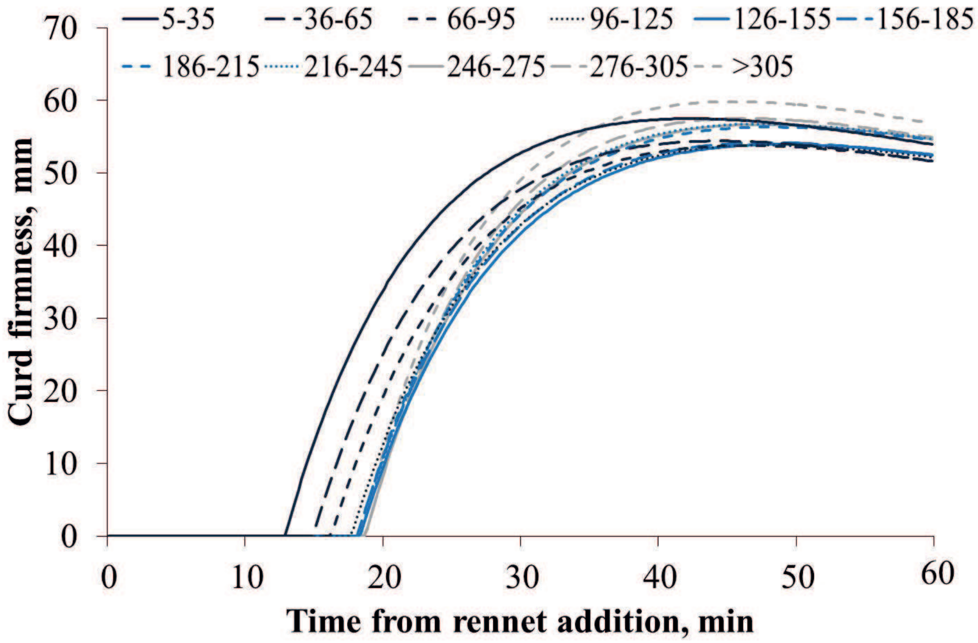


Figure 6. Pattern of curd firming after rennet addition (CF_t modeling) of milk samples according to herd productivity level (High-HP or Low-HP) defined by the herd's average daily milk energy output of the cows (corrected for breed, parity and DIM).

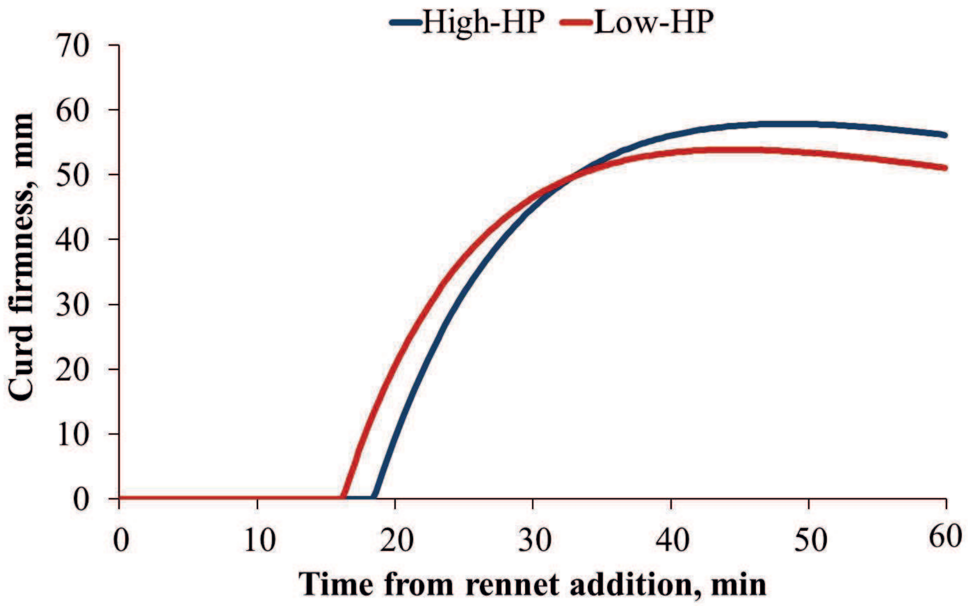
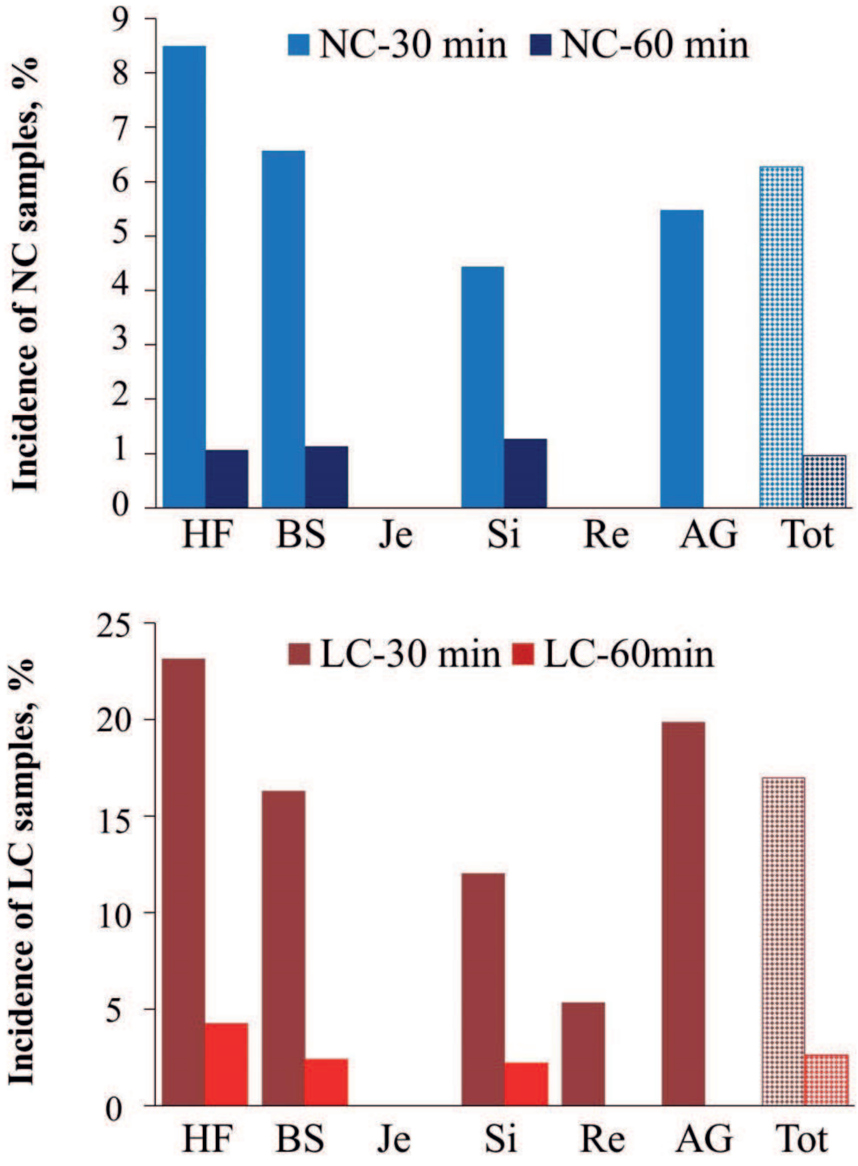
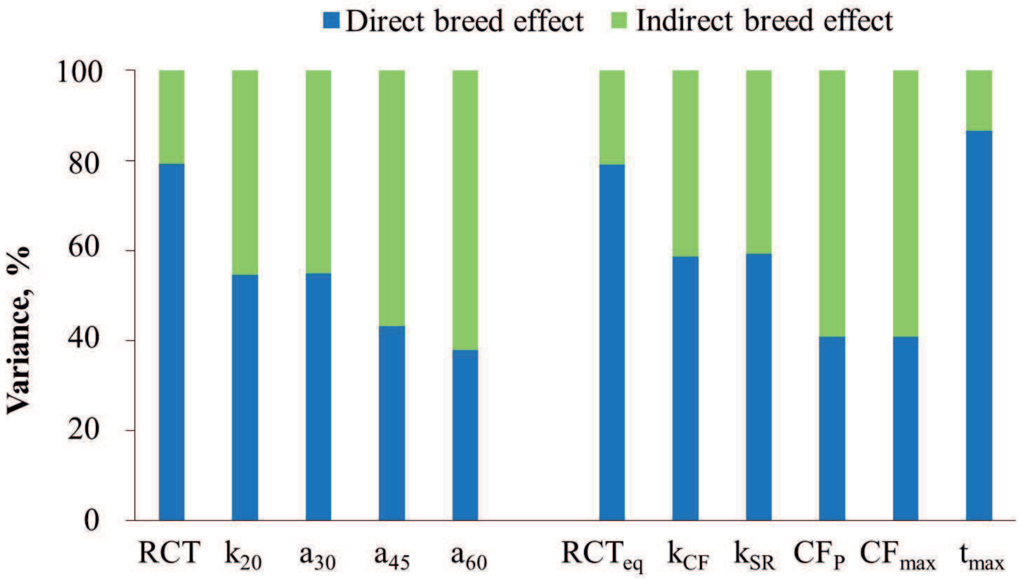


Figure 7. Incidence of: a) non-coagulating (NC) and b) late-coagulating (LC) milk samples in different cattle breeds after 30 or 60 min from rennet addition.



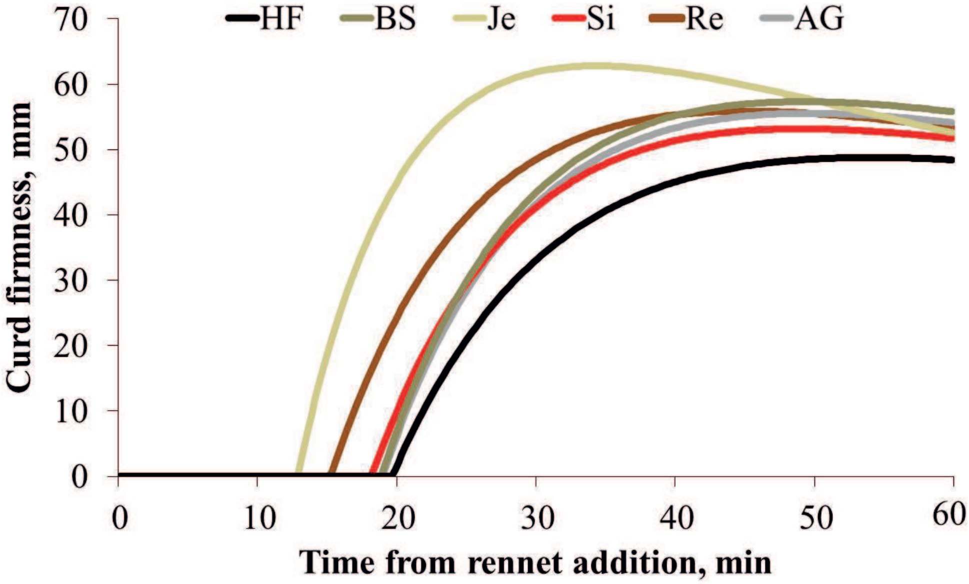
HF = Holstein-Friesian; BS = Brown Swiss; Je = Jersey; Si = Simmental; Re = Rendena; AG = Alpine Grey.

Figure 8. Proportion of total breed variance explained by direct breed effect or by indirect breed effect through differences in milk yield and quality traits on MCP and CF_t equation traits.



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; 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 achieved within 45 min; t_{max} = time at achievement of CF_{max}.

Figure 9. Pattern of curd firmness after rennet addition (CF_t modeling) of milk samples for the 6 breeds compared within herds.



HF = Holstein-Friesian; BS = Brown Swiss; Je = Jersey; Si = Simmental; Re = Rendena; AG = Alpine Grey.

Chapter 2

Six breeds of cows and herd productivity affect milk nutrients recovery in curd and cheese yield, efficiency and daily production

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ABSTRACT

Cheese production is growing world-wide but cheese-making is a very complex process in which several qualitative, technological and productive traits are taken into account. Little is known of these traits at the level of individual lactating females because of the difficulties and costs of producing many individual model cheeses. Moreover, it is often difficult to differentiate the effects of the most important factors - herd and breed - because of the use of data from single-breed herds and the frequent links between specific breeds and specific environmental and management features. The objective of the present study was to differentiate between the effects of herd productivity class, individual herds within productivity classes, and breed of cow within herds by producing and analyzing 508 model cheeses from 508 cows of 6 different breeds reared in 41 multi-breed herds classified into 2 productivity classes (high *vs* low).

For each cow: a) 6 milk composition traits; b) 4 recovery traits (REC) of milk nutrients (fat, protein, solids and energy) in curd, and 3 actual % cheese yield traits (%CY), expressing fresh cheese, cheese solids and cheese water as percentages of the processed milk were analyzed (these traits were obtained during the experimental cheese making); c) 2 theoretical %CYs (fresh cheese and cheese solids) were calculated from the milk composition, and 2 overall cheese-making efficiencies (fresh cheese and cheese solids) were calculated as the % ratio between actual and theoretical %CYs; d) daily milk yield (dMY) was measured and estimates were made of 3 actual daily cheese yield production traits (dCY) per cow (fresh cheese, cheese solids and water retained in the cheese). The data were analyzed using a mixed model in which the following effects were included: 2 herd productivity classes, 27 random herd effects, 4 parity categories, 10 DIM classes, 6 breed effects, 3 interactions, 3 random water-bath effects, 15 random vat effects and the random residual.

Cows reared in high productivity herds yielded more milk with greater nutrient contents and more cheese per day, had greater theoretical %CY, although to a lesser extent, actual %CY, but did not differ from low productivity herds in terms of REC traits (except solids) and had a lower solid cheese-making efficiency. Individual herds within productivity classes were an intermediate source of total variation with respect to REC traits (11.3% to 17.1%), and to actual and theoretical %CY and estimates of efficiency (10.0% to 17.2%), and a major source for milk yield and dCY traits (43.1% to 46.3%). Parity of cows was an important source of variation, especially with respect to productivity traits, whereas DIM affected almost all traits.

Breed within herd greatly affected all traits. Compared with the dual-purpose breeds, the 3 specialized dairy breeds (Holstein, Brown Swiss and Jersey) had, on average, a similar dMY, better milk composition, greater actual and theoretical %CY, similar fat and protein REC, and slightly lower cheese-making efficiency. Of the specialized dairy cow breeds, Holsteins produced more milk, but Brown Swiss cows produced milk with a greater nutrient content, greater nutrient REC, higher actual and theoretical %CY and a higher cheese-making efficiency, so the two large breeds had the same dCY. Small Jersey cows produced much less milk, with much more fat and protein and greater REC traits than the two large-framed breeds resulting in greater actual and theoretical %CY but similar efficiencies. Although the Jersey breed had lower dMY and dCY, the difference was much smaller for the latter. The differences among the 3 dual-purpose breeds (Simmental and the local Rendena and Alpine Grey) were not very large. Compared with medium-framed cows of the local breeds, Simmentals had greater dMY, tended to have better milk composition, REC and %CY traits (but similar efficiencies), and also had much greater dCY. Among the local breeds, the higher dMY of Rendena was offset by the greater nutrient content of milk from the Alpine Greys, so their dCY was similar.

Differentiating the effects of herd productivity class and individual herds from the characteristics of individual cows gave us a better understanding of breed characteristics and provided information of use for better evaluating selective breeding of purebred animals and deciding on breed combinations in crossbreeding dairy programs.

Key words: Cheese-making efficiency, cheese yield, herd effect, fat recovery, protein recovery

INTRODUCTION

Cheese yield (**CY**), expressed as the percentage ratio between the cheese produced and the milk processed (**%CY**), is of global economic importance, and daily yield of cheese, expressed in kilograms of cheese produced daily per cow (**dCY**), is the final direct or indirect production target of many dairy farmers. CY relies primarily on the fat and protein content of milk, particularly casein, and on the technological properties of the processed milk (Law and Tamine, 2010), but also on recovery in the curd of the individual milk components (**REC** traits) that determine the overall efficiency of cheese-making. Measurement or prediction of %CY and dCY of individual cows is important for studies aimed at investigating the existence of a genetic basis for these traits (Othmane et al., 2002), and also for selecting breed combinations in crossbreeding dairy programs. However, most of the studies in the literature involving laboratory cheese-making procedures have used bulk milk, largely because it is very time-consuming and labor-intensive to produce a large number of small model cheeses from milk samples of individual cows. Very few published studies have considered individual %CY traits of bovine milk (Cipolat-Gotet et al., 2013).

The primary genetic characteristic of a cow - its breed - has been shown to have an enormous effect on cheese yield traits (Banks et al., 1986; Verdier-Metz et al., 1995), but this information generally comes from studies using bulk milk from cows grouped into individual experimental herds (Mistry et al., 2002; Hurtaud et al., 2009; Martin et al., 2009), or using bulk milk from different commercial single-breed herds (Malacarne et al., 2006; Bland et al., 2015). Therefore, comparison of breeds may be affected by a lack of representativeness, or by different individual (parity, stage of lactation, etc.) or herd (facilities, feeding, management, etc.) characteristics. In fact, in a large survey on factors affecting the variability of individual cheese-making traits in Brown Swiss cows, Cipolat-Gotet et al. (2013) found that the effect of herd represented 21 to 31% of total variance for the REC traits of milk components in the curd, 24 to 42% in the case of %CY traits, and 51 to 53% for dCY, expressed as the daily production of cheese per cow. It is also possible that very different dairy systems and levels of farm productivity interact with breed.

Far, more studies on milk coagulation and curd firming properties (MCP) using lactodynamography have been carried out with individual cows, in part because of the small quantity of milk needed and the possibility to test several milk samples in a short period of time (often 10 samples in 30 min). Lactodynamography reproduces only some steps of the cheese-making process (i.e., rennet addition, milk coagulation, curd firming) but several studies found a large effect of breed of cow on MCP, as reviewed by Bittante et al. (2012). Although lactodynamography gives no direct measurement of %CY and REC traits, some authors identified MCP as possible predictors of %CY (Ikonen et al., 1999b; Malacarne et al., 2006). In contrast, Bonfatti et al. (2014) produced small experimental cheeses from milk samples that varied widely in their MCP but had similar fat and casein contents, and concluded that the MCP did not affect %CY traits. It should be noted that these studies were not carried out with individual cows. Recently, Cecchinato and Bittante (2016) demonstrated that, at the individual level, traditional MCPs are not very relevant for predicting individual %CY. Evaluating the different REC, %CY and dCY traits while distinguishing between genetic and environmental (herd) factors is, therefore, very important when comparing different dairy and dual-purpose breeds, effectively planning breed combinations in crossbreeding programs, and in order to better define objectives in the selection of purebred animals.

For these reasons, a large research project (Cowplus project) was established, and several cheese-making-related phenotypes have been measured in milk from individual cows from multi-breed herds, thereby allowing for independent evaluation of the effects of farm and breed of cows. The specific aims of this study were: 1) to quantify and characterize the effects of herd productivity (defined on the basis of the average net energy of milk yielded daily by the cows) on 15 REC, %CY, and dCY traits; 2) to quantify the variability of herds within class of herd productivity; and

3) to make a within-herd comparison of 6 dairy and dual-purpose breeds for these cheese-making traits.

MATERIALS AND METHODS

Multi-breed herds

A total of 1508 cows from 41 multi-breed herds (2 to 5 breeds per herd, on average 3) located in Trentino province in the north-eastern Italian Alps were monitored once for daily milk yield and composition. Details of the milk sampling and analysis have been described by Stocco et al. (2016), and of the environmental context and dairy systems involved by Sturaro et al. (2013).

Milk from a subsample of 513 cows (reared in 27 multi-breed herds) were assessed also on cheese-making traits. Six breeds were investigated, 3 specialized dairy breeds: Holstein Friesian (HF = 17 herds, 110 cows), Brown Swiss (BS = 22 herds, 155 cows), and Jersey (Je = 6 herds, 39 cows); and 3 dual-purpose breeds: Simmental (Si = 14 herds, 69 cows), and the two native breeds, Alpine Grey (AG = 13 herds, 71 cows) and Rendena (Re = 8 herds, 69 cows). Initially, 20 cows per herd were selected to include all breeds under study and different parities and days in milk. A few cows and milk samples were discarded because of health problems, milk composition abnormalities or technical problems.

Herd productivity classification

The herds were classified into two categories of herd productivity (**HP**), defined on the basis of the average daily milk energy output (**dMEO**) yielded by all lactating cows in the herd. The net energy content (NE_L) of milk was estimated by means of the following equation, proposed by the NRC (2001):

$$NE_L \text{ (Mcal/kg)} = 0.0929 \times \text{fat,\%} + 0.0547 \times \text{protein,\%} + 0.0395 \times \text{lactose,\%},$$

where NE_L is the energy of one kg of milk. The NE_L values obtained were converted to MJ/kg and multiplied by the daily milk yield of each cow (kg/d) to obtain the individual dMEO of each cow

(MJ/d). The individual dMEO data of all lactating cows were subjected to an ANOVA using the SAS GLM procedure (SAS Institute Inc., Cary, NC) in order to calculate the least square means (LSMs) for dMEO for the selected herds after correcting for breed, DIM and parity of cows. After ranking the dMEO LSMs of the 27 farms, we divided them into high producing (**High-HP**: n = 13, dMEO = 83.82 MJ/d) and low producing (**Low-HP**: n = 14, dMEO = 51.60 MJ/d) herds on the basis of the median value.

All breeds were distributed throughout the high and low productivity herds, with the exception of the Jersey, which was only found in High-HP herds, and the Alpine Grey, found only in Low-HP herds.

Analysis of milk samples

Immediately after collection, individual milk samples of about 2000 mL per cow were stored at 4°C, and processed within 24 hours of sampling at the Milk Quality Laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) of the University of Padova.

All samples were analyzed for pH (Crison portable pH-meter Basic 25; Crison Instruments SA, Barcelona, Spain), and SCC (Fossomatic Minor, Foss Electric A/S, Hillerød, Denmark). Milk SCC was log-transformed to SCS (Ali and Shook, 1980). Total solids, protein, casein, fat and lactose contents were measured with a Milkoscan FT2 infrared analyzer (Foss Electric A/S, Hillerød, Denmark) calibrated in accordance with the reference methods [ISO 6731/IDF 21 for total solids (2010a); ISO 8968–2/IDF 20–2 for protein (ISO-IDF, 2014); ISO 17997–1/IDF 29–1 for casein (ISO-IDF, 2004); ISO 1211/IDF for fat (ISO-IDF, 2010b); ISO 26462/IDF 214 for lactose (ISO-IDF, 2010c)].

Individual model cheese-making procedure

Individual milk samples were processed using a model cheese-making method to assess %CY and REC traits, as proposed by Cipolat-Gotet et al. (2013) with modifications. The cheese-making apparatus consisted of 3 water baths (WB) fitted with a digital temperature controller and pumps for water mixing to ensure homogeneous heat distribution throughout the WB. Five stainless steel vats (capacity 1500 mL) were placed in each WB.

The following procedure, summarized in Figure 1, was performed on each milk sample (1500 mL): milk was heated to 35°C for 30 min and pH was then recorded using a Crison Basic 20 electrode (Crison, Barcelona, Spain). Bovine rennet solution [8 mL per sample consisting of 0.2145 mL of Naturen Plus 215 Hansen with 80 ± 5% chymosin and 20 ± 5% pepsin; 215 IMCU/mL (Pacovis Amrein AG, Bern, Switzerland) diluted in distilled water] was then added. The curd was cut into cubes of about 0.5 cm^3 10 minutes after visual observation of milk gelation by the operator. It was then separated from the whey (10 minutes after cutting) and placed in a cheese mold suspended over the vat containing the whey for 20 min, during which the curd was turned every 2 min to facilitate draining. After draining, the curd was cross-cut into four pieces, shaped into a smaller cheese mold and submerged in its whey for 10 min to encourage additional whey expulsion from the curd. At the end of this phase, the whey was weighed and sampled for analysis of its composition using a MilkoScan FT2 (Foss Electric A/S, Hillerød, Denmark), while the curd was pressed for 30 min at 250 kPa, during which it was turned every 10 min. Finally, the curd was brined for 30 min (saturated solution; 20% NaCl). After brining, the cheese wheel was weighed.

Definition of cheese-making traits

The weights of the milk and whey (g) and their chemical compositions enabled us to calculate cheese-making traits, as proposed by Cipolat-Gotet et al. (2013). The composition of the curd was calculated by subtracting the weight of the nutrient in whey from the corresponding nutrient in the milk processed. Briefly, the measured traits were: **%CY_{CURD}**, **%CY_{SOLIDS}** and

%$\mathrm{CY}_{\mathrm{WATER}}$, calculated as the ratio of the weight (g) of fresh curd, curd dry matter and curd water, respectively, to the weight of the milk processed (g); **$\mathrm{REC}_{\mathrm{PROTEIN}}$**, **$\mathrm{REC}_{\mathrm{FAT}}$** and **$\mathrm{REC}_{\mathrm{SOLIDS}}$**, calculated as the ratio of the weight (g) of the curd component (protein, fat and dry matter, respectively) to the same component of milk (g). Recovery of energy in the curd (**$\mathrm{REC}_{\mathrm{ENERGY}}$**) was determined by estimating milk and curd energy using an equation proposed by the NRC (2001) and converted to MJ/kg. Lastly, daily cheese yields (**$\mathrm{dCY}_{\mathrm{CURD}}$**, **$\mathrm{dCY}_{\mathrm{SOLIDS}}$** and **$\mathrm{dCY}_{\mathrm{WATER}}$**; kg/d) were calculated by multiplying the different %CYs (%$\mathrm{CY}_{\mathrm{CURD}}$, %$\mathrm{CY}_{\mathrm{SOLIDS}}$ and %$\mathrm{CY}_{\mathrm{WATER}}$, respectively) by the daily milk yield (dMY, kg/d).

Definition of cheese-making efficiency

The theoretical %$\mathrm{CY}_{\mathrm{CURD}}$ (*Th*-%$\mathrm{CY}_{\mathrm{CURD}}$) of the milk samples of each cow was estimated using the historical formula of Van Slyke and Price (1949) reported by Emmons and Modler (2010) in their review:

$$Th\ \%CY_{CURD} = (0.93 \times \%fat + \%casein - 0.1) \times 1.09/[(100 - \%M)/100]$$

where 1.09 represents correction for milk minerals and cheese salt and carbohydrates, and %M is the percentage moisture of cheese (100 - %total solids).

A formula for estimating the theoretical %$\mathrm{CY}_{\mathrm{SOLIDS}}$ (*Th*-%$\mathrm{CY}_{\mathrm{SOLIDS}}$) was derived from the previous one by simply deleting the last part, which corrects for cheese moisture:

$$Th\ \%CY_{SOLIDS} = (0.93 \times \%fat + \%casein - 0.1) \times 1.09$$

The efficiencies of %$\mathrm{CY}_{\mathrm{CURD}}$ (*Ef*-%$\mathrm{CY}_{\mathrm{CURD}}$) and of %$\mathrm{CY}_{\mathrm{SOLIDS}}$ (*Ef*-%$\mathrm{CY}_{\mathrm{SOLIDS}}$) were calculated by simply expressing the experimental value in relation to the corresponding theoretical value for each cow:

$$Ef\text{-}\%\mathrm{CY}_{\mathrm{CURD}} = \%\mathrm{CY}_{\mathrm{CURD}} / Th\text{-}\%\mathrm{CY}_{\mathrm{CURD}}, \text{ and}$$

$$Ef\text{-}\%\mathrm{CY}_{\mathrm{SOLIDS}} = \%\mathrm{CY}_{\mathrm{SOLIDS}} / Th\text{-}\%\mathrm{CY}_{\mathrm{SOLIDS}}$$

Statistical Analysis

Experimental data were analyzed using the MIXED procedure (SAS Institute Inc., Cary, NC), according to the following model (base model):

$$y_{ijklmnop} = \mu + HP_m + Herd_n(HP)_m + Breed_k + Parity_j + Breed_k \times Parity_j + HP_m \times Parity_j + DIM_i + HP_m \times DIM_i + Waterbath_l + Vat_o(Waterbath)_l + e_{ijklmnop}$$

where $y_{ijklmnop}$ is the observed trait ($REC_{PROTEIN}$, REC_{FAT}, REC_{SOLIDS}, REC_{ENERGY}, $\%CY_{CURD}$, $\%CY_{SOLIDS}$, $\%CY_{WATER}$, *Th-*$\%CY_{CURD}$, *Th-*$\%CY_{SOLIDS}$, *Ef-*$\%CY_{CURD}$, *Ef-*$\%CY_{SOLIDS}$, dMY, dCY_{CURD}, dCY_{SOLIDS}, and dCY_{WATER},); μ is the overall intercept of the model; HP_m is the fixed effect of the m^th^ herd productivity (m = 2 levels); $Herd_n$ is the random effect of the n^th^ herd (n = 1 to 27) within the m^th^ class of herd productivity; $Breed_k$ is the fixed effect of the k^th^ breed (k = HF, BS, Je, Si, Re and AG); $Parity_j$ is the fixed effect of the j^th^ parity (j = 1 to ≥ 4); DIM_i is the fixed effect of the i^th^ class of days in milk [i = 1 to 10; class 1, 5-35 days (31 samples); class 2, 35-65 d (26 samples); class 3, 65-95 d (41 samples); class 4, 95-125 d (56 samples); class 5, 125-155 d (64 samples); class 6, 155-185 d (58 samples); class 7, 185-215 d (63 samples); class 8, 215-245 d (57 samples); class 9, 245-275 d (32 samples); class 10, > 275 d (84 samples)]; $Waterbath_l$ is the random effect of the l^th^ water bath (l = 4 baths); Vat_o is the random effect of the o^th^ vats (o = 1 to 20) within the l^th^ water bath; $e_{ijklmnopq}$ is the random residual ~ N (0, σ_e^2).

A model that also included the breed × herd productivity interaction was fitted to test the data from all the breeds present in both classes of herds (Jersey and Alpine Grey excluded). As this interaction was never significant, the results of this model analysis are not shown nor discussed.

A further model was used to analyze the direct effects of breed on CY and cheese-making efficiency traits, corrected for the milk yield and quality traits and was obtained from the base model with inclusion of linear covariate of dMY, total solids, protein, fat, lactose, pH and SCS. Moreover, the breed effect was considered random to obtain a correct quantification of breed variance. The indirect effect of breed on the considered traits due to breed differences in terms of milk yield and quality was obtained subtracting the breed variance yielded by the extended model from the breed variance obtained from the base model (with breed as random effect). Both direct and indirect breed variance were represented as percentage of total breed variance.

Orthogonal contrasts were estimated between the LSMs of traits for the effect of breed:

a) specialized dairy (HF, BS and Je) *vs* dual-purpose breeds (Si, AG and Re);

b) within specialized, large-framed *vs* small-framed breeds (HF + BS *vs* Je), and

c) comparison between the two large-framed dairy breeds (HF *vs* BS);

d) within dual-purpose, large-framed breed *vs* medium-framed local breeds (Si *vs* Re + AG), and

e) comparison between the two medium-framed local dual-purpose breeds (Re *vs* AG).

Orthogonal contrasts were also estimated between the LSMs of traits for the effect of parity: a) 1st *vs* ≥2nd, b) 2nd *vs* ≥3rd, c) 3rd *vs* ≥4th.

RESULTS

Descriptive statistics and random effects on the cheese-making ability of milk

Descriptive statistics of milk quality, REC, %CY, *Th-%*CY, and *Ef-%*CY traits, and of dMY and dCY of individual cows are summarized in Table 1. All traits exhibited high variability, due mainly to the diversity of herd productivity and of the six breeds sampled, and an almost normal distribution.

Figure 2 shows the incidence of herd-date variance on total variance of the traits examined in this study. In the case of REC, the herd-date effect was modest, whereas it was intermediate for %CY, *Th-%*CY and *Ef-%*CY traits, and much greater for daily milk and cheese production per cow, varying from 11.3% for $\text{REC}_{\text{PROTEIN}}$ to 41.5 to 46.3% for production traits. Regarding the other random effects in the model (data not shown), both water bath and vat within water bath had little effect on cheese-making ability (in both cases from about 0.0% for the majority of the traits examined to 0.6% of the total variance for $\text{REC}_{\text{ENERGY}}$ (water bath), and $\text{REC}_{\text{PROTEIN}}$ (vat within water bath), highlighting the good reproducibility of the method.

Herd productivity, effects of parity and DIM

The least square means of the herd productivity (HP) classes, and the *F*-values of the same factors of variation and of the HP × parity and HP × DIM interactions on milk quality and cheese-making traits are summarized in Table 2. The daily yield and nutrient contents of milk were both very different in the two herd productivity classes, and greater in the High-HP herds. Although there were no differences in the milk nutrient recoveries in the curd (except for $\text{REC}_{\text{SOLIDS}}$), the greater content of fat and casein is responsible for the greater $\%\text{CY}_{\text{SOLIDS}}$ (+6.3%) in High-HP herds than in Low-HP herds. This difference is slightly lower than the theoretical %CY (+8.3%), so actual cheese-making efficiency is lower (-2.5%) in High-HP than in Low-HP herds, and than the predictions based on milk composition (Table 2). Similar but non-significant nominal differences between the two classes of herds were also found with respect to water retained in the curd ($\%\text{CY}_{\text{WATER}}$). As a result, both the actual $\%\text{CY}_{\text{CURD}}$ and *Th*-$\%\text{CY}_{\text{CURD}}$ were greater in High-HP than in Low-HP herds (+6.7% and +10.8%, respectively), but no significant difference was found for *Ef*-$\%\text{CY}_{\text{CURD}}$ (-3.1%).

As a result of these differences, cows in High-HP herds produced 50% more milk, 58% more cheese, and 63% more cheese solids per day than the cows reared in Low-HP herds.

Parity (Table 3) had a moderate effect on the quality of milk and on cheese-making traits. Milk from primiparous cows had only a slightly greater content of casein and lactose than milk from multiparous cows. $\text{REC}_{\text{PROTEIN}}$ was also slightly greater in primiparous cows, as was actual $\%\text{CY}_{\text{CURD}}$ and *Ef*-$\%\text{CY}_{\text{CURD}}$, although the latter seems due to greater water retention in their model cheeses than to differences in *Ef*-$\%\text{CY}_{\text{SOLIDS}}$ (Table 3).

Milk from second-calvers also had slightly greater contents of total solids, protein, casein, fat and energy than older cows, and correspondingly slightly greater actual and theoretical %CYs, whereas there were no differences in water retention in the cheese and in cheese-making efficiencies.

Daily production per cow was, as expected, lower for primiparous than multiparous cows with respect to milk (-10%), fresh cheese (-8%), and cheese solids (-9%). The lower production of second-calvers compared with older cows (-6%) was in part compensated for by greater %CY traits, so there were no significant differences in daily cheese production. The HP × parity interaction did not affect any traits.

The variation during lactation was highly significant for all the cheese-making traits examined, with the sole exception of REC_{FAT} (data not shown). With respect to the 3 %CY traits, it can be seen from Figure 3 that, after the first month, these increased almost linearly until the end of lactation, when they all reached their greatest values. We also noted a weak, insignificant interaction between DIM and HP class for the three %CY traits, and 2 of the 4 nutrient recovery traits (REC_{FAT} and REC_{SOLIDS}). In fact, we found a greater increase in $\%CY_{CURD}$ from the beginning to the end of lactation with cows from High-HP herds than with cows from Low-HP herds (2.6 *vs* 2.0%). This was also the case for CY_{WATER} (2.3 *vs* 0.8%), but not for CY_{SOLIDS} (0.8 *vs* 1.1%).

Effect of cow breed

Least square means and their orthogonal contrasts (*F*-values) for milk quality and cheese-making traits of the 6 breeds sampled are reported in Table 4. These least square means are corrected for all the other factors of variation included in the model, and particularly for herd productivity class, individual herds within class, and the cows' parity and DIM.

Comparing, firstly, the average of the three specialized dairy breeds (Holstein Friesian, Brown Swiss and Jersey) with that of the dual-purpose breeds (Simmental, Rendena and Alpine Grey) we note that 5 of the 6 milk quality traits and 12 of the 15 cheese-making traits were better with the former group of breeds, although the differences were not, on average, very large.

With respect to almost all the traits examined, the individual values making up the average value of the three specialized dairy breeds covered a greater range than the individual values of the three dual-purpose breeds. Our findings confirmed the lower milk productivity potential of Jersey cows compared with the large-framed dairy breeds, i.e., Holstein Friesian (-40%) and Brown Swiss (-29%), but greater %CY_{CURD} (+35% and +19% compared with HF and BS, respectively). This is due not only to higher milk-fat and protein contents, but also to the greater recovery of all nutrients in the curd of Jersey cows. As a result, the differences between the daily cheese production of Jersey cows on the one hand and Holstein Friesian and Brown Swiss cows on the other (-12% and -10%, respectively, for dCY_{CURD}) are much less than the differences in daily milk yield.

Theoretical cheese yield (both as *Th*-%CY_{CURD} and *Th*-%CY_{SOLIDS}) based on milk composition confirmed the superiority of the Jerseys over the two large dairy breeds. However, it is interesting to take a closer look at the differences between the actual and theoretical cheese-yields. The ratio between them yields an estimate of the global efficiency of different breeds in terms of milk nutrient and water retention in cheese. As shown in Table 4, the cheese-making efficiency (both as *Ef*-%CY_{CURD} and *Ef*-%CY_{SOLIDS}) of Jersey cows did not differ from that of the large-framed cows.

Compared with Holstein-Friesian, the Brown Swiss cows had a lower productivity potential (-16.1%), compensated for by greater CY_{CURD} (+13.5%), CY_{SOLIDS} (+10%) and CY_{WATER} (+15.9%), due to the greater nutrient content of their milk as well as better nutrient recovery in the curd (+3.9% for fat, +1.5% for protein, +6.6% for solids, and +4.6% for energy). The final result was that none of the three dCY traits differed statistically in the two large-framed dairy breeds.

The theoretical %CYs, as expected being based on milk composition, confirmed the large difference between the two breeds, the Brown Swiss having the higher values. However, as the difference between the *Th*-%CYs of the two breeds is less than the difference between the actual values, the cheese-making efficiency (both as *Ef*-%CY_{CURD} and *Ef*-%CY_{SOLIDS}) of the Brown Swiss breed was greater than that of the Holstein Friesians (Table 4).

Regarding the dual-purpose breeds, it is to be noted that, compared with the two medium-framed local breeds (Rendena and Alpine Grey), the large-framed Simmental cows had a greater daily production of milk (+15.9%) and also cheese (+22%, +20% and +24% for dCY_{CURD}, dCY_{SOLIDS} and dCY_{WATER}, respectively). The greater differences in dCY traits than in dMY are mainly due to differences in milk composition and not in cheese-making efficiency.

The differences found between the two local breeds were modest: Rendena cows produced more milk than Alpine Greys, but had lower %CY traits (due to lower fat and protein contents), similar REC_{FAT} and $REC_{PROTEIN}$, and lower REC_{SOLIDS} (Table 4). Taking daily milk yield, milk composition, %CY and REC traits all together, the results were slightly better for the Rendena cows for the three dCY traits, but the difference was not significant (Table 4).

The breed × parity interaction did not affect any of these traits, while breed × DIM affected, in particular, the three actual and 2 theoretical %CY traits, REC_{SOLIDS} and REC_{ENERGY}, but not overall cheese-making efficiency and daily cheese yield. In particular, of the specialized breeds, $\%CY_{CURD}$ from Holstein Friesians decreased the first part of lactation and reached a peak of about 16% at the end; with Brown Swiss cows there was a linear increase from 14.5 to 18.7% during lactation, while with Jersey cows there was a linear increase from 15.6% to 20.7% over 125 days and then a slower increase to 22.1% at the end of lactation. With all the dual-purpose breeds there was a small reduction in CY_{CURD} during the first phase of lactation, then an increase towards the final phase.

DISCUSSION

Effect of environment on cheese-making traits

The effect of herd, parity and DIM on %CY traits, the amount of nutrients recovered in the curd and the cow's daily production (dCY) has been previously studied in 1167 individual Brown Swiss cows reared in 85 single-breed farms (Cipolat-Gotet et al., 2013).

The present study provided confirmation of the finding that the effect of herd-date always represents a lower proportion of total variability than the effect of animal and the residual variability with respect to daily milk and cheese production, and %CY and REC traits (Figure 2).

Given that herd clusters together several management factors (i.e., housing conditions, feed administration and quality), as well as the collection and processing of milk samples, and given that here herd is combined with sample collection date (and therefore also with season), the % of variability in cheese-making traits explained by this factor may be considered low for REC traits, moderate for %CY and moderate-high for daily milk and cheese production per cow. This means that the improvement in REC and %CY traits is basically due to individual animal factors (i.e., breed, genetics, parity, stage of lactation, etc.), while herd (facilities, management, nutrition, health, etc.) plays a much more important role in the level of production. Cipolat-Gotet et al. (2013) found this factor to have a greater effect on the same traits compared with our study, and, in particular, they reported values of variability due to herd-date from 21 to 31% for %REC traits, from 24 to 42% for %CY traits, and from 51 to 53% for dCY traits. However, it is worth noting that in their case herd-date was included in the statistical model as a random effect, but not within class of herd productivity.

To our knowledge, no previous studies have investigated the effects of herd productivity (high or low) on cheese yield and cheese-making traits. The effects of farming conditions, season and cow feeding regime (Summer et al., 2003), and of cheese-making technologies (Bynum and Olson, 1982b) on $REC_{PROTEIN}$ and REC_{FAT} have been investigated, but few studies have examined the effects of individual sources of variation on these traits.

Effect of breed within herd

No previous studies have processed milk obtained from individual animals of several breeds, but information on comparisons of some breeds is available.

Using laboratory model cheeses produced from defatted milk samples from 45 individual cows, Wedholm et al. (2006) compared the cheese yield of Swedish Holstein Friesian, Danish Holstein Friesian and Swedish Red and White specialized dairy breeds. None of the three %CY traits measured was affected by breed, but it should be noted that the milk was defatted before cheese-making, and that the statistical model used also included linear regressions on total casein and on each of the casein fractions and genetic variants. The same authors also found effects of parity and lactation stage similar to the results found in the present study.

More information is available from studies using bulk milk from experimental or commercial farms and processed in small-scale or conventional dairy plants. Among the specialized dairy breeds, in particular, large differences were observed between the small-framed Jersey cows, and the large-framed Holstein Friesians. The former are known for their lower average milk yield, but also higher milk fat and protein contents, and consequently greater %CY traits. Auldist et al. (2004) found the cheese yields from bulk milk from 29 Jersey and 29 Holstein cows reared at pasture to be 12.0 and 10.8%, respectively, i.e., +11% *vs* + 34% for Jerseys in the present study. The difference is therefore smaller than in our study, but, in accordance with Cheddar production norms, the protein:fat ratio was normalized to 0.80 before cheese making so that it reflects the difference between the two breeds in terms of protein content. In another study, the same authors compared the two breeds, also after equalization of the solids content through ultrafiltration of Holstein milk, and, in this case, found no differences between the two breeds, in agreement with our results on overall cheese-making efficiency. Milk composition is not the only reason for the greater cheese yield of milk from Jersey cows. There were no differences either between the two breeds in the ratios between the moisture-adjusted cheese produced for every 100 kg of milk solids. This parameter is apparently similar to our REC_{SOLIDS}, but the major reasons for the differences between Auldist et al (2004) and the present study seem to be partial skimming of the Jersey milk in the former study (because the higher REC_{FAT} found in Jersey milk cannot be fully appreciated), and equalization of the solids content, which also causes equalization of the lactose:total solids ratio in the milk of the two breeds.

Recently, Bland et al. (2015) carried out a study on the effects of blending different proportions of Holstein and Jersey bulk milk on cheese production in a pilot-scale cheese-making facility without protein:fat standardization and total solids equalization (as in the present study).

The $\%CY_{CURD}$ was 12.0% using 100% Jersey milk and 9.5% using 100% Holstein milk , i.e., +26% for Jerseys *vs* + 34% in the present study. The same authors found a moisture-adjusted cheese yield (conceptually similar to our $\%CY_{SOLIDS}$) of 12.1% for Jersey milk and 9.1% for Holstein milk, i.e., +33% for Jerseys *vs* + 35% in the present study. The differences between the two breeds in both studies are very close (+32% for Jersey milk) to those regarded as typical of the two breeds by Lucey and Kelly (1994). Using the traditional van Slyke and Pryce (1949) equation, Bland et al. (2015) calculated a theoretical cheese yield ($\%CY_{CURD}$, obtained on the basis of the fat and casein content of milk) of 12.4% for Jersey and 10.6% for Holstein milk, i.e., +17% for Jerseys *vs* + 29% in the present study. As the $\%CY_{CURD}$ is based on the fat and casein contents of milk, assuming constant recovery rate for both, the difference between the theoretical and actual yields depends on differences in the REC traits. In fact, Bland et al. (2015), found an REC_{FAT} of 99.3 *vs* 76.6%, and an $REC_{PROTEIN}$ of 81.3 *vs* 71.6% for Jersey and Holstein milk, respectively, i.e., differences in the same direction but much larger than those obtained in the present study, probably because of very different cheese-making procedures.

In any case, the better REC traits in Jersey milk could be explained, in part, by milk coagulation, curd firming and syneresis properties. Several authors have found higher traditional MCP levels in Jersey milk than in Holstein milk, as reviewed by Bittante et al. (2012). Similar results also were found when the entire pattern of the curd firming process (CF_t equation) was modeled (Stocco et al., 2016). Rapid milk gelation and, especially, an efficient curd firming process and syneresis have been found to result in favorable genetic and phenotypic correlations with regard to %CY and REC traits, especially for those parameters recorded at maximum curd firmness or later (Cecchinato and Bittante, 2016).

Similar interpretations could be applied when comparing the two large-framed specialized dairy breeds, HF and BS. The superiority of the breed of Alpine origin is, in fact, not only based on greater contents of fat and protein in milk, but also on efficient milk coagulation, curd firming, syneresis (Bittante et al., 2012) and overall cheese-making process leading to lower fat and protein losses in the whey (Cecchinato et al., 2015). Mistry et al. (2002) and Malacarne et al. (2006) found greater REC_{FAT} (but not $REC_{PROTEIN}$), as well as greater actual than theoretical %CY, in milk from Brown Swiss cows than in milk from Holstein Friesians.

Within dual-purpose breeds, we were able to confirm the large-framed Italian Simmental breed, derived from Austrian and German Fleckvieh and from French Montbeliarde (Cecchinato et al., 2015), as having a good technological aptitude, better than Holstein Friesian and closer to Brown Swiss (Bittante et al., 2012; Malchiodi et al., 2014; Stocco et al., 2016). The milk from Montbéliarde cows is known for having a much greater %CY than milk from Holstein cows, as expected on the basis of the fat and protein contents (Martin et al., 2009), although other studies found no differences in the REC_{SOLIDS} of the two breeds (Verdier-Metz et al., 1998).

The differences between the two small local breeds were slight, the Alpine Grey cows performing better with respect to %CY traits as the milk of this breed contains more fat and protein than milk from Rendena cows. This greater milk fat and protein content also explains the higher REC_{SOLIDS} and REC_{ENERGY} of the Alpine Greys, which is due to the lower proportion of lactose compared with total milk solids (and consequently the proportion of solids lost in the whey). The composition of milk from Rendena cows is similar to that from Holstein cows, but has better coagulation and curd firming patterns (Stocco et al., 2016), which could explain the greater REC_{FAT} and greater, although to a smaller degree, $REC_{PROTEIN}$ compared with the specialized dairy breed. It is worth noting that, even after correcting for herd productivity class, the effect of individual herd, parity and DIM, this breed had the highest overall cheese-making efficiency (both as *Ef*-%CY_{CURD} and as *Ef*-%CY_{SOLIDS}) of all the 6 breeds examined in the present study.

Direct and indirect effects of breed

Since the differences among breeds were substantial, to distinguish and quantify the direct effects of breed on cheese-making efficiency and daily cheese-yield traits from the indirect effects mediated by differences in terms of milk yield and composition, we included MY, TS, protein, fat, lactose, pH and SCS as general covariates in the basic model, and calculated the differences in breed variances with and without covariates for each trait. It can be seen from Figure 4 that the proportions of direct and indirect effects are very different for the various traits examined.

Milk yield and composition (indirect effect of breed) accounted for a large proportion of the total breed variance for all REC traits, but the extent of the direct effect of breed ranged from 11% for REC_{FAT} to 52% for REC_{SOLIDS}. The direct effect of breed on %CY traits was, as expected, much lower, because of the dependence of these traits on available fat and casein. Nevertheless, they represent a sizeable proportion of total variability from a technical and economic point of view, being 8.3% for $\%CY_{CURD}$ and representing 2.3% and 13%, respectively, for the constituent traits, $\%CY_{SOLIDS}$ and $\%CY_{WATER}$. The theoretical cheese yields were, as expected, totally dependent on the indirect breed effects, as they were calculated only from milk fat and casein contents (and the moisture content of cheese). However, given the ratio between the actual and the theoretical %CYs, the two cheese-making efficiencies are, as expected, about two thirds dependent on the direct effect of breed.

Moving on to production traits, the total variance of the effect of breed on dMY was only about 30% dependent on milk composition (indirect effect of breed, in this case, of course, as the model with covariates did not include dMY). In the case of dCY traits, the indirect proportion of breed variance was substantial, including in the model the covariate with both dMY and milk composition traits. In any case, it is worth noting that the direct effect of breed represented proportions of variance similar to or greater than those observed for %CY traits (11% for dCY_{CURD}, 2.4% for dCY_{SOLIDS} and 28% for dCY_{WATER}).

No direct comparison is possible with other studies, as this is the first study to attempt to differentiate between the direct and indirect effects of breed on cheese-making traits. The approach that we took to examining milk coagulation, curd firming and syneresis traits (Stocco et al., 2016) showed that, for these traits, the direct effect of breed, i.e., not mediated by milk yield and composition, represented a substantial proportion of total breed variance, ranging from about 40%

to 80%. These traits are important in explaining REC and %CY traits at the phenotypic, genetic, herd and residual levels, as demonstrated in a previous paper (Cecchinato and Bittante, 2016). It is also worth noting that a variable fraction of the breed effect on coagulation properties is explained by genetic variants of milk proteins (Ikonen et al., 1999a; Auldist et al., 2002). Both milk coagulation traits and milk protein genetic variants could be a part of the factors influencing the direct effect of breed, as defined in the present study.

In the case of *Ef*-%CY traits, it is interesting to see that about a third of breed variance is due to indirect effects of breed (MY and composition), even though they represent the ratio between actual %CY and theoretical %CY predicted on the basis of the fat and casein contents of milk. It is evident that this proportion is explained by a different relationship between %CY and milk fat and protein compared with the van Slyke and Price (1949) formula (a greater effect of casein and slightly lower effect of fat, data not shown), and by other factors included here as covariates. In particular, the constituents that could be considered indicators of subclinical mastitis (SCS, lactose and pH) were often significant for several cheese-making traits (data not shown), in agreement with Bobbo et al. (2016) for SCS.

Implications for crossbreeding and selection

The breed effect, when corrected for common (herd) and individual (parity, DIM, etc.) phenotypic sources of variation, may be considered the major genetic difference between animals and may also be an indicator of possible genetic variation between and within populations. Knowledge of the cheese-yield traits of milk from different breeds could be important for planning crossbreeding programs meeting industry requirements, especially in areas where a large part of the milk produced is destined for cheese making. No direct information is available on comparisons of different breed combinations or on the role of heterosis on these traits, but a study carried out on milk coagulation and curd firming traits (Malchiodi et al., 2014) showed that crossbred cows from different breed combinations may sometimes have different milk properties to those expected. If knowledge of breed effects is important for crossbreeding programs, it is evident that further improvements to their overall efficiency requires new knowledge on the effects of heterosis and specific breed combinations.

Moving to within-breed variability, no genetic studies have used data from processed milk from several breeds at the individual level, but the heritability of cheese-yield traits was estimated in the Brown Swiss breed by Bittante et al. (2013). As dMY and milk fat and protein (casein) are included in the selection indices of dairy breeds in almost all developed countries (Miglior et al., 2005), it could be said that *Th*-%CY is selected worldwide, although with weights different from those indicated by van Slyke and Price (1949) and adapted to local industry requirements. It seems clear from the results of the present study that the dairy industry could gain an economic advantage by, first of all, including the recovery of nutrients, and particularly of fat and protein, in the selection indices. The genetic indices in use, which only include the percentages of fat and protein in milk, implicitly assume that the REC of these nutrients is constant, or not heritable. These traits are not only variable, but $REC_{PROTEIN}$ and REC_{FAT} were found to have larger heritabilities than milk protein and fat contents (Bittante et al., 2013). Moreover, a definition of the weights in the selection indices of milk traits indicating subclinical mastitis (not only SCS but also lactose and pH) that takes into account their negative effects on cheese-yield traits could improve the economic efficiency of dairy cattle populations. A more general alternative would be to add the *Ef*-%CY traits to the selection indices.

The major problem with implementing cheese-yield traits in selection programs is how to evaluate the animals for these traits, as laboratory analyses are not feasible at the population level. A promising approach is to predict them using Fourier-transform Infrared (FTIR) spectra of the milk samples routinely collected during milk recording. It has been proven possible to predict the %CY and REC traits with acceptable to good accuracy, with the sole exception of REC_{FAT} (Ferragina et al., 2013 and 2015). The heritability of predicted traits is characterized by values comparable to or greater than those of the corresponding laboratory-measured traits (Bittante et al., 2014), and, more importantly, the genetic correlations between predicted and measured traits have always been greater than calibration accuracy. The feasibility of FTIR prediction of %CY and REC traits at the population level was tested on Holstein Friesian, Brown Swiss and Simmental breeds with good results (Cecchinato et al., 2016). Only complex traits, like *Ef*-%CY, have not yet been evaluated.

Another promising alternative is to predict breeding values directly at the genetic level instead of predicting phenotypes. A genome-wide association study on these traits was carried out by Dadousis et al. (2016a) revealing the complex genetic pathways leading to milk coagulation and cheese-making traits (Dadousis et al., 2016b) The results open new perspectives on direct genomic selection for milk-yield efficiency of dairy cattle. Egger-Danner et al. (2015) concluded their review paper by stating that a combination of phenotyping and genotyping would be a highly suitable option for the new phenotypes.

CONCLUSIONS

In conclusion, the study carried out on 27 multi-breed herds belonging to two classes of herd productivity (high *vs* low) revealed cheese-making to be a complex phenomenon, the end result is of which is driven by several factors. The quality of the milk processed in terms of nutrient contents (mainly fat and casein), the recovery of these nutrients in the curd (affected by technological traits like those involved in milk coagulation, curd firming and syneresis), the retention of water in cheese, and overall cheese-making efficiency all contribute to the percentage cheese yield.

Increasing herd productivity increases milk yield and quality, percentage cheese yields, and daily cheese production per cow, but has only a slight effect on nutrient recovery and a negative effect on overall cheese-making efficiency, i.e., the actual cheese yield is somewhat lower than expected. The factors responsible for this lower efficiency need to be identified. Within herd productivity classes, variability among different herds is much lower for recovery traits, percentage cheese yields and cheese-making efficiency than for the daily production of milk and cheese.

Within individual herds, animal factors are responsible for the greater part of the variability in all traits, and among these factors the breed of cow has proven to be the most important. The differences among different breeds are the result not only of the well-known differences in production potential and nutrient concentrations, but also of the differences in nutrient recovery ability and overall cheese-making efficiency. While the Holstein Friesian breed seemed to be the most productive but to have the least cheese-making efficiency, the most efficient out of the dairy breeds appeared to the Brown Swiss, and out of the dual-purpose local breeds the Rendena. When reared under the same environmental and management conditions, the greater percentage cheese yield and efficiency of the Brown Swiss breed, and also of the Simmental, meant they were able to overcome their lower milk productivity and to yield a daily quantity of cheese per cow similar to the Holstein Friesians. The Jersey cows, despite their small body size, were also able to partly compensate for their low milk productivity with the high fat and protein contents and recovery rates of their milk, so that the daily cheese production per cow was only about a tenth lower than the large-framed cows. Analysis of the differences between the various breeds also provided new insights into the possibilities and directions of genetic selection within breed and of breed combinations in crossbreeding programs.

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TABLES AND FIGURES

Table 1. Descriptive statistics of milk composition, of cheese-making traits and production.

Trait	N	Mean	P1[2]	P99	Kurtosis	Skewness
Milk composition:						
Total Solids, %	520	13.50	10.7	17.0	0.14	0.04
Protein, %	518	3.62	2.9	4.7	0.59	0.14
Casein, %	516	2.81	2.1	3.8	0.31	0.23
Fat, %	513	4.18	1.4	7.1	0.42	-0.05
Lactose, %	515	5.00	4.4	5.5	0.59	-0.09
Milk energy, MJ/kg	515	3.29	2.2	4.5	0.35	0.00
pH	515	6.49	6.3	6.7	-0.02	0.13
SCS[1]	518	2.70	-0.6	7.1	-0.14	0.09
Nutrients recovery, %:						
REC_{FAT}	508	84.71	70.0	91.5	0.52	-0.58
$\text{REC}_{\text{PROTEIN}}$	512	79.33	74.1	82.7	0.01	-0.22
$\text{REC}_{\text{SOLIDS}}$	514	53.39	43.1	64.8	0.13	-0.13
$\text{REC}_{\text{ENERGY}}$	512	68.91	58.3	78.0	0.35	-0.46
Cheese Yields, %:						
%CY_{CURD}	512	15.71	10.4	23.4	-0.03	-0.09
%$\text{CY}_{\text{SOLIDS}}$	508	7.23	4.8	10.7	0.01	-0.05
%CY_{WATER}	512	8.48	5.3	12.4	-0.14	-0.05
Theoretical CY, %:						
Th-%CY_{CURD}	514	15.66	9.5	23.4	0.20	0.08
Th-%$\text{CY}_{\text{SOLIDS}}$	515	7.21	4.4	11.0	0.32	0.10
Efficiency of CY, %:						
Ef-%CY_{CURD}	513	101.0	77.3	123.2	0.13	-0.06
Ef-%$\text{CY}_{\text{SOLIDS}}$	512	101.0	87.9	112.4	0.50	-0.27
Daily production, kg/d:						
dMY	510	20.3	6.0	41.1	-0.24	0.02
dCY_{CURD}	504	3.16	0.8	6.0	0.02	-0.06
$\text{dCY}_{\text{SOLIDS}}$	504	1.46	0.4	2.9	0.15	0.02
$\text{dCY}_{\text{WATER}}$	504	1.71	0.4	3.4	0.05	0.05

[1] SCS= 3 + $\log_2$ (SCC/100,000); [2] P1 = 1st percentile; P99 = 99th percentile.

Table 2. Effect of herd productivity level and of its interactions with parity and days in milk on milk composition, and on cheese-making traits and production of individual cows.

	Herd productivity (HP):			Interactions (F-value):		
	High-HP (LSM)	Low-HP (LSM)	F-value	HP × Parity	HP × DIM	RMSE[1]
Milk composition:						
Total Solids, %	13.83	13.13	17.4^{***}	1.6	2.2^{*}	0.8
Protein, %	3.67	3.50	8.1^{**}	0.9	2.1^{*}	0.2
Casein, %	2.85	2.70	9.2^{**}	0.7	1.9	0.2
Fat, %	4.45	3.99	9.1^{**}	0.7	1.4	0.7
Lactose, %	5.00	4.99	0.0	0.4	1.6	0.2
Milk energy, MJ/kg	3.42	3.17	15.3^{***}	1.5	2.6^{**}	0.3
Curd nutrients recovery, %:						
REC_{FAT}	84.69	85.31	0.6	0.5	2.6^{**}	4.1
$REC_{PROTEIN}$	79.51	79.23	0.5	0.9	1.3	1.6
REC_{SOLIDS}	54.13	52.68	4.4^{*}	1.0	2.3^{*}	3.1
REC_{ENERGY}	69.44	68.49	2.8	0.8	2.1^{*}	2.9
Cheese yields, %:						
$\%CY_{CURD}$	16.15	15.13	5.9^{*}	1.7	2.4^{*}	1.5
$\%CY_{SOLIDS}$	7.46	7.02	6.7^{*}	1.4	2.2^{*}	0.7
$\%CY_{WATER}$	8.65	8.18	3.1	0.9	2.1^{*}	0.9
Theoretical %CY, %:						
$Th\text{-}\%CY_{CURD}$	16.46	14.86	16.3^{***}	1.4	2.3^{*}	1.9
$Th\text{-}\%CY_{SOLIDS}$	7.57	6.83	16.3^{***}	1.4	2.3^{*}	0.9
Efficiency of %CY, %:						
$Ef\text{-}\%CY_{CURD}$	99.42	102.57	4.2	0.5	1.3	8.4
$Ef\text{-}\%CY_{SOLIDS}$	99.95	102.44	7.8^{**}	0.7	1.3	4.0
Daily production, kg/d:						
dMY	25.1	16.7	36.0^{***}	2.2	1.2	4.0
dCY_{CURD}	3.99	2.52	34.8^{***}	0.2	1.1	0.6
dCY_{SOLIDS}	1.87	1.15	43.0^{***}	0.6	1.6	0.3
dCY_{WATER}	2.13	1.37	30.5^{***}	0.1	1.3	0.4

[1] RMSE= Root Mean Square Error; *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3. Effect of parity on milk composition, and on cheese-making traits and production of individual cows.

	Parity (LSM):				Parity Contrasts (*F*-value):		
	1st	2nd	3rd	≥4th	1st vs ≥2nd	2nd vs ≥3rd	3rd vs ≥4th
Milk composition:							
Total Solids, %	13.61	13.69	13.25	13.38	3.2	11.0^{**}	0.8
Protein, %	3.62	3.63	3.55	3.53	3.6	6.6^{*}	0.3
Casein, %	2.82	2.80	2.74	2.73	5.9^{*}	4.6^{*}	0.1
Fat, %	4.27	4.31	4.04	4.25	0.7	2.6	2.8
Lactose, %	5.07	5.01	4.94	4.97	17.6^{***}	3.6	1.1
Milk energy, MJ/kg	3.33	3.35	3.20	3.28	2.3	6.2^{*}	2.6
Curd nutrients recovery, %:							
REC_{FAT}	85.29	84.74	85.21	84.77	0.6	0.2	0.4
$\text{REC}_{\text{PROTEIN}}$	79.94	79.42	79.36	78.75	15.6^{***}	2.4	4.7^{*}
$\text{REC}_{\text{SOLIDS}}$	53.55	53.81	53.03	53.21	0.3	2.3	0.1
$\text{REC}_{\text{ENERGY}}$	69.53	69.15	68.78	68.40	4.7^{*}	1.7	0.6
Cheese yields, %:							
$\%\text{CY}_{\text{CURD}}$	16.07	15.82	15.29	15.38	10.3^{*}	4.9^{*}	0.1
$\%\text{CY}_{\text{SOLIDS}}$	7.35	7.36	7.07	7.19	2.7	4.5^{*}	0.9
$\%\text{CY}_{\text{WATER}}$	8.72	8.44	8.24	8.25	13.4^{***}	2.0	0.0
Theoretical %CY, %:							
$Th\text{-}\%\text{CY}_{\text{CURD}}$	15.80	16.10	15.22	15.52	0.7	7.2^{**}	0.8
$Th\text{-}\%\text{CY}_{\text{SOLIDS}}$	7.27	7.41	7.00	7.14	0.7	7.2^{**}	0.8
Efficiency of %CY, %:							
$Ef\text{-}\%\text{CY}_{\text{CURD}}$	102.5	99.9	101.5	100.1	4.4^{*}	0.5	0.9
$Ef\text{-}\%\text{CY}_{\text{SOLIDS}}$	101.5	100.9	101.4	100.9	0.9	0.3	0.5
Daily production, kg/d							
dMY	19.3	20.5	22.1	21.5	19.0^{***}	4.7^{*}	0.8
dCY_{CURD}	3.06	3.29	3.41	3.25	10.8^{*}	0.1	2.0
$\text{dCY}_{\text{SOLIDS}}$	1.41	1.54	1.58	1.52	13.8^{***}	0.1	1.1
$\text{dCY}_{\text{WATER}}$	1.67	1.77	1.82	1.73	5.6^{*}	0.0	2.0

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 4. Effect of breed and of interactions between breed and parity and DIM on milk composition, and on cheese-making traits and production of individual cows.

	Breed (LSM):						Contrasts (*F*-value):					Interactions (*F*-value):	
	Holstein-Friesian (HF)	Brown Swiss (BS)	Jersey (Je)	Simmental (Si)	Rendena (Re)	Alpine Grey (AG)	HF BS Je vs Si AG Re	HF BS vs Je	HF vs BS	Si vs Re AG	Re vs AG	Breed × Parity	Breed × DIM
Milk composition:													
Total Solids, %	13.06	13.51	14.81	13.41	12.73	13.37	28.0^{***}	65.5^{***}	9.4^{**}	4.2^{*}	10.5^{**}	0.6	1.5^{*}
Protein, %	3.34	3.67	3.91	3.55	3.38	3.65	9.8^{**}	50.0^{***}	50.2^{***}	0.4	20.8^{***}	0.9	1.6^{*}
Casein, %	2.55	2.84	3.10	2.76	2.56	2.83	11.4^{***}	62.8^{***}	49.0^{***}	1.9	24.0^{***}	0.7	1.7^{**}
Fat, %	4.03	4.13	5.46	4.22	3.60	3.86	37.1^{***}	66.2^{***}	0.5	9.4^{**}	2.2	0.5	1.4^{*}
Lactose, %	4.99	5.01	4.85	4.96	5.08	5.07	9.4^{**}	10.3^{**}	0.7	6.5^{*}	0.1	1.5	1.4
Milk energy, MJ/kg	3.15	3.30	3.81	3.26	3.01	3.22	1.2	70.1^{***}	30.2^{***}	11.9^{**}	7.3^{*}	0.6	1.1
Curd nutrients recovery, %:													
REC_{FAT}	81.30	84.51	88.39	85.63	85.43	84.75	0.8	32.3^{***}	17.7^{***}	1.4	0.5	1.3	1.4
$\text{REC}_{\text{PROTEIN}}$	78.46	79.64	79.99	79.37	79.49	79.26	0.0	5.7^{*}	14.2^{***}	0.0	0.3	1.1	1.0
$\text{REC}_{\text{SOLIDS}}$	50.42	53.78	58.86	53.56	50.85	52.93	16.6^{***}	82.7^{***}	32.4^{***}	5.8^{*}	7.3^{*}	0.7	1.8^{**}
$\text{REC}_{\text{ENERGY}}$	66.12	69.14	73.63	69.39	67.16	68.36	9.4^{**}	74.2^{***}	30.5^{***}	6.4^{*}	2.9	0.8	1.8^{**}
Cheese yields, %:													
$\%\text{CY}_{\text{CURD}}$	13.95	15.84	18.82	15.68	14.15	15.40	24.4^{***}	117.2^{***}	41.9^{***}	7.2^{*}	10.5^{**}	0.6	1.8^{**}
$\%\text{CY}_{\text{SOLIDS}}$	6.58	7.24	8.90	7.26	6.47	6.99	35.3^{***}	122.5^{***}	20.8^{***}	9.7^{**}	7.9^{*}	0.5	1.8^{**}
$\%\text{CY}_{\text{WATER}}$	7.40	8.58	10.10	8.52	7.58	8.30	15.3^{***}	86.2^{***}	41.1^{***}	7.4^{*}	8.7^{**}	1.0	1.5^{*}
Theoretical %CY, %:													
$\mathit{Th}\text{-}\%\text{CY}_{\text{CURD}}$	14.65	15.74	18.93	15.48	13.90	15.26	38.1^{***}	87.0^{***}	12.1^{**}	5.2^{*}	9.5^{*}	0.5	1.7^{**}
$\mathit{Th}\text{-}\%\text{CY}_{\text{SOLIDS}}$	6.74	7.24	8.71	7.12	6.39	7.02	37.8^{***}	85.5^{***}	11.8^{**}	5.1^{*}	9.5^{*}	0.5	1.7^{**}
Efficiency of %CY, %:													
$\mathit{Ef}\text{-}\%\text{CY}_{\text{CURD}}$	96.1	102.4	100.9	101.0	103.5	102.2	4.0^{*}	0.7	16.8^{***}	1.0	0.4	0.8	1.0
$\mathit{Ef}\text{-}\%\text{CY}_{\text{SOLIDS}}$	98.6	101.4	101.5	101.0	102.6	102.0	5.3^{*}	2.5	13.6^{***}	2.1	0.4	1.3	1.3

Daily production, kg/d:													
dMY	26.1	21.9	15.6	22.6	20.9	18.1	1.2	70.1^{***}	30.2^{***}	11.9^{***}	7.3*	1.5	1.1
dCY_{CURD}	3.57	3.47	3.14	3.55	2.94	2.85	7.9^{**}	5.4^{*}	0.6	20.1^{***}	0.3	1.7	0.9
dCY_{SOLIDS}	1.71	1.59	1.43	1.62	1.38	1.33	8.2^{**}	8.0^{**}	3.5	14.9^{***}	0.4	1.8	0.8
dCY_{WATER}	1.89	1.89	1.71	1.92	1.58	1.51	7.5^{*}	3.9^{*}	0.0	19.2^{***}	0.5	1.5	1.0

*$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure 1. Cheese-making procedure adopted to obtain the 508 model-cheeses from individual cows.

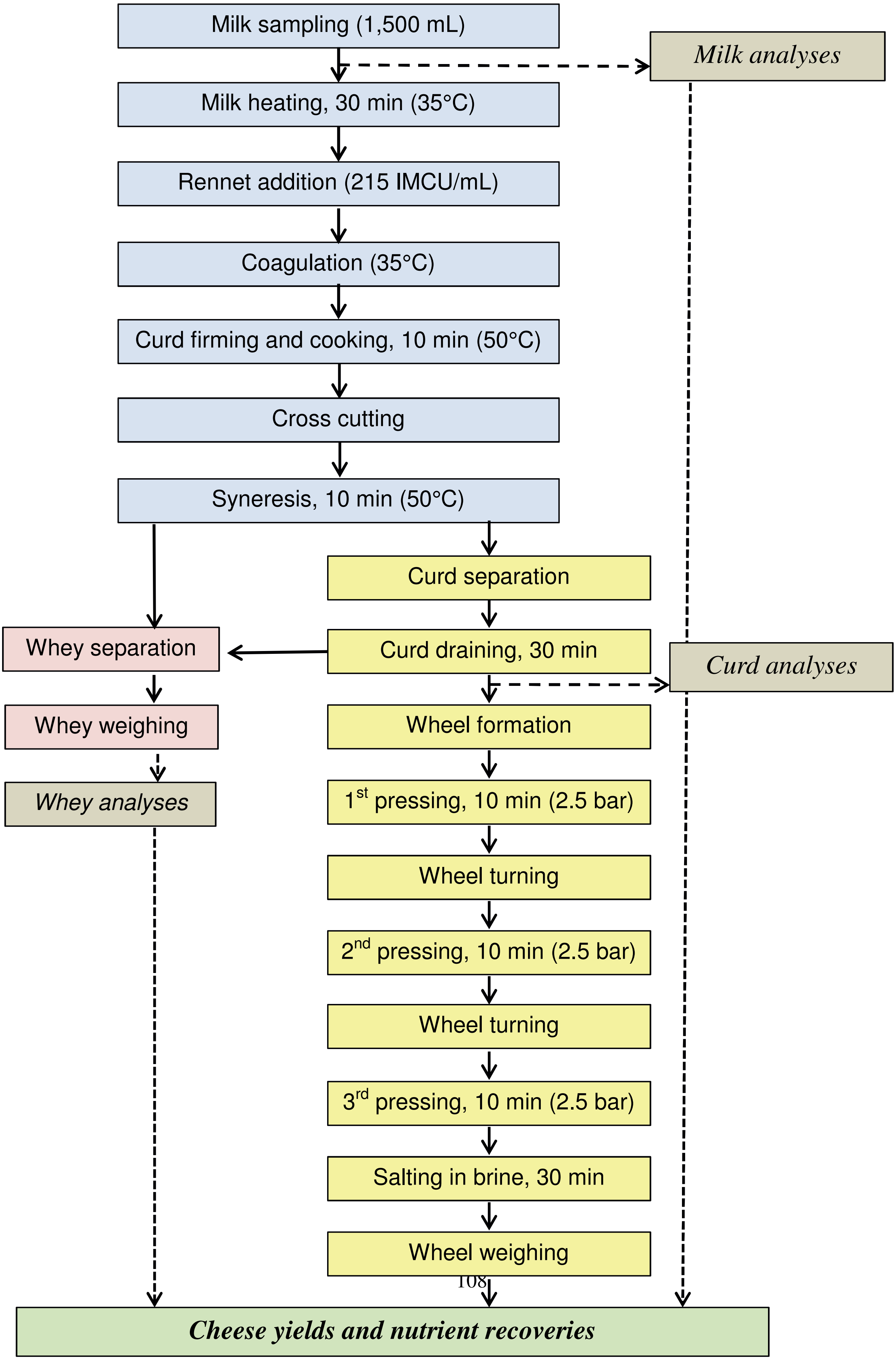


Figure 2. Incidence of herd-date variance on total variance of milk composition, and on cheese-making traits and production of individual cows.

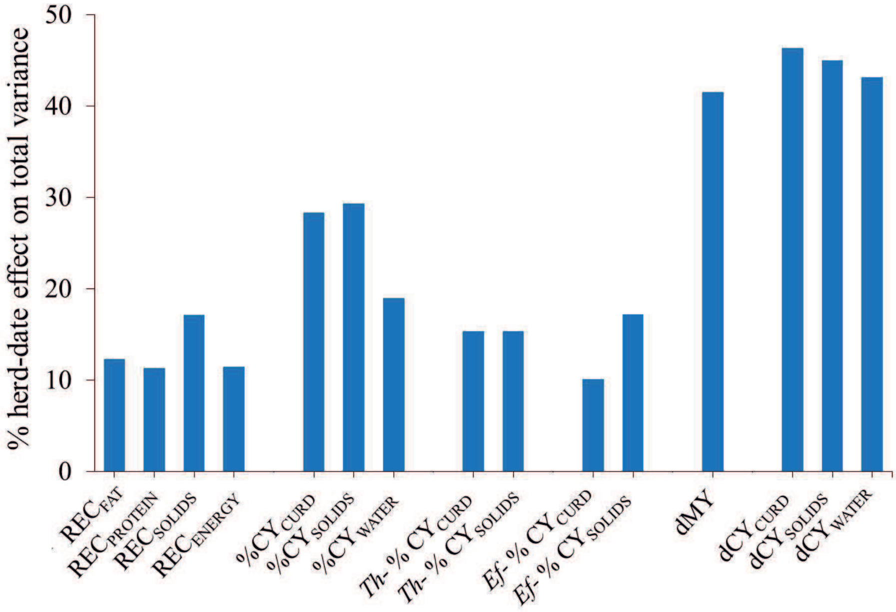


Figure 3. Effect of DIM on the three actual %CY traits

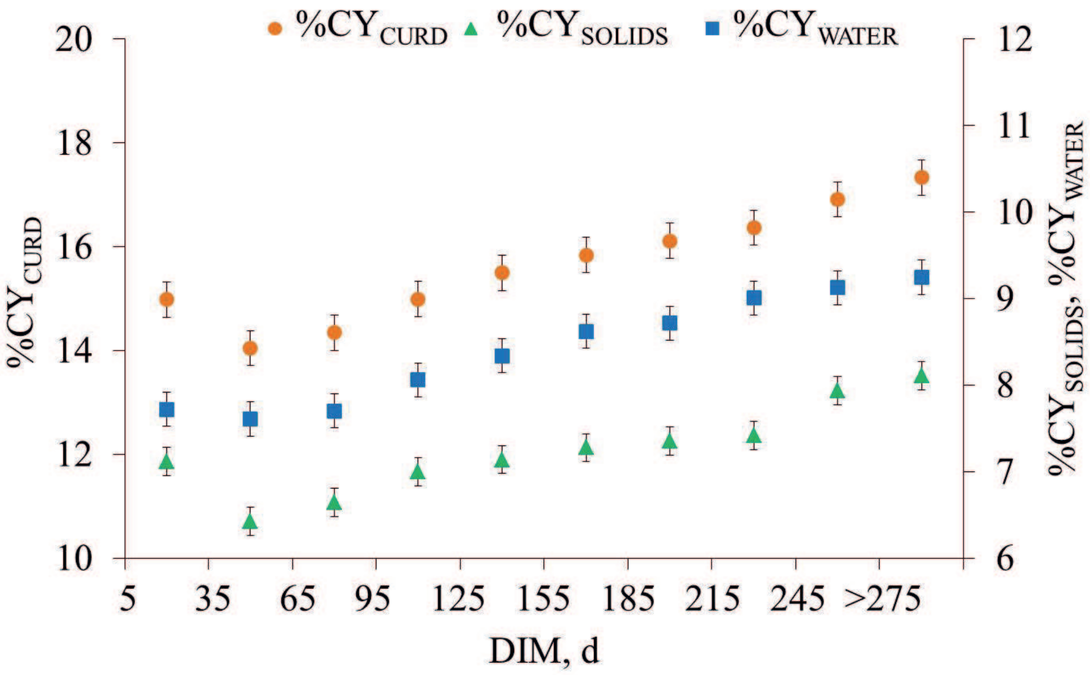
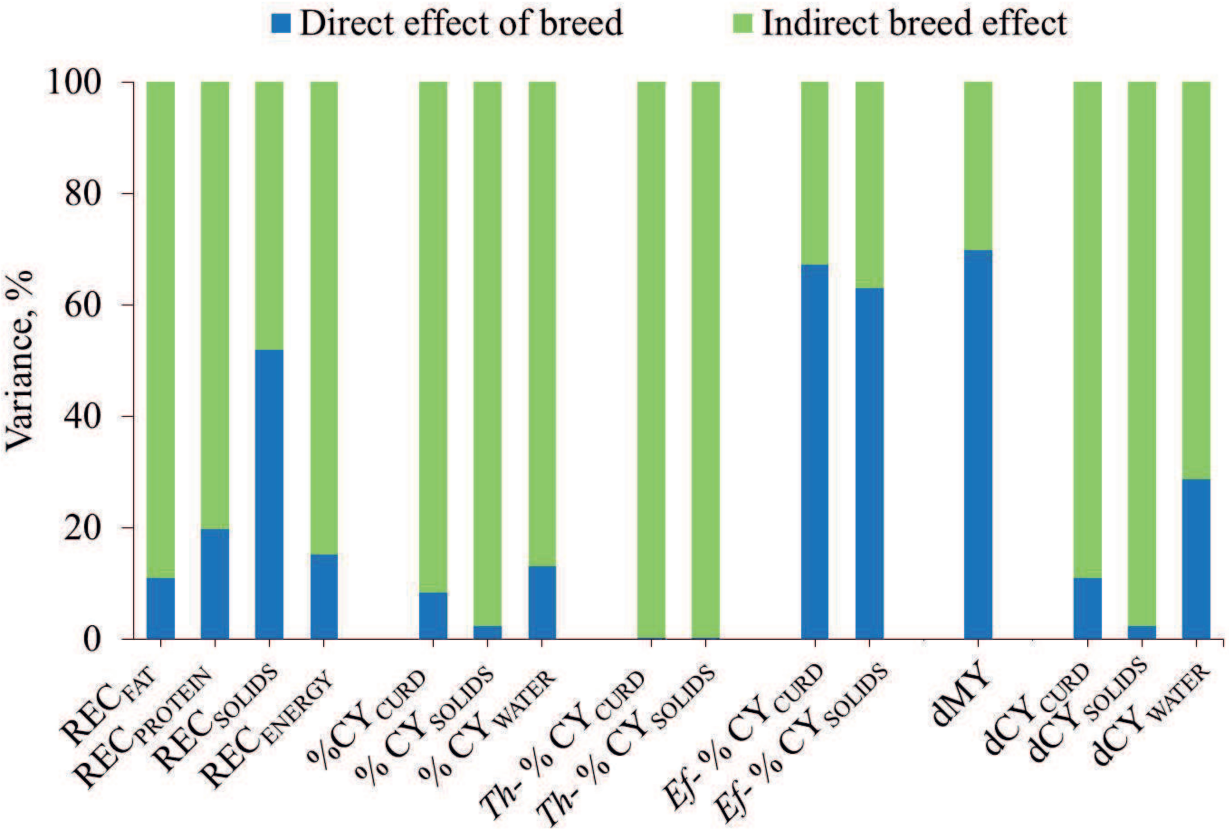


Figure 4. Proportion of total breed variance explained by direct breed effect or by indirect breed effect through differences in milk yield and quality traits on REC, %CY and dCY traits.



Chapter 3

Detailed macro- and micro-mineral profile of milk is affected by herd productivity, 6 breed of cows, parity and stage of lactation

Giorgia STOCCO, Andrea SUMMER,

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ABSTRACT

Milk macro- and micro-minerals are essential for animal's growth and reproduction. To our knowledge, no previous studies have assessed the content of a large number of minerals in many individual milk samples collected from several multi-breed herds with different level of milk production, allowing the separation of the effects of herd and of breed of cows. Herds (n = 27) were classified into two categories based on milk productivity [HP (high production) *vs* LP (low production)], defined according to the average net energy of milk yielded daily by the lactating cows. Milk samples were collected from 240 cows of 6 different breeds, 3 dairy specialized (Holstein-Friesian, Brown Swiss, and Jersey) and 3 dual-purpose (Simmental, Rendena, and Alpine Grey), and they were analyzed for macro- (Ca, P, Na, K, Mg, S), essential micro- (Cu, Fe, Mn, Se, Zn), and environmental micro- (B, Si, Sn, Sr) elements, using Inductively Coupled Plasma - Optical Emission Spectrometry (**ICP-OES**). Results showed that the effect of herd-date varied across minerals, however it was large especially for environmental minerals (ranging from 47 to 91% of the total variance), while for macro- and micro-minerals it ranged from 11% to 61% . Milk samples collected in farms characterized by high level of productivity had a richer mineral profile compared to milk samples collected from low productivity herds. Parity influenced exclusively macro-minerals, with the exception of Ca and S, while DIM influenced almost all minerals, with few exceptions related to the environmental elements. Large differences were observed among breeds, both between specialized and dual-purpose, and within the two groups. Differences remained even after adjusting for milk quality and yield. In comparison to the milk collected from Holstein-Friesian cows, milk samples from Jersey and Brown Swiss cows had better composition and a more valuable mineral profile; the other breeds of Alpine origin produced milk of intermediate quality. Our findings suggested that the effects of breed on macro- and on some of the essential micro-minerals are stronger than the effects of herd productivity, parity and DIM. Moreover, the cow's individual variance was greater than the variance of individual herds within herd productivity level.

We can conclude that improvement of milk macro- and micro-mineral elements seemed to depend on genetics (breed, selection, etc.) rather than on environmental and management factors.

Key words: minerals, breed, herd productivity, days in milk

INTRODUCTION

Cow milk is a valuable source of minerals (Cashman, 2006; Zamberlin et al., 2012), even if they represent a small portion of milk composition (about 8-9 g/L). Minerals in milk occur in different chemical forms: inorganic ions and salts or as part of proteins, nucleic acids, fats and carbohydrates (Cashman, 2006). The demand of dairy products, and especially of fortified products, is increasing in the global market, since consumers are paying more and more attention to the nutritional composition of food products. Some studies reported that minerals have better functional effects when combined to each other (Huth et al., 2006), or with other compounds such as vitamins, proteins and fatty acids (Soyeurt et al., 2009). However, minerals in milk (especially Ca, P and Mg) are studied not only to evaluate their effects on human health, but also for their effects on milk technological properties, in particular for the efficiency of cheese-making (Cooke and McSweeney, 2014; Gustavsson et al., 2014; Malacarne et al., 2014). Furthermore, some elements like Na and K are involved in diagnosis of specific diseases such as mastitis in dairy cows (Hamann and Krömker, 1997). It is recognized that milk mineral content varies among breeds. Mariani et al. (2002) reported that Ca and P contents of milk from Holstein-Friesian cows averaged 112 mg/100 ml and 89.6 mg/100 ml, respectively. The same authors reported that milk of Holstein-Friesian cows had lower concentrations of all colloidal mineral elements, except for colloidal Mg, than Brown Swiss and Reggiana breeds; nevertheless, it contained more colloidal Ca phosphate than milk of Modenese breed. The phenotypic variability of minerals within breed can be partly due to additive genetic effects. However, few studies dealt with the genetics of milk mineral composition because of the high analytical costs. However, results from a study conducted by Buitenhuis et al. (2015) demonstrated the high heritability of minerals, in particular of Ca (0.57), P (0.62), Mg (0.60) and Zn (0.41). The lack of fair comparisons among breeds on the mineral profile persists. In fact, the studies reported in literature are mostly based on a small number of cows of two-three different breeds reared in one or two farms (Mariani et al., 2002; Summer et al., 2004), or on a large number of cows housed in many single-breed farms (Van Hulzen et al., 2009), so that the effect of breed on mineral elements continues to be confounded with the effects of farm, feeding strategy and sampling date. Moreover, since dairy farms have moved towards larger and more industrialized setups in which cows are fed high-energy diets, and dairy breeds have been persistently selected to improve productivity and milk quality, the majority of analytical studies were applied to elucidate any possible relationship between diets, season, manufacturing process and mineral elements in milk and dairy products of cows (Coni et al., 1995), sheep and goats (Coni et al., 1996), and not for the evaluation of the direct breed and herd productivity effect on these elements.

For these reasons we have carried out a large study involving 27 multi-breed herds characterized by variable levels of productivity, allowing for independent evaluation of the effects of farm and of 6 different cattle breeds. The specific aims of this study were: 1) to quantify and characterize the effects of high or low herd productivity (defined according to the milk net energy yielded daily by the cows) on 15 minerals; 2) to quantify the variability of herds within herd productivity class; 3) to make a within-herd comparison of 3 dairy and 3 dual-purpose breeds for their milk mineral composition, and 4) to quantify the effects of DIM and parity on several minerals determined by Inductively Coupled Plasma - Optical Emission Spectrometry (**ICP-OES**).

MATERIALS AND METHODS

Multi-breed herds

The present study is part of the Cowplus project. A total of 1,508 cows from 41 multi-breed herds (2 to 5 breeds, with an average of 3) located in the Trentino Alto Adige region, north-eastern Italian Alps, were controlled once for daily milk production and sampled during the evening milking for milk quality analyses. The details of the milk sampling have been described by Stocco et al. (2016) and the environmental context and dairy systems involved by Sturaro et al. (2013).

A subsample of 240 cows from 27 multi-breed herds were enrolled in the study on determination of mineral composition of milk. Six breeds were considered, 3 of them specialized dairy breeds: Holstein Friesian (HF = 15 herds and 50 cows), Brown Swiss (BS = 16 herds and 50 cows), and Jersey (Je = 7 herds and 35 cows); and the remaining 3 dual purpose breeds: Simmental (Si = 11 herds and 35 cows), and the two autochthonous breeds Rendena (Re = 8 herds and 34 cows) and Alpine Grey (AG = 9 herds and 34 cows).

Herd productivity classification

The herds were classified into two categories of productivity (**HP**), defined according to the average daily milk energy output (dMEO) of the lactating cows. The net energy content (NE_L) of milk was estimated with the following equation, proposed by the NRC (2001):

$$NE_L \text{ (Mcal/kg)} = 0.0929 \times \text{fat,\%} + 0.0547 \times \text{protein,\%} + 0.0395 \times \text{lactose,\%},$$

where NE_L is the energy of one kg milk. The NE_L values obtained were converted to MJ/kg and multiplied by the daily milk yield of each cow (MJ/d) to obtain the individual dMEO of each cow. Individual dMEO data were subjected to an ANOVA using the GLM procedure in SAS (SAS Institute Inc., Cary, NC) in order to estimate the least square means (LSMs) of the dMEO for the selected herds after correcting for breed, DIM, and parity of the cows. After ranking the dMEO LSMs of the 27 farms, we divided them in high producing (**High-HP**: n = 13, dMEO= 82.41 MJ/d) and low producing (**Low-HP**: n = 14, dMEO= 50.63 MJ/d) herds on the basis of the median value.

Large-framed breeds (Holstein Friesian, Brown Swiss and Simmental) were found in herds of both high and low productivity, Jerseys only in High-HP herds, and local breeds (Rendena and Alpine grey) only in Low-HP herds.

Analysis of milk samples

Milk samples (without preservative) were adjusted to 4°C immediately after collection, and analyzed within 24 hours of sampling at the Milk Quality Laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) of the University of Padua.

All samples were analyzed for SCC (Fossomatic Minor, Electric A/S, Hillerød, Denmark). Milk SCC was log-transformed to SCS (Ali and Shook, 1980). Protein and fat contents were measured by a Milkoscan FT2 infrared analyzer (Foss Electric A/S) calibrated according to reference methods [ISO 8968–2/IDF 20–2 for protein (ISO-IDF, 2014); ISO 1211/IDF for fat (ISO-IDF, 2010)].

The ICP-OES, Arcos EOP (Spectro A. I. GmbH, Kleve, Germany) was employed to determine 15 elements: Ca, P, Na, K, Mg, S, Cu, Fe, Mn, Se, Zn, B, Si, Sn, Sr. All instrument operating parameters were optimized for nitric acid 10% solution as follows. Plasma observation axial, nebulizer Crossflow, spray chamber Scott doublepass, torch injector quartz diameter 3.0 mm, plasma power 1400 W, coolant gas 12.0 L/min, auxiliary gas 0.6 L/min, nebulizer gas 0.85 L/min, additional gas 0.20 L/min, sample uptake rate 2.0 mL/min, replicate read time 28 s, replicates 3, pre-flush time 60 s. The milk samples were analyzed after microwave closed vessel digestion (Ethos 1600 Milestone S.r.l. Sorisole, BG, Italy). For each sample was weighted between 1.950 and 2.050 g of milk and placed in a TFM vessel with 2 mL of 30% hydrogen peroxide and 7 mL of concentrated (65%) nitric acid both Suprapur quality (Merck Chemicals GmbH, Darmstadt, Germany). The so prepared sample was subject to a microwave digestion as follows: Step 1 25–200 1°C in 18 min at 1500 W with P max 45 bar; Step 2 200 1°C for 15 min at 1500 W with P max 45 bar; Step 3 200–110 1°C in 15 min. After cool down to room temperature, the dissolved sample was diluted with ultrapure water (resistivity 18.2 M Ω cm at 25 1°C) to a final volume of 20 mL. Calibration standards were prepared using multi element and single elements standards solutions (Inorganic Ventures Inc. Christiansburg, VA, USA) in 10% Suprapur nitric acid (Merck Chemicals GmbH, Darmstadt, Germany) to get similar matrix as the samples. Concentrations of 0, 0.002, 0.005, 0.02, 0.05, 0.2, 0.5 and 2 mg/L of the analytes were prepared. The concentrations of the calibration solutions for Calcium, Potassium, Magnesium, Sodium, Phosphorous and Sulphur were the same like other analytes plus 5, 20, 50 and 200 mg/L respectively. The accuracy and precision of both methods were investigated analyzing blank solution, low level control solution (recovery limits ± 30%), medium level control solution (recovery limits ± 10%) and the international standard reference material BCR – 063R "Skim milk powder" [Institute for Reference Materials and Measurements (IRMM), Geel, Belgium] prepared like above described. The measured values and the certified values were in excellent agreement for all the elements.

Statistical Analysis

Experimental data were analyzed using the MIXED procedure (SAS Institute Inc., Cary, NC) according to the following model (base model):

$$y_{mnopqr} = \mu + HP_m + Herd_n(HP)_m + Breed_o + Parity_p + DIM_q + e_{mnopqr}$$

where y_{mnopqr} is the observed trait (Ca, P, Na, K, Mg, S, Cu, Fe, Mn, Se, Zn, B, Si, Sn, and Sr); μ is the overall intercept of the model; HP_m is the fixed effect of the mth herd productivity (m = 2 levels); $Herd_n$ is the random effect of the nth herd (n = 1 to 27) within the mth class of herd productivity; $Breed_o$ is the fixed effect of the kth breed (k = HF, BS, Je, Si, AG and Re); $Parity_p$ is the fixed effect of the jth parity (j = 1 to ≥ 3); $DIMq_i$ is the fixed effect of the ith class of days in milk (i = 1 to 8; class 1, 8-49 days (25 samples); class 2, 50-91 d (27 samples); class 3, 92-133 d (39 samples); class 4, 134-175 d (42 samples); class 5, 176-217 d (43 samples); class 6, 156-259 d (32 samples); class 7, 186-301 d (18 samples); class 8, > 302 d (12 samples); e_{mnopqr} is the random residual ~ N (0, σ_e^2).

Orthogonal contrasts were estimated between the LSMs of traits for the effect of breed:

a) dairy specialized (HF, BS and Je) *vs* dual purpose breeds (Si, AG and Re);

b) within specialized, large-framed *vs* small-framed breeds (HF + BS *vs* Je), and

c) comparison of the two large-framed dairy breeds (HF *vs* BS);

d) within dual-purpose, large-framed breed *vs* medium-framed local breeds (Si *vs* Re + AG), and

e) comparison of the two medium-framed local dual-purpose breeds (Re *vs* AG) .

Orthogonal contrasts were also estimated between the LSMs of traits for the effect of parity: a) 1st *vs* ≥2nd, b) 2nd vs ≥3rd; and the effect of dim: a) linear, b) quadratic and c) cubic trend.

A further model (extended model) was used to analyze the direct effects of breed on mineral elements corrected for the milk yield and quality traits and was obtained from the base model with inclusion of linear covariate of milk yield, fat%, protein%, and SCS. The indirect effect of breed on minerals due to breed differences in terms of milk yield and quality was obtained subtracting the breed variance yielded by the extended model from the breed variance obtained from the base model (with breed as random effect). Both direct and indirect breed variance were represented as percentage of total breed variance.

RESULTS

Milk mineral profile and effects of herd productivity

Of the 31 minerals analyzed, 11 were above the limit of detection for all milk samples, 4 were detected in 45 to 66% of milk samples and 16 were mainly below the limit of detection, and were not considered. Descriptive statistics of milk yield, of protein, fat and SCS content of milk samples, and of the mineral content of the 15 elements detected are summarized in Table 1. Minerals exhibited large variability due to the diversity of herd productivity and of the six sampled breeds.

Least square means of herd productivity (HP), *F*-values and significance are summarized in Table 2. Milk yield was obviously very different in the two herd productivity levels, and also milk protein content was higher in High-HP than in Low-HP, whereas milk fat and SCS did not reached significant differences because of their greater residual variability.

Regarding macro-minerals, milk samples from farms characterized by High-HP presented higher content of Ca, Mg and S compared with milk samples from Low-HP farms and among essential micro-minerals, Cu and Zn were greater in High-HP farms. No effect of HP was observed on environmental micro-minerals. The variability among different herds within HP classes is also presented in Table 2 as incidence of herd/test date variance on total variance. Considering macro-minerals, P and S were characterized by a large variability among different herds (greater than other milk traits), Ca and S by an intermediate herd variability (similar to that of milk traits) and Na and K by a low variability (similar to that of SCS). Essential micro-minerals were characterized by an intermediate herd variability with the exception of Zn (low) and Mn and Se (high and very high). Environmental micro-minerals were all characterized by a very high variability among different herds/sampling dates. This high herd within HP class variability is also to be considered the main reason of the non-significance of HP effect, even though large differences between the LSM of the two HP classes were present, as the herd variance was considered the error line for testing HP effect.

Effect of parity

Parity effect on production traits was limited to a greater daily milk production in multiparous respect to primiparous cows and to SCS ($P < 0.001$). Moving to mineral content, milk from primiparous cows was characterized by a higher content of P, Mg and Cu than multiparous cows. A opposite pattern with parity was observed for Na content that increased not only moving from 1st to 2nd lactation but further also to 3rd and more lactations. Environmental minerals did not modify their concentrations across parities.

Effect of days in milk

The variation of the milk content of the 15 minerals analyzed during lactation was highly significant for all the essential macro- and micro-minerals, except for Se, while among the environmental micro-minerals only B was affected by lactation stage. (data not shown). Figure 1 shows that among the macro-minerals, the concentration of Ca and P (corrected for all the other sources of variation considered in statistical model) increased almost linearly (P) and curvilinearly (Ca) during lactation period (+6% and +10%, respectively for Ca and P between the first and the last class of DIM). The pattern of K and Na in milk was different, the former followed a quadratic and the latter a cubic pattern. Milk K, in fact, tended to decrease during the second part of lactation, while Na principally increased during the central part of lactation (Figure 2). Both milk content of S and Mg increased almost linearly during the lactation period, respectively of 17% and 13% (Figure 3).

Among essential micro-minerals, milk content of Fe increased linearly of about 27% during lactation, while Cu had a curvilinear trend decreasing rapidly at first, and then increasing toward the end of lactation (Figure 4). Taking into account that about half of the milk samples presented a content of Cu below the instrumental limit of detection, it is particularly important to consider if the proportion between samples above and below the limit of detection was affected by stage of lactation. The pattern of the incidence of samples with a Cu content above limit of detection on total milk samples (Cu ISAL%) followed a pattern very similar to that observed for Cu content (data not shown). Milk content of Se and Mn had different patterns (Figure 5), as the former decreased during lactation of about 24% (with a non-significant pattern, common also to Se ISAL%, data not shown), while milk content of Mn raised almost linearly of about 48%. The milk content of Zn showed a cubic pattern with a decrease during the first third of lactation, followed by an increase thereafter (Figure 7), while an almost opposite cubic pattern was observed for B, the only environmental micro-mineral showing a variation during lactation.

Effect of breed

Least square means of the effects of breed and their orthogonal contrasts (*F*-value) for all the observed traits are reported in Table 3. All these least square means were corrected for all the other factors of variation included in the model (herd productivity class, individual herds, and parity and DIM of the cows).

Comparing first the group of the three specialized dairy breeds (Holstein Friesian, Brown Swiss and Jersey) with that of the dual-purpose breeds (Simmental, Rendena and Alpine Grey), it will be noted that for all traits analyzed there are large differences among different breeds within each group, so that the orthogonal comparison between the dairy specialized and the dual-purpose breeds is seldom significant. In fact, the difference in milk yield did not attain statistical difference. Regarding milk quality traits, on average specialized dairy breeds outyielded dual-purpose breeds in protein and fat content of milk. The productive aptitude of cows on average did not affect the milk content of any mineral element, except for P content, which was higher in dual-purpose breeds, and Se and Sr, which were higher in specialized dairy breeds.

Very large differences were observed among the three specialized dairy breeds for the production traits and for the majority of the minerals analyzed, while the differences were smaller within the three dual-purpose breeds. In particular, comparing the small-framed breed (Jersey) with the two large-framed breeds (Holstein Friesian and Brown Swiss), results confirmed that the former have a lower productive potential (daily milk yield: -37%) than the latter breeds, but that had also higher milk protein (+13%) and fat (+33%) contents and lower SCS. Among macro-minerals, milk from Jersey cows was characterized by much higher content of Ca (+10%), Mg (+8%) and S (+9%) and lower content of K (-5%). Regarding essential micro-elements Mn, Se and Zn were all much higher in Jersey milk compared with Holstein-Friesian and Brown Swiss milk (Table 3). Among environmental micro-elements, only B content was different in milk from Jersey cows (lower) compared to milk from the two large-framed dairy breeds.

Moving to the comparison between the two large-framed dairy breeds, compared with Holstein Friesians, Brown Swiss cows had a lower productive potential (-17%), compensated by greater milk protein content (+12%) and by favorable milk Ca (+6%), P (+9%), Mg (+6%) and S (+13%) macro-elements, and also by much more favorable content in Fe (+23%), Mn (+36%) and Zn (+24%) in milk among essential micro-minerals. For the Se it is interesting to observe that milk from Holsteins and Brown Swiss cows is characterized by almost the same LSM values for milk content but by a greater incidence of samples above limit of detection for Brown Swiss cows (it depends by the fact that for Holstein cows milk samples above the limit are less frequent but are characterized by higher amount of the mineral). Among environmental micro-minerals, only Si was different in the two large dairy breeds (higher in Brown Swiss milk).

Moving to dual-purpose breeds, it will be noted that the large-framed Simmental cows produce, on average, more milk (+30%) than the two medium-framed local breeds (Rendena and Alpine Grey), with a greater fat content (+16%). The only differences found between the Simmental breed and the averages of the two local breeds for minerals in milk were due to Na (-5%) and Sr (+64%) contents. Lastly, the only differences recorded between the two local breeds were that Rendena cows produced milk with less protein (-7%), and S (-8%).

DISCUSSION

Effect of environment on mineral profile of milk

The findings of the present study on mineral profile of milk revealed strong differences in the effect of different sources of variation for different individual minerals, and also among the three main groups of minerals considered: the essential macro-minerals, the essential micro-minerals and the environmental micro-minerals.

The effect of herd-date within class of HP (High-HP vs Low-HP) represented a very variable proportion of total variability of milk content according to the mineral considered, going from 11-12% in the case of Na and K to 91% for Sn (Table 2). Given that herd groups together several management factors (i.e., housing conditions, feed supplements and diet quality, water quality, milking procedure and equipment, etc.), as well as the different geographical area of herds, the % of variability explained by this factor may be considered low to moderate for the majority of macro-minerals, moderate for the majority of essential micro-minerals (Se excluded), and very all high for environmental micro-minerals. This means that the variation of the content of macro-minerals in milk is basically due to individual animal factors (i.e., breed, genetics, parity, stage of lactation, health status, etc.), while herd (diet, location, etc.) plays a much more important role in determining the concentration level in milk of environmental micro-elements (B, Si, Sn and Sr), that do not play a specific role in animal metabolism and, than, are not strictly regulated in terms of absorption, retention and excretion (Klasing et al., 2005).

Essential macro- and micro-minerals are mainly provided to animals by supplementation, however for some of them the concentration in milk is strongly regulated by animal factors. Among macro- and essential micro-minerals, those scarcely (< 25%) influenced by herd-date effect were Ca, Na, K, Mg, Cu, Fe and Zn. In fact, Ca, Mg and Zn are associated to casein micelles in milk, so their variation is correlated with casein content, while Na and K are involved in the maintenance of osmotic equilibrium blood-milk (Holt, 2011), so the low herd-date effect was expected. In the case of Mg, this element follows the metabolism of Ca and P, as confirmed by correlations reported by Pilarczyk et al. (2013).

Van Hulzen et al. (2009) in their study on 1,948 Dutch Friesian cows included herd (N = 398) as random effect in the model, and observed a herd effect lower than 16% for Ca, Zn, Mg and P. Only in the case of K their herd effect was much greater than our (24% vs 11%, respectively). They supposed that herd variance could result from differences among herds in housing, and especially in feed, but probably the farms in the Dutch planes are more homogeneus for environmental, management, and genetic conditions respect to those in the Italian Alps. In the case of Zn, the low effect of herd-date was probably due to the influence of other metal ions and the presence of organic chelating agents or competing mineral elements (i.e., copper) on the efficiency of absorption of this element. It worth noting that, with the only exception of S, only minerals with a low proportion of variability explained by the herd-date (Ca, Mg, Cu and Zn) exhibited a significant effect of HP on their milk content, in every case with higher content in the milk sampled from cows reared in High-HP *vs* Low-HP herds (Table 2). For these elements it seems reasonable to assume that diet fed to cows could represent an important source of variation, and in particular the forage:concentrate ratio (much lower in Low-HP herds) and mineral-vitamins supplementation (greater in High-HP) herds.

The high effect of herd-date (34 to 41% of total variance) on P, S, and Mn, milk content could be explained by the fact that their content in the feedstuffs is relatively high and variable (Meyer et al., 2014), especially in the case of S, which content in feedstuffs is related to protein concentration. In the case of Mn, its content in feedstuffs is quite variable and is influenced by soil type and plant species (Hurley and Keen, 1987).

Among the essential minerals, only Se showed a very high variability among herd-dates within HP classes (49 and 61% of total variance when expressed as milk content or as incidence of samples exhibiting values above detection limit, respectively, Table 2). This high herd variability was confirmed by the large survey carried out by Van Hulzen et al. (2009) that observed that herd explained 65% of the phenotypic variance of Se content in milk. They supposed that herd variance could result from differences among herds in housing, and especially in feed. It is known that Se content in milk is directly proportional to the organic Se content in the feedstuffs (Ceballos et al., 2009; Meyer et al., 2014)

As previously seen, all environmental micro-minerals presented a very high variability among different herds and sampling dates. Available literature on these micro-minerals in bovine milk is really dated, but their variability in milk could be the result of their different natural content in feed and water consumed by cows, in relation also on the different soil characteristics in different valleys of the sampled province. Boron enters the environment mainly through the weathering of rocks, but also through fertilizers, while forages and cereal grains in fiber (i.e., oats, barley) are the major sources of Si (Pennington, 1991). Most fresh feeds naturally contain less than 1 mg/kg of Sn (Greger, 1988), thus the amount of Sn in the diets of livestock is small. However contaminated, water is a valid source of this element. In general, feedstuffs of plant origin are rich sources of Sr, especially leguminous rather than gramineous forages (Klasing et al., 2005).

Effect of breed within herd

Our study outlined that the breed of cows affected the content in milk of all essential macro- and micro-mineral, with the only exception of Cu. Few studies have analyzed detailed mineral profile of milk obtained from individual animals of several breeds, but none analyzed data only from multi-breed herds and used a model correcting for herd productivity class and herd effect within class, and also for parity and DIM of cows. From this study it appears also that the main differences are not due to the attitude (specialized *vs* dual purpose breeds) but to the different breeds within the attitude group, and especially within the dairy specialized group of breeds.

Barlowska et al. (2006) determined 8 mineral elements in 147 milk samples from five breeds, and the results showed that the milk from the three breeds, including the two native breeds, Whiteblacks, Polish Red and Simmental, contained more essential macro-elements (K, Ca, Na and Mg) and some micro-elements (Zn, Fe) compared to Polish Holstein-Friesian black-white and red-white varieties. Also in our study, milk samples from Holstein Friesian cows were characterized by the lowest content of almost all essential minerals.

More information is available regarding the comparison between two specialized dairy breeds: the Holstein and the jersey. Hermansen et al. (2005) studied 51 mineral elements in 480 milk samples from Danish-Holstein and Jersey cows, and found that Jersey milk was richer in Ca, P, Cu, Fe, Mg, Mn and Zn, compared to the milk of Danish-Holstein breed. Recently, Buitenhuis et al., (2015) studied 10 mineral elements in 892 milk samples from cows of the same two breeds, and their results confirmed the superiority of the Jersey milk on the Holstein milk for all the minerals considered, except for K. Also in our case, Jersey cows showed the lowest K content compared to the other breeds. Differences between these two breeds were found also studying the susceptibility to Cu toxicity (Du et al., 1996): Jersey cattle fed the same diet as Holstein-Friesian accumulated more Cu in their livers. It was not clear whether this reflects differences in feed intake, efficiency of Cu absorption, or biliary excretion of Cu. In any case, Jersey had the best mineral profile, that could be explained, in part, by its higher protein (casein) and fat content in milk. Most of Ca, S and Zn are bound to casein micelles (Neville et al., 1995), so this could explain their higher concentrations in Jersey milk compared to the two large-framed dairy breeds. The idea that milk composition could explain differences among breeds with regard to mineral profile could be applied also when comparing the two Holstein-Friesian and Brown Swiss.

Moving to dual-purpose breeds, Pilarczyk et al. (2013) studied the concentrations of 10 mineral elements (2 toxic heavy metals and 8 trace elements) in milk of Simmental and Holstein-Friesian cows reared in organic farms, and concluded that the milk of Simmental breed was characterized by a more advantageous mineral composition and lower concentration of noxious metals compared to the milk of Holstein-Friesian cows. Also in our study milk from Simmental cows showed a macro-mineral profile close to that of milk from Brown Swiss cows and better than that of milk from Holstein Friesian cows. Also in our study, like in the survey carried out by Barlowska et al. (2006), the two local breeds produced a milk with a mineral profile similar to that characterizing Simmental breed.

Direct and indirect effects of breed

Since the differences among breeds were substantial, and since some authors related these differences to the different composition of milk (Neville et al., 1995), it appears important to distinguish and quantify the direct effects of breed on detailed mineral profile, independent from its effect on milk yield and composition, from the indirect effects, mediated by differences in terms of milk yield and composition. As no information is available, we are aware of, we try to reach this objective through a modification of the basic statistical models used: we included MY, protein, fat, and SCS as general covariates, and calculated the differences in breed variances (treated as random factor) with and without covariates for each mineral trait. It can be seen from Figure 7 that the proportions of direct and indirect breed effects are very different for the various minerals considered.

Practical diets would not be expected to result in a severe deficiency of any of the essential elements. Most of these elements can be toxic when provided in large amounts and this is occasionally a problem in dairy cattle (NRC, 2001), and even less for human health. In our study, milk yield and composition (indirect effect of breed) accounted for a large proportion of the total breed variance for Ca, Mg, S among macro-minerals (81 to 94%), and Mn and Zn among trace elements (76 and 68%, respectively). The direct effect of breed on Ca and S was much lower because of the dependence of these elements on available casein, on the contrary Mg exists mainly as citrate, phosphate and free ions; only 35% of Mg is bound to casein micelles (Gaucheron, 2005), therefore, the casein content might not be so important in determining Mg variation in milk. The 67% of Mn in cow milk is bound to casein, 1% to globular fat membranes, 14% to whey proteins, and 18% to molecular weight fraction, while less than 0.1% of total Se content is bound to fat (Cashman, 2002). Zn is bound for 1-3% to lipid fraction, the rest can be found in the skim milk fraction.

The other essential minerals, with the exclusion of Fe and included environmental B, showed to depend in similar proportions from milk composition and yield and from the direct, independent, effect of breed (Figure 8). Only Fe, among the essential micro-minerals, and the other environmental minerals were not much affected by milk yield and composition and showed a specific independent effect of breed (in the case of Sn, the breed variance was observed to even increase after correction for milk yield and composition). These results on direct breed effect (so mainly due to genetic factors) is confirmed by the study of Du et al. (1996) on different absorption of Cu, Zn and Fe by Jersey and Holstein-Friesian cows. Differences in trace elements metabolism between different breeds have been reported.

CONCLUSIONS

In conclusion, this study on multi-breed herds allowed the effects of farm and of breed of cow to be independently evaluated. In particular, there was a relatively low incidence of the effect of herd within class of farm productivity on the essential macro- minerals whose blood level is strictly regulated (Na, K, and, in part, Mg) while this effect was higher for the other essential macro- and micro-minerals, and very high for Se and the environmental micro-minerals. Comparison of farms with high or low average daily milk energy output revealed significant differences only for minerals with a moderate variability of herd effect within productivity class.

Breed remained a major source of variation of milk level of all essential minerals, with the only exception of Cu. The major differences were not noted between dairy specialized and dual-purpose breeds, but especially within the dairy breeds. In particular, results confirmed the much greater concentration in milk from Jersey cows of almost all essential minerals (Na and K excluded), and also of milk from Brown Swiss (and the dual-purpose cows) respect to milk from Holstein-Friesian cows. Moreover, it was noted that the combined effects milk yield and composition explains a proportion of breed variance very variable according to the mineral considered. This indirect effect of breed is dominant in the case of Ca, Mg, S, Mn, and Zn, and intermediate for P, Na, K, Cu, Se, and B. For all these minerals it appears very important to try to analyze the reason of the dependency of their milk level with milk yield and composition, as well as of their independency, as a base for further understanding of the genetic and physiological mechanisms regulating milk minerals. In the case of Fe and of the environmental micro-minerals the breed effect seems not depend on milk yield and composition, but also the direct breed effect seems not much important. It is also important to deepen the relationships between milk mineral profile and cheese-making traits and cheese quality.

ACKNOLEDGMENTS

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TABLES AND FIGURES

Table 1. Descriptive statistics of milk yield, protein, fat, somatic cell score and mineral contents.

Item	**N**	**Mean**	**SD**	**Min**	**Max**
MY, kg/d	238	20.8	8.1	4.5	46.0
Protein, %	238	3.59	0.44	2.77	4.85
Fat, %	238	4.23	1.20	1.02	7.97
SCS[1], unit	238	2.64	1.83	-1.06	7.56
Macro-minerals, mg/kg					
Ca	234	877	93	679	1109
P	236	657	73	481	834
Na	235	372	48	292	509
K	233	1304	81	1115	1494
Mg	233	85	11	58	114
S	238	181	23	135	246
Essential micro-minerals, µg/kg					
Cu	107	46	18	13	108
Cu, ISAL%[2]	238	47	50	0	100
Fe	235	165	54	67	357
Mn	229	17	6	4	35
Se	110	162	46	100	287
Se, ISAL%	238	48	50	0	100
Zn	233	2877	584	1788	4281
Environmental micro-minerals, µg/kg					
B	236	158	48	50	301
Si	147	1306	304	738	2088
Si, ISAL%	238	63	48	0	100
Sn	158	588	459	103	1434
Sn, ISAL%	238	69	46	0	100
Sr	235	327	140	84	733

[1]SCS= 3 + $\log_2$ (SCC/100,000); [2]ISAL%: Percentage incidence of samples above the limit of detection

Table 2. Effect of class of herd productivity and of parity on milk yield, protein, fat, somatic cell score and mineral contents.

	Herd productivity (HP)			Herd (HP)	Parity (LSM)			Parity Contrasts (*F*-value):		RMSE
	High-HP (LSM)	Low-HP (LSM)	*F*-value	% of total variance	1st	2nd	≥3rd	1st *vs* ≥2nd	2nd *vs* ≥3rd	
MY, kg/d	24.0	14.9	51.1***	23.7	17.8	19.9	20.7	13.8***	0.9	4.6
Protein, %	3.74	3.54	8.8*	25.5	3.67	3.65	3.59	2.3	2.8	0.2
Fat, %	4.50	4.15	2.5	19.1	4.37	4.27	4.33	0.3	0.2	0.9
SCS[1], unit	2.94	2.38	2.7	9.3	2.40	2.33	3.25	2.8	11.2**	1.6
Macro-minerals, mg/kg										
Ca	907	866	4.9*	22.4	890	888	881	0.2	0.4	68
P	676	645	2.6	40.8	685	653	643	22.2***	1.3	51
Na	374	376	0.0	12.0	355	375	395	27.7***	9.1**	39
K	1289	1300	0.5	10.6	1305	1301	1278	2.1	3.4	70
Mg	90	84	8.1**	17.6	89	87	84	9.0**	3.3	8
S	189	178	5.3*	33.8	185	185	182	0.5	1.6	14
Essential micro-minerals, µg/kg										
Cu	33	23	4.4*	21.9	32	25	26	6.9**	0.0	17
Cu, ISAL%[2]	57	42	1.7	25.3	52	47	50	0.3	0.2	39
Fe	175	160	1.5	24.5	171	168	163	0.8	0.4	43
Mn	19	16	3.7	38.0	18	17	18	1.9	1.9	4
Se	112	96	0.9	49.2	102	105	105	0.2	0.0	41
Se, ISAL%	60	37	2.2	61.0	45	48	52	1.0	0.7	30
Zn	3151	2708	19.4***	13.6	2935	2942	2911	0.0	0.2	427
Environmental micro-minerals, µg/kg										
B	149	168	1.4	67.5	158	160	157	0.1	0.5	27
Si	699	882	0.9	46.9	763	824	783	0.3	0.2	484
Si, ISAL%	57	61	0.1	54.2	58	60	60	0.1	0.0	34
Sn	526	262	2.4	90.6	410	393	379	1.2	0.3	142

Sn, ISAL%	72	55	1.0	76.0	68	62	61	3.3	0.0	24
Sr	333	335	0.0	78.1	324	344	334	2.3	0.7	63

[1] SCS= 3 + $\log_2$ (SCC/100,000); [2] ISAL%: Percentage incidence of samples above the limit of detection

Table 3. Effect of breed of cows on milk yield, protein, fat, somatic cell score and mineral contents.

	Breed of cows (LSM):						Breed Contrasts (*F*-value):				
	Holstein Friesian (HF)	Brown Swiss (BS)	Jersey (Je)	Simmental (Si)	Rendena (Re)	Alpine Grey (AG)	HF BS Je *vs* Si AG Re	HF BS *vs* Je	HF *vs* BS	Si *vs* Re AG	Re *vs* AG
MY, kg/d	24.3	20.2	14.7	22.8	17.9	16.9	0.5	46.1***	14.2**	21.5***	0.4
Protein, %	3.35	3.75	4.02	3.60	3.43	3.68	9.8**	66.9***	50.7***	0.6	10.0**
Fat, %	4.08	4.10	5.45	4.52	3.73	4.05	7.6*	43.5***	0.0	8.7*	1.3
SCS^1, unit	2.99	3.19	2.20	2.05	2.49	3.04	0.9	5.9*	0.3	3.6	1.4
Macro-minerals, mg/kg											
Ca	841	895	950	900	868	864	1.8	23.2***	10.6**	3.7	0.3
P	611	665	660	681	660	684	8.7*	2.8	17.3***	0.4	1.7
Na	373	381	371	361	391	373	0.0	0.6	0.8	5.1*	2.3
K	1298	1316	1244	1299	1307	1302	1.7	14.6***	1.3	0.2	0.1
Mg	83	88	92	87	84	86	1.6	10.5**	7.8*	1.1	0.6
S	170	192	198	185	172	187	3.5	23.5***	45.9***	2.3	8.8**
Essential micro-minerals, µg/kg											
Cu	24	30	33	24	30	27	0.2	2.3	2.2	1.1	0.3
Cu, ISAL%^{2}	46	55	64	32	51	49	2.1	2.1	0.9	3.3	0.0
Fe	150	184	170	163	167	170	0.0	0.1	11.2**	0.3	0.1
Mn	14	19	20	18	16	18	0.3	11.3**	18.3***	0.3	3.0
Se	98	98	152	93	94	90	8.4**	25.7***	0.0	0.0	0.1
Se, ISAL%	36	56	58	45	45	50	0.3	2.2	6.4**	0.1	0.2
Zn	2482	3077	3171	3078	2868	2899	0.2	14.9***	34.9***	3.5	0.1
Environmental micro-minerals, µg/kg											
B	160	171	147	167	147	156	0.2	7.3**	2.5	3.9	0.8
Si	685	979	831	894	824	528	0.7	0.0	5.8*	2.7	2.7
Si, ISAL%	55	70	67	64	59	40	2.1	0.3	2.9	2.4	2.3

Sn	398	362	370	382	464	390	1.5	0.1	0.9	1.2	1.7
Sn, ISAL%	60	61	62	69	65	65	1.1	0.1	0.0	0.3	0.0
Sr	326	357	363	373	288	299	5.1^{*}	1.7	3.3	19.5^{***}	0.2

[1]SCS= 3 + $\log_2$ (SCC/100,000); [2]ISAL%: Percentage incidence of samples above the limit of detection

Figure 1. Milk content of calcium (Ca, $P < 0.001$) and phosphorous (P, $P < 0.001$) during lactation.

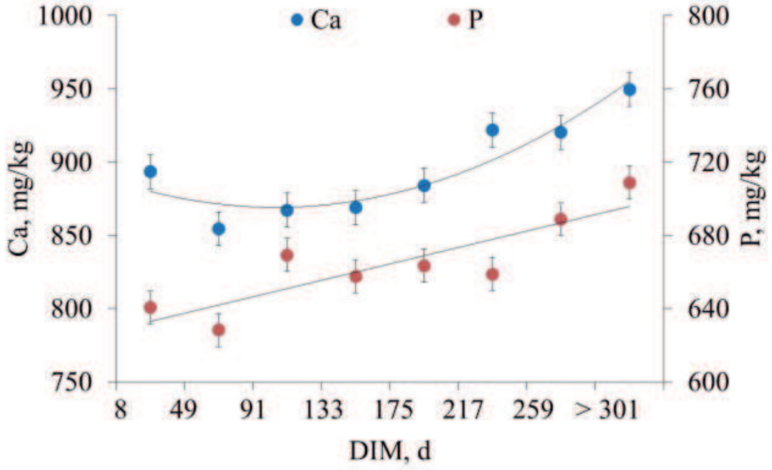


Figure 2. Milk content of potassium (K, $P < 0.001$) and sodium (Na, $P < 0.001$) during lactation.

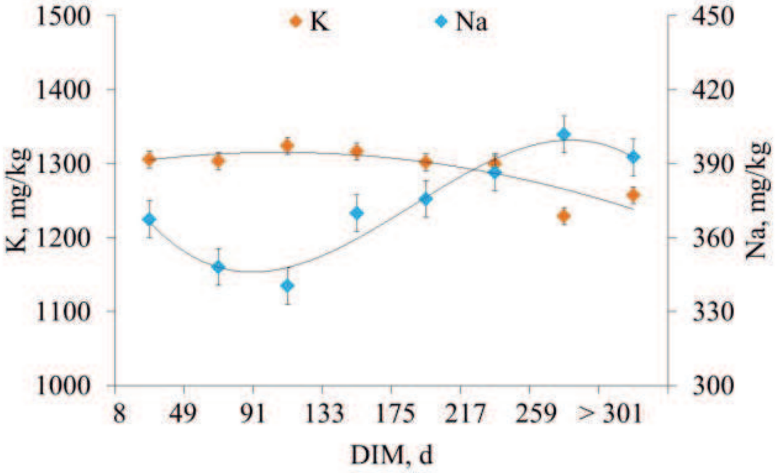


Figure 3. Milk content of sulphur (S, *P* < 0.001) and magnesium (Mg, *P* < 0.001) during lactation.

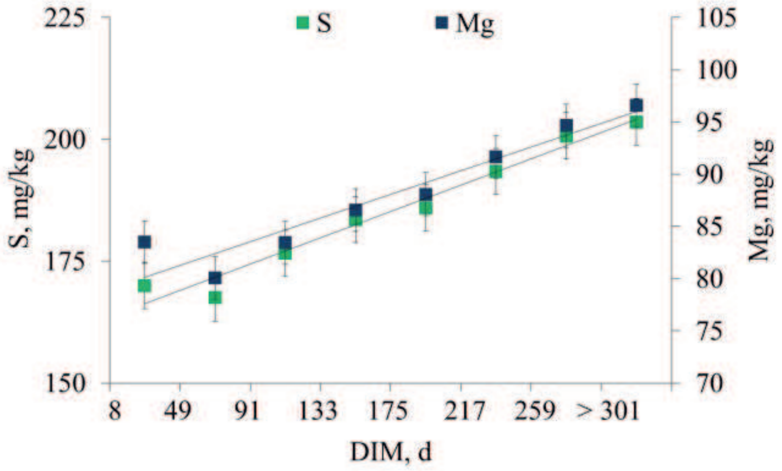


Figure 4. Milk content of iron (Fe, *P* < 0.001) and copper (Cu, *P* < 0.001) during lactation.

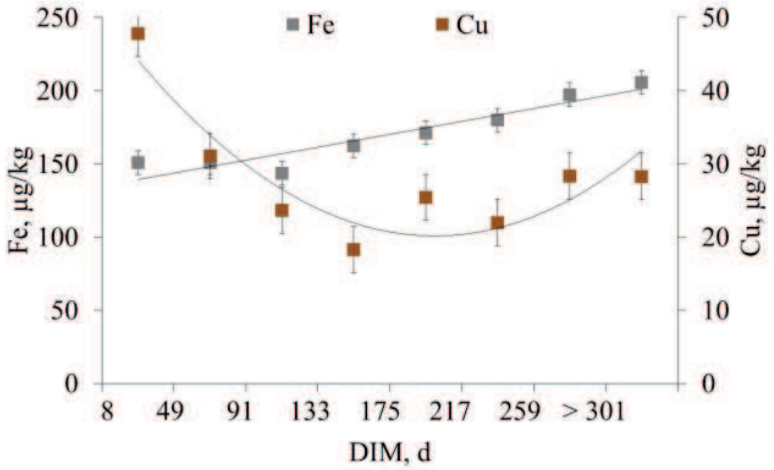


Figure 5. Milk content of selenium (Se) and manganese (Mn, $P < 0.001$) during lactation.

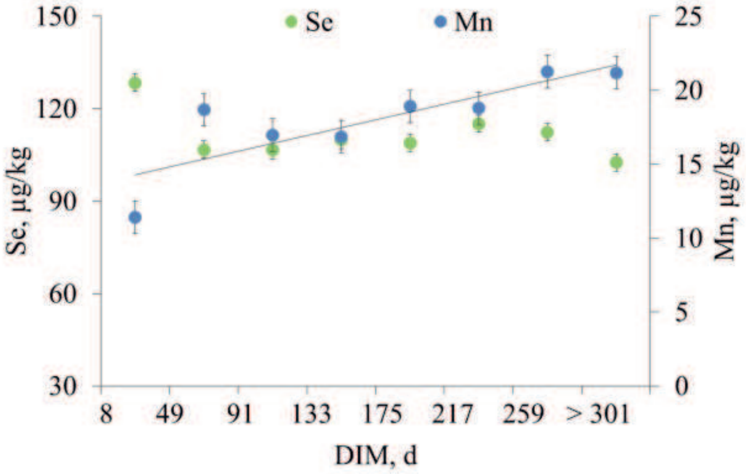


Figure 6. Milk content of boron (B, $P < 0.05$) and zinc (Zn, $P < 0.01$) during lactation.

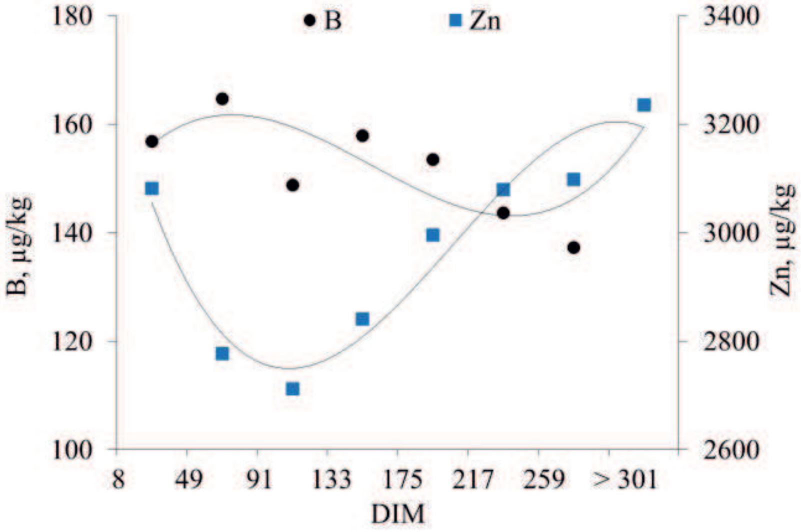
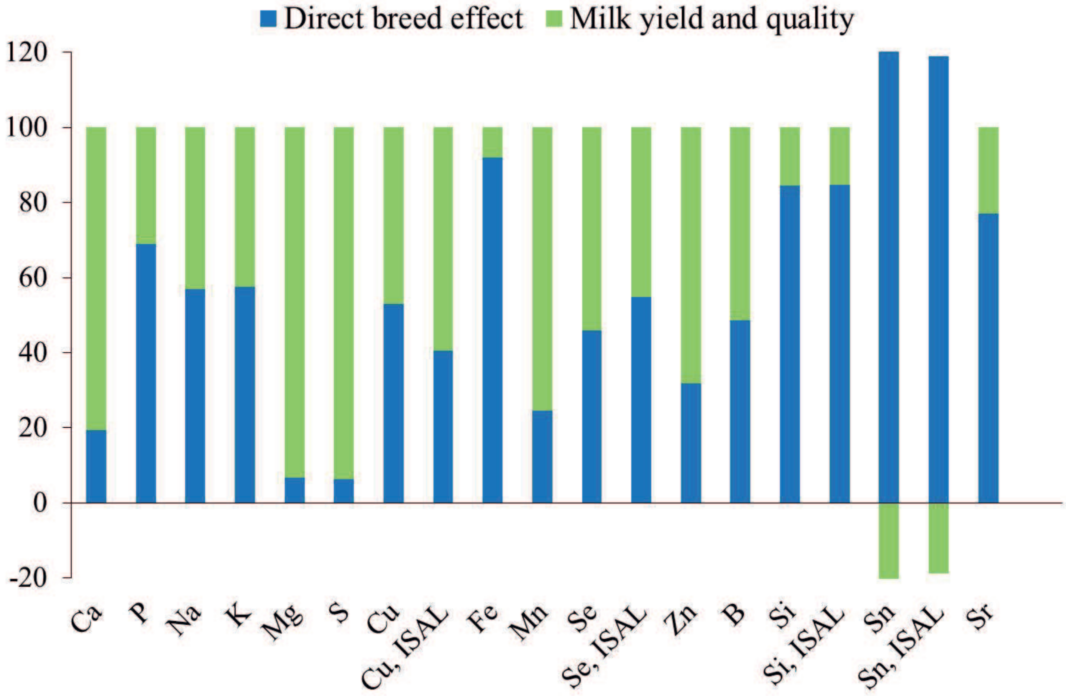


Figure 7. Proportion of total breed variance explained by direct breed effect or by indirect breed effect through differences in milk yield and quality traits on macro-minerals, essential and environmental micro-minerals.



Chapter 4

Productivity and efficiency of cows of 6 dairy and dual-purpose breeds reared in multi-breed herds of high and low production level

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ABSTRACT

The concepts of production, productivity and efficiency are interrelated but different. Productivity relates production to a scaling factor often associated to the dimension or cost of the producing unit. As a major cost of production of milk is the feed needed for the maintenance of the animal, often the productivity is expressed by a ratio between the output of production, in the nominator, and the "size" of productive animal (scaling unit), at the denominator. Efficiency of production implies a complete balance (for example, energetic or economic efficiency) of production activity, and it could be expressed as the ratio between production output, at the nominator, and the sum of all production inputs, at the denominator, or by the difference between total revenue and the costs, or the major cost of production "income over feed costs" (**IOFC**). As breed of cows and herd characteristics are the most important factors affecting milk productivity and efficiency, to obtain independent evaluation of these factors, the data (body size and production) and milk samples from 41 multi-breed herds (3 breeds per farm on average) on a total of 1,508 lactating cows from three dairy (Holstein, Brown Swiss and Jersey) and three dual-purpose (Simmental, Rendena and Alpine Grey) breeds were collected. The milk samples were processed to obtain milk composition and coagulation properties, while cheese-making traits were predicted on the basis of 508 measured individual model cheeses. Nine productivity indicators and two simplified indicators of cow efficiency for cheese production, one energetic and one economic, were calculated. The data were analyzed using a mixed model including: the class of herd production level (high vs low), the individual herd (random), the breed, parity and DIM class of the cow.

Breed within herd greatly affected all traits. On average the 3 dairy breeds were not much different from the 3 dual-purpose breeds, but large differences characterized both groups of cows. Jersey cows were the less productive, but, after correcting for herds effect and scaling for body size, they showed the highest efficiency among the dairy breeds. Holstein was the most productive dairy breed, but Brown Swiss cows had better milk quality and more efficient cheese-making aptitude and thus produced more cheese per day than Holsteins. Dual-purpose breeds were less variable than dairy ones, with Simmental with larger body size and production, but not productivity and efficiency respect to local Rendena and Alpine Grey breeds.

If within-herd comparison and correctly scaling of production traits reduce strongly herd differences in productivity, they did not reduce very much the differences in terms of milk composition, technological properties and efficiency of cheese-making (recovery of milk nutrients in cheese) so that the differences among breeds remain strong and their importance on the overall efficiency evaluation of the breeds increased. The knowledge and correct quantification of the importance of productive, qualitative and technological properties of different breeds offers interesting new insights for modifying selection indices within dairy and dual purpose breeds and for projecting crossbreeding plans across breeds.

Key words: Dairy breeds efficiency, milk productivity, energy requirements, body condition score, income over feed costs.

INTRODUCTION

The concepts of production, productivity and efficiency are not synonyms even though they are correlated. The high milk production of Holstein cows in the past decades promoted a worldwide diffusion of this breed. Productivity relates production to a scaling factor, often related to the dimension or cost of the producing unit. As a major source of "fixed" cost of production of food of animal origin for humans is the feed needed for the maintenance of the animal. The productivity is often expressed by the ratio of the food produced, in the nominator, and the "size" of productive animal (scaling unit), at the denominator, where the size could be represented simply by the body weight (**BW**) of the animal, or by some predictor of its nutrients requirement for maintenance of which the metabolic weight (**MW**) and the body protein weight (**PW**) are examples. The Jersey cows produces much less milk than Holsteins, but their size is much smaller, so if their productivity per unit BW or MW be lower or higher respect to Holsteins is questionable (). Efficiency of production is another commonly used index, but more complicated to be defined and measured. It implies a complete balance (for example material, or energetic, or environmental, or economic, etc.) of production activity and it could be expressed as a ratio between production output, at the nominator, and the sum of all production inputs, at the denominator. A simplified way of representing economic efficiency is not a ratio but a difference between total revenue and the costs or the major cost of production. Often used in animal production is "income over feed costs" (**IOFC**).

The primary genetic characteristic of a cow - its breed - has been shown to have an enormous effect on milk yield, and on its main destination: cheese yield (Banks et al., 1986; Verdier-Metz et al., 1995), but the comparisons of different breeds in scientific papers could go from few dozens of cows with a lot of precise data obtained in an experimental farm (Mistry et al., 2002; Hurtaud et al., 2009; Martin et al., 2009), to few data obtained from whole cattle populations underwent production recording systems with a large majority of single-breed herds (Malacarne et al., 2006; Bland et al., 2015). In this last case the effect of individual herds and dairy systems are confused with the effect of breed.

Therefore, comparison of breeds may be affected by a lack of representativeness, or by different individual (parity, stage of lactation, etc.) or herd (facilities, feeding, management, etc.) characteristics. It is also possible that very different dairy systems and levels of farm productivity interact with breed.

For these reasons, a large research project (Cowplus project) was established, and several milk yield, quality and coagulation properties (Stocco et al., 2016a) and cheese-making-related phenotypes (Stocco et al., 2016b) have been measured in individual cows from multi-breed herds, thereby allowing for independent evaluation of the effects of farm and breed of cows. The specific aims of this study were: 1) to quantify and characterize the effects of herd productivity (defined on the basis of the average net energy of milk yielded daily by the cows) on several indicators of cow's productivity and milk-processing efficiency in cheese-making; 2) to quantify the variability of herds within class of herd productivity; and 3) to make a within-herd comparison of 6 dairy and dual-purpose breeds for these productivity and efficiency indicators.

MATERIALS AND METHODS

Multi-breed herds selection and their classification

Fourty-one multi-breed herds (2 to 5 breeds per herd, on average 3) located in Trentino region in the north-eastern Italian Alps were selected for evaluating body characteristics, daily milk yield and composition, and cheese-yield of cows of 6 dairy and dual purpose breeds. The herds were selected from a total of 610 herds enrolled in milk recording system in the Trento province previously analyzed for their environmental and management characteristics and clustered according to 5 different dairy systems (Sturaro et al., 2013). The selection was based on the following criteria: a) interest and willingness of farmers in participating to the proposed research; b) number of breeds present in each herd (about two thirds of the herds of the area are multi-breed herds), with special emphasis on the less represented breeds and the proportions among different breeds; c) representativeness of all different dairy systems and geographical areas of the province.

The selected herds were classified into two categories of herd production level (**HP**) according to the procedure described by Stocco et al. (2016a) on the basis of the average daily milk energy output (**dMEO**) yielded by all lactating cows in the herd. In brief, the net energy content (NE_L) of milk was estimated by means of the following equation, derived from that proposed by the NRC (2001):

$$NE_L \text{ (MJ/kg)} = 0.3887 \times \text{fat,\%} + 0.2289 \times \text{protein,\%} + 0.1653 \times \text{lactose,\%},$$

where NE_L is the gross energy of one kg of milk. The NE_L values obtained were multiplied by the daily milk yield of each cow (kg/d) to obtain the individual dMEO of each cow (MJ/d) that were analyzed through ANOVA using the SAS GLM procedure (SAS Institute Inc., Cary, NC) in order to calculate the least square means (LSMs) for dMEO for the selected herds after correcting for breed, DIM and parity of cows. After ranking according to the dMEO LSM, the 41 farms were divided, on the basis of the median value, into high producing (**High-HP**: n = 20, dMEO = 90.86 MJ/d) and low producing (**Low-HP**: n = 21, dMEO = 57.33 MJ/d) herds, that means a daily energy output for High-LP cows of + 58% respect to Low-HP cows.

Table 1 shows the main features differentiating the High-HP and Low-HP farms. Respect to Low-HP, High-LP farms were larger (+59% of utilized agricultural area), with larger herds (+64% cows). The large majority of High-HP farms (15 out of 20) were characterized by modern barns with loose cows, milking parlor and, with only 2 exceptions, total mixed rations. In the case of Low-HP farms the large majority (15 out of 21 farms) were characterized by very traditional dairy systems with tied animals and cow's feeding based on farm hay and some compound feed. On average lactating cows of the former group of herds were receiving a daily quantity of concentrates almost double than the cows of the latter (Table 1). Moreover 9 of the Low-HP farms, against none of the High-HP farms, were practicing summer transhumance to temporary farms in the Highland Alpine pastures.

Every selected herd was visited for animal evaluation, body recording and milk and blood sampling once (generally one herd per week with few exceptions) by technician and veterinarians of Padova University and technicians of the Breeders Federation of Trento Province.

Cows selection and breed characteristics

The selection of individual cows within each herd was based on the following criteria: a) only lactating cows within 8 and 301 DIM were considered; b) all cows with clinical symptoms of any diseases were excluded from sampling; c) all crossbred cows, purebred cows not registered in the Herd Book, and registered cows of breeds sporadically present were excluded; d) all eligible cows of the smaller herds (< 20 lactating cows) were included; e) in larger herds (> 40 lactating cows) until 80 cows were selected, excluding the cows belonging to the more represented breeds, parity and DIM classes, without any selection based on daily milk yield; f) all sampled cows with incomplete data were excluded.

At the end of recording and sampling, data from 1,508 lactating cows of 3 specialized dairy: Holstein Friesian (HF = 31 herds and 471 cows), Brown Swiss (BS = 36 herds, 663 cows), and Jersey (Je = 7 herds, 40 cows); and 3 dual-purpose breeds: Simmental (Si = 20 herds, 158 cows), and two autochthonous breeds, Alpine Grey (AG = 13 herds, 73 cows) and Rendena (Re = 8 herds, 103 cows) were analyzed. All breeds were distributed throughout the high and low productivity herds, with the exception of the Jersey, which was found only in High-HP herds, and the local breeds Rendena and Alpine Grey, found only in Low-HP herds.

The 41 mixed-breed dairy farms selected for the study had only cows enrolled in the Italian Herd Books of the 6 breeds studied and were practicing almost exclusively artificial insemination (AI) using national or imported semen from proven bulls or progeny testing young bulls.

The dairy large-framed Holstein Friesian cows in the province of Trento were obtained from semen mainly from Italian, German, American and Dutch bulls (Cecchinato et al., 2015). In this study, the cows were characterized by a parity of 2.4±1.6, and DIM of 197±140. The cows of the other dairy large-framed breed, Brown Swiss, were obtained from semen from Italian, Austrian, German, American and Swiss bulls. Parity (2.6 ± 1.6) and DIM (188 ± 139) were very close to those of Holstein Friesians. The dairy small-framed Jersey breed has been recently introduced in the area, and the cows came from semen imported mainly from the USA and Denmark. The parity and DIM were similar to those of the other two specialized dairy breeds (parity 2.9 ± 2.1, DIM 214 ± 116).

Beyond the genetic evaluation for milk traits that, like for specialized dairy breeds, is based on progeny testing of cows in milk recorded herds, all the three dual purpose breeds are selected for meat production (mainly growth rate and muscularity evaluated subjectively by experts) through a performance testing of all young bulls to be destined to AI. The large-framed Simmental cows in the area belong to the dual-purpose strains of this breed reared mainly in the Alpine regions, and came from inseminations using semen from Italian, German, and Austrian bulls, and also from French Montbéliarde bulls. Parity (2.7 ± 1.9) was similar to that of the dairy breeds, while DIM was lower (177 ± 118). The medium-framed local breeds, Rendena and Alpine Grey, are both dual-purpose breeds of Alpine origin. The Rendena breed has a dark chestnut coat, while the other has a grey coat, and they are similar to the Simmental breed in parity (2.8 ± 1.8 and 2.5 ± 1.7) and DIM (189 ± 94 and 158 ± 75).

Body characteristics evaluation

Every cow was measured for hearth girth and height at withers and evaluated subjectively for body-weight (**BW**, in kg) and body condition score (**BCS**) by the same skilled operator. For BCS scoring, the method proposed by Edmonson et al. (1989) for Holstein Friesian cows from 1 (lean) to 5 (fat) with increments of 0.25 was adapted for the other breeds (Gallo et al. 2016; Zendri et al., 2016). Metabolic weight (**MW**, kg) was derived from BW ($MW=BW^{0.75}$) for all cows, independently from their breed (NRC, 2001).

Body composition of cows was estimated from starting equations 2-20, 2-21 and 2-22 of Nutrient requirements of dairy cattle (NRC, 2001). As these equations estimate composition of empty body weight (**EBW**) and are referred to the BCS evaluated in 9 classes, we have modified them to express the composition of BW, considering EBW=0.85×BW (NRC, 2001), from BCS evaluated in 5 classes. Thus, the modified equations to estimate fat (**BW_{fat}**), protein (**$BW_{protein}$**) and water-ash (**$BW_{w\text{-}a}$**) proportion of BW of each cow were:

$$BW_{fat} = 0.06397 \times BCS - 0.0320;$$

$$BW_{protein} = -0.01134 \times BCS + 0.1764;$$

$$BW_{w\text{-}a} = -0.05262 \times BCS + 0.7056;$$

The total content (kg/cow) of fat, protein and water+ash of each cow was obtained multiplying its BW time its estimated BW_{fat}, $BW_{protein}$ and $BW_{w\text{-}a}$, respectively.

Assuming an energy value of 38.49 MJ/kg and 23.22 MJ/kg for body fat and body protein, respectively (Andrew et al., 1991), the equations to derive fat (**E_{fat}**, MJ/kgBW), protein (**$E_{protein}$**, MJ/kgBW) and total (**E_{total}**, MJ/kgBW) energy per kg BW of each cow were obtained:

$$E_{fat} = 2.516 \times BCS - 1.258;$$

$$E_{protein} = -0.264 \times BCS + 4.097;$$

$$E_{total} = 2.252 \times BCS + 2.839;$$

The total body energy (MJ/cow) of each cow was obtained multiplying its BW (kg/cow) for its E_{total} (MJ/kg).

Milk sampling and analysis

Daily milk yield (**dMY**, kg/d) was recorded and a milk sample was taken from all selected cows for the analysis of milk composition and technological properties. All details of the milk sampling and analysis and of cheese-making have been reported by Stocco et al. (2016a).

Immediately after collection, individual milk samples of about 2000 mL per cow were stored at 4°C, and processed within 24 hours of sampling at the Milk Quality Laboratory of the

Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) of the University of Padova. In the present study only milk yield, and its fat, protein and lactose content was considered. Fat, protein, and lactose contents were measured with a Milkoscan FT2 infrared analyzer (Foss Electric A/S, Hillerød, Denmark) calibrated in accordance with the reference methods [ISO 8968–2/IDF 20–2 for protein (ISO-IDF, 2014); ISO 1211/IDF for fat (ISO-IDF, 2010a); ISO 26462/IDF 214 for lactose (ISO-IDF, 2010b)].

The energy content of milk (NE_L, MJ/kg) was estimated as previously described for herd production level classification. The daily production of fat, protein and energy of each cow was obtained multiplying its dMY (kg/d) time its fat, protein and NE_L content per kg.

Cheese yield and cheese-making efficiency

The theoretical cheese yield expressing the kg of fresh cheese obtainable from every 100kg milk processed (***Th-*$\%\mathbf{CY_{CURD}}$**) was estimated as reported in detail in a previous study (Stocco et al., 2016b) using the historical formula of Van Slyke and Price (1949) reported by Emmons and Modler (2010) in their review:

$$Th\ \%CY_{CURD} = (0.93 \times \%fat + \%casein - 0.1) \times 1.09/[(100 - \%M)/100]$$

where 1.09 represents correction for milk minerals and cheese salt and carbohydrates, and %M is the percentage moisture of cheese (100 - %total solids).

In a previous study (Stocco et al., 2016b) we have carried out on 508 of the 1,508 cows used in the present study an individual cheese-making procedure for the production of a model-cheese from each cow and the measurement of actual $\%CY_{CURD}$, and we have defined the efficiency of $\%CY_{CURD}$ (***Ef-*$\%\mathbf{CY_{CURD}}$**) by simply expressing the experimental value in relation to the corresponding theoretical value for each cow:

$$Ef\text{-}\%CY_{CURD} = \%CY_{CURD} / Th\text{-}\%CY_{CURD}$$

In that study the *Ef-*$\%CY_{CURD}$ was found to be highly affected by the breed of cow and significantly by the comparison between primiparous and multiparous, so in the present study the

LSM of *Ef*-%$\mathrm{CY_{CURD}}$ for breed and parity effects were used for correcting *Th*-%$\mathrm{CY_{CURD}}$ and estimating actual %$\mathrm{CY_{CURD}}$ of different cows.

The daily yield of fresh cheese (**dCY**, kg/d) of each cow was calculated as:

$$\mathrm{dCY = dMY \times \%CY_{CURD}}$$

Productivity of lactating cows

Productivity of each lactating cow was expressed as a ratio between an output and a scaling unit, that represents a predictor of inputs. The output in the numerator of the productivity ratio was expressed: as dMY, as dMEO or as dCY. The scaling factor of inputs at the denominator of the productivity ratio was expressed: as individual cow, or as unit of MW, or as unit of PW. A total of 9 productivity ratios were calculated.

Energy requirements and efficiency

In many commercial dairy farms, being not possible to control the daily individual feed intake of lactating cows, the energy efficiency of each cow was estimated as the ratio between the daily energy output with milk (dMEO, MJ/d, as previously defined) and the sum of energy requirements for maintenance, lactation, pregnancy, growth and activity expressed as $\mathrm{NE_L}$ (MJ/d).

Energy requirement for lactation, being expressed as $\mathrm{NE_L}$, is dMEO.

Energy requirement for maintenance (MJ/d) was expressed as 0.305 × MW according to NRC (2001).

Energy requirement for activity was assumed to be 10% of maintenance requirement for cows kept in free stall herds and null for the cows in tied stalls. No farm was practicing pasture.

Energy requirement for variation of body reserves was not considered, assuming that the BCS at parturition (not known) should be very similar within and across breeds and parities and that the energy mobilized at the beginning of lactation need to be restored before next calving.

Energy requirement for pregnancy considered for calculation of efficiency indicators was not the specific requirement estimated in the day of milk recording and sampling but the average daily requirement during a calving interval to avoid biased due to large casual variation in the number of cows in the last couple of month of lactation, and to take into account also the pregnancy needs during dry period. The calculation was based on the total energy increase of the gravid uterus and the udder (NE_{Preg}) of each cow, expressed in terms of NE_L (MJ), and divided by the calving interval assumed to be 400 days for all cows, according to the following steps:

1. Calculation of **NE_{Preg}** by modifying the equation: dNE_{Preg} (Mcal/d) = [0.00318 × (GAge-190) – 0.0352] (Bell et al., 1995, NRC, 2001) as in the next step;
2. NE_{Preg} (Mcal)= [(0.00318 × (235-190) – 0.352) × 90], where 90 (190 to 280 days) represents the number of days during the last part of pregnancy, assumed to last 280 days, during which energy retention is appreciable, and 235 is the central day of this period characterized by an average energetic cost (the equation assume a linear increase through the last part of pregnancy);
3. NE_L for the average calf born (MJ) = $NE_{Preg}/0.218\times4.184$; this estimate, according NRC (2001) is referred to an average Holstein calf weighing 45 kg at birth;
4. Prediction of the calf weight (**CalfW**, kg) from the BW of each cow according to: CalfW (kg) = 0.0625×BW;
5. NE_L for the specific calf (MJ) = $NE_L\times$ CalfW/45;
6. Daily pregnancy requirement of each cow (MJ/d) = NE_L for the specific calf (MJ) / 400 days.

Statistical Analysis

Experimental data were analyzed using the MIXED procedure (SAS Institute Inc., Cary, NC), according to the following model (base model):

$$y_{ijklmn} = \mu + HP_m + Herd_n(HP)_m + Breed_k + Parity_j + Breed_k \times Parity_j + HP_m \times Parity_j + DIM_i + HP_m \times DIM_i + e_{ijklmn}$$

where y_{ijklmn} is the observed trait; μ is the overall intercept of the model; HP_m is the fixed effect of the m^th^ herd productivity (m = 2 levels); $Herd_n$ is the random effect of the n^th^ herd (n = 1 to 41) within the m^th^ class of herd productivity; $Breed_k$ is the fixed effect of the k^th^ breed (k = HF, BS, Je, Si, Re and AG); $Parity_j$ is the fixed effect of the j^th^ parity (j = 1 to ≥ 4); DIM_i is the fixed effect of the i^th^ class of days in milk [i = 1 to 10; class 1, 5-35 days (31 samples); class 2, 35-65 d (26 samples); class 3, 65-95 d (41 samples); class 4, 95-125 d (56 samples); class 5, 125-155 d (64 samples); class 6, 155-185 d (58 samples); class 7, 185-215 d (63 samples); class 8, 215-245 d (57 samples); class 9, 245-275 d (32 samples); class 10, > 275 d (84 samples)]; e_{ijklmn} is the random residual ~ N (0, σ_e^2).

A model that also included the breed × herd productivity interaction was fitted to test the data from all the breeds present in both classes of herds (Holstein Friesian, Brown Swiss, and Jersey). As this interaction was never significant, the results of this model analysis are not shown nor discussed.

Orthogonal contrasts were estimated between the LSMs of traits for the effect of breed:

a) specialized dairy (HF, BS and Je) *vs* dual-purpose breeds (Si, AG and Re);

b) within specialized, large-framed *vs* small-framed breeds (HF + BS *vs* Je), and

c) comparison between the two large-framed dairy breeds (HF *vs* BS);

d) within dual-purpose, large-framed breed *vs* medium-framed local breeds (Si *vs* Re + AG), and

e) comparison between the two medium-framed local dual-purpose breeds (Re *vs* AG).

Orthogonal contrasts were also estimated between the LSMs of traits for the effect of parity: a) 1st *vs* ≥2nd, b) 2nd *vs* ≥3rd, c) 3rd *vs* ≥4th.

RESULTS

Body size, condition and estimated composition of lactating cows

The LSMs of all the body size, condition and estimated composition traits of cows reared in the High-HP and in the Low-HP herds, from the model correcting for breed, parity, DIM and relative interactions, were different (Table 2), being the former respect to the latter:

1. +8%, +5%, +10%, and +2% in terms of BW, MW, BCS, and hearth girth, respectively:
2. -2%, +11%, and- 3% in terms of estimated body protein, fat, and water+ash proportions, respectively;
3. +6%, +20%, and +5% in terms of estimated protein, fat and water+ash content of the whole body;
4. +16% in terms of estimated total energy content of the body.

It worth to note that the incidence of the variability of individual herds (corrected for HP class, breed, parity and DIM class of cows) on total variability of all body size and composition traits is small, going from only 7.8% for hearth girth to 14.6% for BCS.

The 6 breeds showed large differences for all the body traits considered (Table 2). In particular: the 3 specialized dairy breeds were, on average, smaller (lower average BW, MW, hearth girth, estimated body content of protein, fat, water+ash and energy), and leaner (lower BCS and estimated body fat proportion, and higher estimated body protein and water+ash proportions) than the 3 dual-purpose breeds; within the specialized dairy breeds, Jersey cows were much smaller than the two large-framed breeds (Holstein and Brown Swiss), but similar in terms of BCS and estimated body proportion of protein, fat and water+ash. Between the two large-framed dairy breeds, Holsteins were similar in size, but leaner than Brown Swiss cows. Among the dual-purpose breeds, the Simmental cows were heavier but with similar BCS and estimated body proportions than cows of the two local breeds. Between these, Rendena cows were heavier than Alpine Grey cows only in terms of BW, MW, and estimated body content of water and ash (Table 2).

Also parity affected body traits because, as expected, primiparous cows were smaller than multiparous ones, but, being the former slightly fatter than the latter, they had similar total estimated body content of energy. Among multiparous cows, the secundiparous were slightly smaller than the older ones, but with similar body composition (Table 2).

Regarding the effect of the lactation stage, the body size traits were noted to increase linearly with DIM, while fatness traits (BCS, estimated body fat proportion and total content, as well as the total body energy content) were characterized by a quadratic pattern with a decrease at the beginning of lactation and an increase afterward, compensated by an opposite pattern of estimated body protein and water+ash proportions.

Interactions were generally not significant with the exception of that between herd production class and parity for BCS and estimated body proportion traits, because the effect of parity was more evident in High-HP than in Low-HP cows, and of that between breed and parity, only for BW, because the differences of BW among cows of different parities were larger for large-framed breeds than for mid- and small-framed breeds (Table 2).

Milk yield, composition and estimated energy output

The LSMs of all the milk yield and composition traits of cows reared in the High-HP and in the Low-HP herds, are illustrated in Table 3. The daily yields of cows of the High-HP herds, as expected, out yielded cows of the Low-HP herds by 54% to 58%, for milk, protein, fat and energy productions. Also the composition of milk tended to be superior for cows of the High-HP herds than for those of the Low-HP herds (significantly for protein content).

The incidence of individual herds variability within HP class on total variability was low for composition traits (7.2 to 20.1%), while was much larger for dMY (31.9%) and intermediate for the other daily yield traits (21.1 to 29.9%).

The 6 breeds showed large differences also for all the milk yield and composition traits considered (Table 3). Taking into consideration the orthogonal contrasts: the 3 specialized dairy breeds were characterized, on average, by similar daily milk and protein production and slightly greater daily fat and energy yield, because of the higher content of protein, fat and energy of their milk (lactose content was slightly lower) respect to the 3 dual-purpose breeds; within the specialized dairy breeds, Jersey cows were characterized by a much lower dMY with higher nutrients and energy content than the cows other two large-framed breeds (Holstein and Brown Swiss) so that the daily yield of nutrients and energy of Jersey cows was still lower, but to a smaller extent (and not significantly in the case of fat yield). Between the two large-framed dairy breeds, Holsteins were superior to Brown Swiss cows for all daily yield traits, but inferior for milk composition traits (lactose excluded); among the dual purpose breeds, the Simmental cows were superior to the cows of the two local breeds for daily yield traits and, to a lesser extent, for milk composition (protein excluded). Between the local breeds, Rendena cows produced more milk than Alpine Grey cows but with a lower content of nutrient and energy, so that the daily production of nutrients and energy were not different (Table 3).

Parity had a minor effect on milk traits because, beyond the lower daily production traits of primiparous respect to multiparous cows, only a decrease of lactose content with increasing parity and a small decrease of protein content in older cows was noted (Table 3).

The effect of the lactation stage was important for all milk traits (with a quadratic for all traits excepted a linear trend of milkfat daily yield and a cubic pattern of lactose percentage) because, as expected, the daily yield traits decreased slowly during the first part of lactation and much more rapidly thereafter, whereas the composition traits showed an almost opposite pattern.

Interactions were generally not significant or, if significant, not relevant (Table 3).

Cheese yield

The LSMs of the percent and daily cheese-yield traits of cows reared in the High-HP and in the Low-HP herds, are illustrated in Table 3. The theoretical, and actual cheese yields were 5% higher for cows reared in High-HP herds than in Low-HP herds and thus, taking into account their superior dMY, they produced 58% more cheese per day. The incidence of individual herds variability within HP class on total variability was low for theoretical and actual %CY (10.5 and 10.6%, respectively), and much larger for dCY (27.8%).

The 6 breeds showed large differences also for all the cheese-yield traits considered (Table 3). On the basis of the orthogonal contrasts: the 3 specialized dairy breeds were characterized, on average, by slightly higher percent and daily cheese-yield respect to the 3 dual-purpose breeds. Within the specialized dairy breeds, Jersey cows were characterized by a much greater theoretical and actual %CY, but by a lower dCY than the cows other two large-framed breeds (Holstein and Brown Swiss). Holsteins were superior to Brown Swiss cows in terms of dMY, but inferior for theoretical (based on milk composition) and especially actual %CY, and as a consequence, by similar dCY. Among the dual-purpose breeds, the Simmental cows were superior to the cows of the two local breeds for theoretical but not actual %CY and for dCY. Between the local breeds, Rendena cows produced more milk than Alpine Grey cows but with lower %CY, so that the dCYs were not different (Table 3).

Parity had a minor effect on %CY traits (in favor of primiparous cows) and did not affect the dCY (Table 3).

The effect of the lactation stage (linear and quadratic) was important for all cheese yield traits, with an accelerated increase of both theoretical and actual %CY during lactation and a correspondent decrease of dCY.

Interactions were not significant with the only exception of breed × parity effects on dCY (Table 3).

Estimated productivity ratios

Table 4 summarizes the LSMs of the 9 productivity ratios, obtained by dividing three measures of daily cow's output (milk yield, milk energy output, or fresh cheese yield) by three scaling units to take into account different size and feeding costs of cows (BW, MW and PW).

All the productivity ratios of cows were profoundly affected by herd production level. The superiority of productivity ratios of the High-HP herds respect to the Low-HP herds was not much different according the different output measures or scaling unit used (+36% to +43%). Also the incidence of individual herds variability within HP class on total variability was similar across different ratios (26% to 31%).

Also the breed of cow affected profoundly all the productivity ratios, but, in this case, different productivity ratios yielded different ranking of the 6 breeds compared, even though all data were corrected for herd, parity and DIM effects. Taking into consideration the orthogonal contrasts, the 3 specialized dairy breeds were characterized, on average, by higher productivity ratios respect to the dual-purpose breeds, and this superiority was greater than those found for the 3 daily yield per cow traits used as output measures (milk, energy and cheese daily yields; Table 3), because of the lower average values of the 3 scaling units used in the denominator of the ratios (BW, MW and PW; Table 2) of dairy respect to dual-purpose breeds. This differences are mainly due to the fact that the very low values of scaling units (BW, MW, and PW) typical of Jersey cows cause productivity ratios greater than those of the large-framed dairy cows, with the only exception of milk yield per kg of metabolic weight. Having the two large-framed dairy breeds very similar body size, the superiority of Holstein-Friesian cows respect to Brown Swiss ones, in terms of milk yield, was reflected by all the three productivity ratios based on this output measure. The differences between the two breeds become much smaller when the ratios took into account the energy and the cheese output measures, because of the greater content of nutrients and better technological properties of the milk of the latter breed respect to the former. The differences within dual-purpose breeds were small. As the Simmental, respect to local breeds, was characterized by greater daily output measures, but also by greater body size (i.e., by greater numerators and also denominators). However, its productivity ratios were not different from those of the two local breeds. Within these latter breeds, Rendena was characterized by a higher milk productivity only when this is related to BW and MW (Table 4).

Parity had a minor effect on productivity ratios because the lower daily output of primiparous cows were partly compensated by their lower body size (Table 4). On the contrary, the effect of the lactation stage was accentuated, because the decrease of daily output measures during lactation faced an increase in body size (Table 4). Interactions were generally not significant or, when significant, not relevant (Table 4).

Estimated efficiency indicators

The LSMs of the estimated daily energy requirements of individual cows are reported in Table 5. The daily requirements of cows of the High-HP herds, as expected, were slightly greater for maintenance, much greater for lactation and very similar for pregnancy, so their total requirements were greater than those of the cows in Low-HP herds. The efficiency of cows from High-HP farms was higher than for cows from Low-HP farms, in terms of energy (+13%) and economic (+15%) efficiency, and especially as IOFC (+84%). The incidence of individual herds variability within HP class on total variability was large (27 to 30%).

The 6 breeds showed large differences also for all the energy requirements, especially, as expected, when comparing breeds very different in terms of body size and production traits (Table 5). The differences among breeds in terms of efficiency indicators were much lower than in terms of energy requirements.

Considering the orthogonal contrasts: the 3 specialized dairy breeds were characterized, on average, by greater energy and economic efficiencies and also IOFC respect to the 3 dual-purpose breeds. Within the specialized dairy breeds, Jersey cows were characterized by similar IOFC and energy efficiency but by greater economic efficiency than the two large-framed breeds (Holstein and Brown Swiss). Between the two large-framed dairy breeds, Holsteins were superior to Brown Swiss cows for energy efficiency, but inferior for both monetary indicators. Among the dual-purpose breeds, the Simmental cows had a slightly greater energetic efficiency than the cows of the two local breeds. Whereas between the two local breeds no difference in efficiency indicators was noted (Table 5).

Parity affected (in favor of primiparous cows) only the economic efficiency indicator (Table 3), whereas the effect (linear and quadratic) of the lactation stage was important for all the 3 indicators, because of their progressive decrease with DIM.

Interactions were significant only in the case of breed × parity (Table 5).

DISCUSSION

The effects of herd production level, herd variability within production level, cow's breed, parity and stage of lactation on milk yield, composition and coagulation properties and those on cheese yield and milk nutrient recovery in cheese have been discussed in our previous studies (Stocco et al., 2016a and b). The effects of individual factors within breed (parity and lactation stage) on productivity and efficiency indicators will not be discussed because, even though interesting from the theoretical point of view, they are not much useful for the evaluation and improvement of dairy farm efficiency. The discussion will be focused on the effects of herd characteristics and of breed of cows on milk and cheese productivity and efficiency.

Efficiency of milk and cheese production in relation to herd characteristics

Obviously cows from High-HP herds were characterized by greater values for daily milk yield (+53%) and milk fat and protein contents (+6% and +5%, respectively) and daily productions (+58% and +58%), actual cheese yield (+5%) and daily production (+57%), but also by heavier BW (+8%) and body fat mass (+20%), and, as a consequence, of all individual and total energy requirements (+37%).

The superiority of productivity indicators of High-HP vs Low-HP farms ranged from +35% to 43% (Table 4), whereas the superiority of High-HP farms was greater for IOFC (+84%) and much lower when expressed as ratio efficiency indices: +13% in terms of energy utilization, and +15% when daily milk value is divided for feed costs (Table 5).

Efficiency of milk and cheese production of cows of different breeds

The results showed that, within herd, there is a very large variation in terms of size of the cows and of their milk yield, composition and cheese yield.

Specialized dairy and dual purpose breeds

Both groups of specialized dairy and dual-purpose breeds presents a large variability among different breeds within group. Anyway, the three specialized dairy breeds, respect to the three dual-purpose breeds, are characterized by a smaller body size and by a leaner estimated body composition. Within herd, on average the specialized dairy breeds do not produce more milk than the dual-purpose ones.

At national level, the three dairy breeds here studied presented an average milk production much greater than the three dual-purpose breeds, as evidenced by Italian milk recording system (AIA, 2015). It is evident that the national differences in average milk production are affected by the different geographical distribution of the single breeds, and by the different proportion among the various dairy systems present in the country. Restricting the area of comparison to a more homogeneous environment, like when only the province of Trento (the area of this study) is considered, the differences between dairy and dual-purpose breeds are slightly lower, but remain substantial (AIA, 2015). Also, at local level many herds are single-breed herds with a different distribution of these herds among the different dairy systems present in the province, from the very small and traditional to the very modern ones (Sturaro et al., 2013). But a large difference is evident also in the 41 one multi-breed herds of the present study. In fact, if the raw means of daily milk yield of different breeds are considered (data not shown), the dairy breeds present an average superiority of 48% respect to dual-purpose breeds. Even though all the considered herds were multi-breed, it is evident that the distribution of breeds is different, and different is also the proportion of different breeds within herd. In particular, the specialized dairy breeds are reared especially in modern farms, using many concentrates, while dual-purpose breeds are more present in traditional low input farms. As shown in Table 1, only Holstein, Brown Swiss and Simmental cows are present (in different proportions) in both the High-HP and Low-HP farms, while Jerseys are only present in the former, and the local breeds in the latter group of farms. A preliminary statistical analysis carried out only on the three breeds present in both production level (data not shown) evidenced not significant or not relevant interactions between herd production level and breed, all data have been analyzed using the model shown in material and methods section, with inclusion of herd category and breed of cow. Correcting the least squares means for the different presence and proportion of breeds, and also for the parity and DIM of the cows, within category of herd and individual herds, the average superiority of production of dairy breeds on dual purpose breeds is only +3% (Table 3, not significant).

On average, the specialized dairy breeds were superior to the dual-purpose ones in terms of milk content of fat, protein and energy, and consequently also of theoretical and actual percent cheese yield (Table 3). This explain why the average daily production of cheese of the three dairy breeds was greater than the corresponding value of the three dual-purpose breeds (+7%, $P < 0.05$, Table 3).

Taking into account the average smaller body size of dairy breeds, respect to dual-purpose ones, all the productivity indicators, per unit of BW, MW or PW, on average were in favor of dairy breeds, especially for those referring to milk energy or cheese daily production (+21% to +26%, Table 4) rather than milk yield (+12% to +16%; Table 4).

Regarding the estimation of energy requirements of the lactating cows, the dairy breeds were characterized, on average, by greater lactation requirements, but by smaller requirements for maintenance, activity and pregnancy (because of their lower average size) than the dual-purpose breeds. So, the total energy requirements of the two groups of breeds was not different and the efficiency of energy utilization (proportion of lactation requirement on total requirement) was in favor of dairy breeds (+7%; Table 5). The differences between specialized and dual-purpose breeds from the economic point of view were similar, and on average, in favor of dairy breeds, both in terms of IOFC (+13%) and economic efficiency (+6); Table 5).

Jersey breed

Among the three dairy breeds, Jersey is the most peculiar. Even though its least squares means should be considered with prudency, being this the breed with the lowest number of cows controlled, as known, Jersey was the breed with the smallest body size, the lowest daily milk yield and the greatest fat, protein and energy content of milk among the three dairy breeds tested, but in general, among all the six breeds controlled. The milk produced by Jersey cows is very peculiar also from the technological point of view (Auldist et al., 2002 and 2004; Bittante et al., 2012). A previous study on the same cows demonstrated that the Jersey milk is characterized by the shortest time from addition of rennet to coagulation, by a fast curd firming, by the greatest maximum curd firmness and also by a rapid syneresis among all the 6 breed compared (Stocco et al., 2016a). Due to both high content of fat and protein and the excellent coagulation and curd firming properties, Jersey milk was characterized by the greatest theoretical (on the basis of its composition) and actual %CY (taking into account also differences in the efficiency of retention of nutrients in the cheese) (Stocco et al., 2016b).

The excellent composition and cheese-making properties of its milk explain while Jersey cows produced 30% less milk per day, but only 12% less full-fat fresh cheese than the average of Holstein and Brown Swiss cows (Table 3).

A correct comparison among breeds should not only take into consideration the production of milk and cheese per cow, but also their productivity after having corrected the production for a measure of the “fixed” costs due to energy requirement different from production (maintenance, activity, growth, pregnancy). If we use, as scaling factor to compare different breeds, the body weight of cows, that is on average 40% lighter for Jerseys than for the other two large-framed dairy breeds (Table 2), we obtain an indicator of productivity (grams of milk produced daily per kg of body weight maintained) greater for Jersey than for the other two breeds (+20%; Table 4).

It is well known that the energy requirement is not directly proportional to body weight of animals, and neither to their empty body weight (corrected for the content of gastro-intestinal tract). Generally, it is assumed that smaller animals consume more energy per kg of body weight for their maintenance respect to larger animals, and so the metabolic weight ($MW = BW^{0.75}$) is a curvilinear function increasing less than proportionally respect to body weight. The American National Research Council in the "Nutrient requirement of dairy cattle" (NRC, 2001) adopted value of 80 kcal/kg MW for the maintenance of dairy cows (included a 10% allowance for cow's activity), independently of their breed.

Differently than when using BW, using MW as a scaling factor yielded a milk productivity indicator not different between the Jersey and the large-framed breeds (Table 4).

For beef cattle, the NRC (NRC, 2000) reviewed the scientific literature regarding the maintenance requirements of cattle belonging to different breeds and adopted specific multiplicative "breed adjustment factors" for different breeds. Respect to the British beef breeds, set as reference (breed factor 1.00) the dairy and dual-purpose breeds (Ayrshire, Brown Swiss, Braunvieh, Holstein-Friesian, Simmental) apparently require 20% more energy (breed factor 1.20). But, also in this case, the curvilinear pattern of metabolic weight is used for comparing animals of different BW. This pattern is justified in mono-gastric animals and in ruminant reared in very cold environment with the fact that animal heat dispersion is roughly proportional to animal's body surface (and not BW) whose relationship with BW is a function of $BW^{0.67}$ (NRC, 2000). For ruminants in areas characterized by temperate climate, the maintenance of body temperature is not a great source of energetic cost, because of the large heat production of pre-stomachs. The other justification to the curvilinear pattern of MW is that, within breed, and in growing animals, fatty depots grow more than proportionally respect to BW, while the opposite is true for lean tissues, and it is well known that lean tissues are characterized by higher energetic costs for maintenance than fatty tissues.

When comparing adult animals of breeds differing widely in mature BW, adoption of MW as a scaling factor for estimating maintenance requirements seems not to be justified. Jerseys cows, on average, had a BCS very close to that of Holsteins, and only slightly lower respect to Brown Swiss cows (Table 2). Adopting the equation derived from those proposed by NRC (2001), we predicted a proportion among body fat, protein and water + ash not different in jerseys and in large-framed dairy breeds (Table 2).

Comparing growing beef cattle from breeds characterized by very different pattern of fat deposition (Schiavon and Bittante, 2012) we demonstrate that, a curvilinear pattern like MW, one could be used to express energy demand of early-maturing cattle breeds (like British breeds), but not for very lean breeds (like double muscled Piemontese), that are characterized by more linear pattern of energy demand. According to the models proposed by Emmans et al. (1994), we proposed to use body protein mass as the scaling factor for estimating maintenance requirements in both early and late maturing animals.

For these reasons, we calculated a third group of productivity indicators using the body protein (BP) mass as a scaling factor. In the case of the comparison between Jersey and the other two large-framed breeds, this indicator yielded results not much different from the indicator based on BW (Table 4), because the percentage body composition estimated from animals BCS was similar across dairy breeds, independently from their average body size.

When, as an output measures, we replaced the dMY with daily yield of milk energy or fresh cheese, Jersey breed maintained its superiority respect to the other two dairy breeds with all scaling factors (Table 4), even though the differences among breeds were greater when outputs were scaled on BW and BP than on MW (Table 4).

Moving from productivity indicators to efficiency indicators (Table 5), it could be seen that Jersey breed was not different from the other two dairy breeds, because the former is characterized by lower milk production but also by lower energy requirements for maintenance, activity and pregnancy because of its small size. Of the two economic indicators, IOFC, that is not scaled on size of cow (is a difference and not a ratio) not different in Jersey and in the other two dairy breeds, despite the large difference in body size. Scaling the income allowed for milk production adjusted for cheese-making ability on the total feed costs, Jersey breed was again superior to other dairy breeds.

Holstein and Brown Swiss breeds

At Italian national level, Holstein cows produce much more milk than Brown Swiss cows (AIA, 2015). In a less heterogeneous environment (Trento Province), on average, Holstein cows produced 18% more milk than Brown Swiss cows. Using the raw averages of all the cows of the present study across the 41 multi-breed farms, the Holsteins produced 14% more milk than Brown Swiss cows (results not shown). After correcting dMY for HP class, individual herds, parity and DIM, Holstein cows still produced 14% more milk than Brown Swiss cows (both breeds are present especially in the modern farms). Taking into consideration the higher fat and protein content of milk from Brown Swiss cows, the daily production of fat and protein of Holsteins was only 7% and 5% greater than for Brown Swiss cows, respectively (Table 3).

Based on milk composition, the theoretical %CY of Brown Swiss cows was 8% higher than that of Holsteins, while the actual %CY, measured through individual model cheese-making, was 16% higher in the former than in the latter breed (Table 3). Brown Swiss cows, not only produced milk with more fat and protein than Holsteins, but produced a milk which was able to retain in the curd a greater percentage of milk-fat and protein, reducing the losses in the whey, as it was proved in these same cows (Stocco et al., 2016b), but also in other studies carried out in Italy (Cassandro et al., 2005), and other countries (Mistry et al., 2002). As a result of the greater nutrients recovery, the daily production of cheese was not different in the two major breeds, even if nominally higher for Brown Swiss cows (Table 3).

Being the two major breeds very similar in terms of body size, the indicators of milk productivity ranked the two breeds about in the same way, as the output measures, independently of the size scaling factor (Table 4), with Holsteins superior to Brown Swiss in terms of milk indicators and also of milk energy (at a lower extent), but not in terms of cheese productivity (on the contrary the latter breed was superior to the former when the dCY was scaled on the BP mass).

Moving to efficiency indicators, both energetic efficiency of milk production and IOFC were not different between the two large-framed breeds, whereas the Alpine breed was characterized by a higher economic efficiency respect to the Holstein breed (Table 5).

Simmental breed

At national level, Simmental cows produce less milk than Holsteins and also than Brown Swiss cows (AIA, 2015). Within Trento Province, on average Simmental cows produce -23% and -9% less milk than Holstein and Brown Swiss cows, respectively (Cecchinato et al., 2015). Limiting the comparison to the present study, on the basis of the raw means, the Simmental cows produced 29% and 19% less milk than Holstein and Brown Swiss cows (results not shown), respectively. On the same cows, after correcting dMY for HP class, individual herds, parity and DIM, Simmental cows produced only 11% less milk than Holsteins, and about the same daily quantity as Brown Swiss cows (a large proportion of Simmental cows are reared in low-HP farms). Taking into consideration the fat and protein content of milk, the daily production of fat and protein was very similar to that of Brown Swiss cows, and only 7% and 6% lower than those of Holstein cows, respectively (Table 3).

Milk from Simmental cows showed coagulation and curd firming properties close to those of Brown Swiss milk, and better than those characterizing Holstein milk (Bittante et al., 2012; Stocco et al., 2016a). Better coagulation and curd firming properties respect to purebred Holsteins were showed also by differing crossbred combination involving Montbéliarde and Brown Swiss breeds (Malchiodi et al., 2014). These technological aspects, together with the different proportion and frequency of genetic variants of milk protein fractions, explain the similarity of cheese-making efficiency of the two breeds of Alpine origin, and their superiority respect to Holstein breed (Stocco et al., 2016b). The combined effect of correcting data for herd factors, milk protein and fat contents, milk coagulation and curd firming properties and cheese-making efficiency, explain the fact that these three large-framed breeds produced a daily quantity of fresh cheese very similar from each other (Table 3).

Moving to productivity ratios, it should be taken into consideration that Simmental cows, respect to the two large-framed dairy breeds, were characterized by slightly greater BW (+4%, table 2), and MW, but not BP mass. In fact, the higher BCS values of dual-purpose breeds respect to specialized dairy ones, led to a predicted lower proportion of body protein and water + ash, and a higher proportion of fat. The predicted lean body mass of the three large-framed breeds are quantitatively very similar, whereas the superiority of BW of Simmental cows respect to the other two breeds was almost entirely explained by a greater weight of fatty tissues (Table 2). This is the reason why Simmental cows presented productivity ratios slightly lower than Holsteins (and similar to Brown Swiss) when scaling milk production on BW and on MW, whereas were very similar when scaling cheese production on PW (Table 4). Considering efficiency indicators, Simmental cows were inferior respect to Holsteins in terms of energetic efficiency, whereas they were similar to both the large-framed dairy breeds, in terms of both economic efficiency indicators (Table 5).

Rendena and Alpine Grey local breeds

Both local breeds were characterized by a daily milk production much lower than the large-framed dual-purpose breed (Simmental) when averaged across herds (-16% and -35%, respectively for Rendena and Alpine Grey cows, data not shown). Once corrected for herds, parity and DIM effects, the differences fell respectively to -5% and -19% (Table 3).

It worth to note that Rendena cows were characterize by a content of protein similar to Holsteins, and by a milk-fat content even lower than Holsteins, so that the theoretical %CY of the local dual-purpose breed was lower than that of the international dairy breed (Table 3). But, as reviewed by Bittante et al. (2012) for traditional milk coagulation properties, and as reported by Stocco et al. (2016a) for curd firming modeling, milk from Rendena cows performed better than milk from Holsteins and this could explain the fact that, differently from theoretical %CY, actual %CY was superior for the local breed than for the international one (Table 3).

Alpine Grey cows yielded a milk with a composition not much different from that of the major dual-purpose breed, nor from that of the Alpine dairy breed, so that %CY was similar. As a result, respect to Simmental cows, Rendena cows produced 10% less cheese per day and Alpine Grey 16% less cheese per day (Table 3). These differences were very similar to those regarding BW (and MW, and PW). In fact BW was 12% lower for Rendena cows, and 18% lower for Alpine Grey cows, respect to Simmental ones. The consequence is that no one of the productivity indices was different for local breeds *vs* Simmental one, and that between the two local breeds, only milk yield scaled for BW and MW was greater for Rendena than for Alpine Grey cows (Table 4). In terms of efficiency of milk production, the energetic indicator was superior for Simmental breed than for the local breeds, while the economic indices were not different, as any of the efficiency indices when the two local breeds were compared from each other (Table 5).

Implications for crossbreeding and selection

The results of the present study, especially for comparisons among different breeds, allow to focus the different role and relative importance of various production, qualitative and technological traits in relation to a dairy sector based on cheese production and not on fluid or dried milk. On the other hand, it has to be considered that cheese production is growing world-wide and nowadays represents the most important milk destination (IDF, 2013).

A more direct utilization of this knowledge is on projecting crossbreeding schemes for dairy sectors. Crossbreeding among dairy breed is increasing and several studies showed the positive results obtainable especially on milk production and fertility (Blöttner et al., 2011; Heins et al., 2012; Malchiodi et al., 2014a) traits. The properties of milk were generally studied only in terms of milk composition (Dechow et al., 2007). Only few studies focused on the milk coagulation properties of crossbred cows in comparison with purebreds (Malchiodi et al., 2014b), and none, the authors are aware of, analyzed the cheese-yield traits. The knowledge of cheese-making specific traits and overall efficiency can be, therefore, very valuable for crossbreeding design, provided that heterosis and recombination effects be similar for different crossing.

But the knowledge of different components of productivity and efficiency of different breeds in a cheese-making based dairy system offers also interesting information and stimulus for the evaluation of current selection indices and the need for their upgrading. Now it is much more clear the important role of traits different from milk production and contents in relation to efficiency, and then to profitability of the productive dairy chain. It is clear that model cheese procedure cannot be applied routinely for genetic evaluation, but indirect prediction of nutrients recovery in cheese through infrared spectroscopy (Ferragina et al., 2013 and 2015; Bittante et al., 2014) and genomics (Dadousis et al., 2016a and 2016b) are available and could further developed.

CONCLUSIONS

In conclusion, the study demonstrated that an important part of the productive differences among dairy and dual purpose cattle breeds is due to differences in dairy systems and herd characteristics. When the comparison among different breeds is carried out within dairy system and within individual herds (using multi-breed herds) the differences in productivity traits are much reduced and depends a lot on the differences in body size of animals. Scaling production traits on the basis of body size indicators allow to obtain a more correct indication of breeds' productivity (instead of production). Among different scaling factors, metabolic weight could be criticized when used to compare breeds of very different size but similar body composition (like Holstein and Jersey) while the use of body protein mass weight allows for a more correct comparison, also when breeds are characterized by different body condition and, consequently, body composition.

If within-herd comparison and correctly scaling of production traits reduce strongly herd differences in productivity, they do not reduce the differences in terms of milk composition, technological properties and efficiency of cheese-making (recovery of milk nutrients in cheese). The differences among breeds remains strong and their importance on the overall efficiency evaluation of the breeds increase. The knowledge and correct quantification of the importance of productive, qualitative and technological properties of different breeds offers interesting new insights for modifying selection indices within dairy and dual purpose breeds and for projecting crossbreeding plans across breeds.

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TABLES AND FIGURES

Table 1. Characteristics of mixed breed herds in North-East Italy based on classification as high or low herd production (HP)[1]

Item	High-HP	Low-HP
Number of herds	20	21
Number of cows	920	588
Average number of cows/herd	46	28
Utilized agricultural area, ha[2]	38.2±26.3	24.0±13.2
Concentrates, kg/d[2]	12.9±4.9	6.7±2.8
Breeds[3]	HF, BS, Je, Si	HF, BS, Si, Re, AG
Milk yield, kg/d[2]	28.0±8.3	18.5±6.9
Milk composition[2]		
Fat, %	4.19±1.00	3.75±0.80
Protein, %	3.73±0.48	3.48±0.50
Casein, %	2.92±0.37	2.73±0.36
Lactose, %	4.84±0.23	4.85±0.24
Urea, mg/100g	21.7±7.9	29.2±9.9
pH	6.51±0.11	6.51±0.10
SCS[5], U	2.89±1.81	2.79±1.94
Milk energy production, MJ/d	90.86	57.33

[1] according to average daily milk energy yield of the cows corrected for breed, DIM and parity.

[2] Mean±SD

[3] Holstein Friesian (HF), Brown Swiss (BS), Jersey (Je), Simmental (Si), Rendena (Re) and Alpine Grey (AG).

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

Table 2. Body size, condition and estimated composition of lactating cows

	Body size			BCS	Empty body % composition			Body composition			Body Energy
	BW kg	MW kg	Hearth girth cm	(1-5)	Protein %EBW	Fat %EBW	Water+ash %EBW	Protein kg	Fat kg	Water+ash kg	MJ
Herd productivity:											
High, (LSM)	604	121	197	3.1	16.6	19.8	63.7	85	102	325	6035
Low, (LSM)	557	115	192	2.9	16.9	17.9	65.2	80	85	309	5221
F-value	70.4***	58.2***	17.9***	35.6***	33.3***	33.3***	33.3***	48.4***	51.8***	43.1***	57.2***
Herd variance (% of total)	14.0	11.2	7.8	14.6	13.1	14.0	14.0	9.8	14.4	9.3	14.4
Breed LSM:											
Holstein Friesian (HF)	645	128	202	2.7	17.1	16.8	66.1	94	92	362	5850
Brown Swiss (BS)	643	127	200	2.9	16.9	18.2	65.0	92	100	353	6106
Jersey (Je)	384	87	174	2.7	17.1	16.9	66.1	56	52	218	3378
Simmental (Si)	669	132	204	3.2	16.5	20.1	63.4	94	115	361	6736
Rendena (Re)	592	120	195	3.2	16.5	20.1	63.4	83	102	317	5974
Alpine Grey (AG)	552	113	190	3.3	16.4	20.9	62.7	76	100	292	5724
Breed contrasts (F-value):											
HF+BS+Je *vs* Si+Re+AG	119.8***	98.76***	12.6***	156.1***	146.5***	146.5***	146.5***	41.5***	161.5***	30.0***	159.9***
HF+BS *vs* Je	1080.0***	880.8***	174.1***	1.8	1.6	1.6	1.6	1155.0***	159.7***	1171.5***	296.8***
HF *vs* BS	0.4	2.6	3.6	81.8***	67.8***	67.8***	67.8***	29.8***	38.7***	39.6***	22.0***
Si *vs* Re+AG	344.1***	278.8***	68.8***	2.5	2.1	2.1	2.1	413.4***	35.8***	426.7***	77.9***
Re *vs* AG	27.6***	23.12***	6.2	3.2	2.9	2.9	2.9	38.4	0.5	40.6***	2.9
Parity LSM:											
1st	571	116	191	3.1	16.7	19.4	64.0	81	95	310	5632
2nd	580	118	194	3.0	16.8	18.6	64.6	82	92	317	5580
≥3rd	591	120	197	2.9	16.8	18.4	64.8	84	93	325	5672
Parity contrasts (F-value):											
1st vs ≥2nd	17.4***	12.3**	30.0***	20.0***	19.3***	19.3***	19.3***	32.8***	1.4	37.1***	0.0

2nd vs ≥3rd	6.9***	6.4*	8.5**	0.6	0.4	0.4	0.4	10.4**	0.4	10.9**	1.2
DIM LSM:											
8-49 d	566	116	191	2.91	16.9	18.1	65.0	81	87	313	5356
50-91 d	563	115	191	2.85	17.0	17.7	65.4	81	85	313	5243
92-133 d	571	116	193	2.90	16.9	18.0	65.1	81	87	314	5364
134-175 d	574	117	192	2.91	16.9	18.2	65.0	82	89	317	5441
176-217 d	584	118	194	3.01	16.7	18.9	64.4	83	94	319	5674
218-259 d	591	119	196	3.04	16.7	19.2	64.2	84	97	322	5780
260-301 d	593	120	196	3.12	16.6	19.7	63.7	83	100	320	5917
≥302 d	604	121	199	3.26	16.4	20.8	62.8	84	108	321	6249
DIM contrasts (F-value):											
Linear	142.9***	112.6***	72.7***	190.7***	183.8***	183.8***	183.8***	48.4***	209.8***	33.7***	206.2***
Quadratic	3.3	3.8	4.9*	29.8***	30.3***	30.3***	30.3***	0.0	24.2***	0.4	19.9***
Cubic	1.4	2.4	0.0	0.2	0.4	0.4	0.4	2.9	0.3	3.0	0.6
Interactions (F-value):											
Herd prod. × parity	0.4	0.0	0.4	3.5*	3.2*	3.2*	3.2*	0.4	2.2	0.6	1.5
Herd prod. × DIM	1.1	0.6	1.1	0.8	0.6	0.6	0.6	0.5	0.8	0.5	0.8
Breed × parity	1.8*	1.4	1.0	1.7	1.7	1.7	1.7	1.3	1.4	1.2	1.5
RMSE	38.1	6.6	10.0	0.3	0.4	2.3	1.9	5.2	16.8	19.9	726.5

Table 3. Milk composition, percentage cheese yield and daily milk and cheese yield

	Milk composition and energy:				Cheese yield			Daily yield per cow:					
	Protein %	Fat %	Lactose %	Energy MJ/kg	theor. %	actual %	relative index[1]	Milk kg/d	ChCM[2] Kg/d	Protein g/d	Fat g/d	Energy MJ/d	Cheese kg/d
Herd productivity:													
High, (LSM)	3.82	4.50	4.99	3.42	14.2	14.5	1.05	26.1	26.3	959	1129	86.5	3.64
Low, (LSM)	3.63	4.24	4.99	3.28	13.5	13.8	0.99	17.0	16.7	606	714	55.1	2.30
F-value	13.6***	4.9*	0.0	6.1*	8.1**	8.2**	8.2**	63.2***	63.9***	66.1***	57.4***	63.9***	63.9***
Herd variance (% of total)	20.1	10.0	7.2	10.2	10.5	10.6	10.6	31.9	27.8	29.9	21.3	28.0	27.8
Breed LSM:													
Holstein Friesian (HF)	3.51	4.06	4.99	3.18	12.8	12.4	0.90	25.9	22.8	890	1035	81.2	3.15
Brown Swiss (BS)	3.79	4.32	4.98	3.34	13.9	14.4	1.04	22.7	23.5	849	967	75.2	3.25
Jersey (Je)	4.08	5.54	4.88	3.89	16.7	17.1	1.24	17.0	20.3	662	914	62.3	2.81
Simmental (Si)	3.67	4.38	4.97	3.34	13.7	14.0	1.01	23.0	22.5	826	970	74.9	3.11
Rendena (Re)	3.52	3.77	5.11	3.07	12.4	13.0	0.94	21.8	20.5	763	841	67.6	2.83
Alpine Grey (AG)	3.79	4.17	5.00	3.28	13.6	14.1	1.02	18.7	19.4	704	800	62.3	2.67
Breed contrasts (F-value):													
HF+BS+Je *vs* Si+Re+AG	16.8***	25.6***	7.8*	26.3***	29.2***	17.5***	17.5***	1.5	4.8*	2.5	8.6*	5.6*	4.8*
HF+BS *vs* Je	51.7***	48.5***	4.1*	52.7***	61.2***	69.9***	69.9***	42.2***	5.3*	24.2***	1.8	13.5***	5.3*
HF *vs* BS	158.4***	13.5***	0.1	24.2***	50.4***	157.7***	157.7***	67.5***	2.5	7.1*	8.4*	17.5***	2.5
Si *vs* Re+AG	0.1	10.1*	5.2*	8.2***	5.5*	2.4	2.4	14.7***	9.8**	10.8**	12.0**	14.0***	9.8**
Re *vs* AG	19.8***	4.5*	4.6*	6.4*	8.4**	6.8**	6.8**	8.7**	0.9	2.0	0.4	1.9	0.9
Parity LSM:													
1st	3.74	4.37	5.06	3.35	14.1	14.6	1.06	20.1	21.2	749	877	67.5	2.92
2nd	3.76	4.44	4.98	3.39	13.9	14.1	1.02	21.8	21.4	793	934	71.6	2.95
≥3rd	3.67	4.30	4.92	3.31	13.5	13.8	1.00	22.6	22.0	805	953	73.4	3.03
Parity contrasts (F-value):													
1st vs ≥2nd	1.0	0.0	25.4***	0.0	4.7*	12.8***	12.8***	21.3***	0.9	1.8*	5.9*	8.9**	0.9

2nd vs ≥3rd	7.0*	1.7	4.6*	2.2	3.5	1.1	1.1	1.5	0.7	0.3	0.3	0.7	0.7
DIM LSM:													
8-49 d	3.45	4.11	5.04	3.17	12.8	13.1	0.95	25.9	23.9	862	1043	80.8	3.31
50-91 d	3.47	3.99	5.10	3.14	12.7	13.0	0.94	25.5	23.4	868	1001	79.1	3.24
92-133 d	3.56	4.17	5.07	3.23	13.2	13.5	0.98	24.0	23.0	840	980	76.4	3.17
134-175 d	3.66	4.15	5.05	3.25	13.4	13.7	0.99	23.0	22.4	833	941	74.0	3.09
176-217 d	3.74	4.44	4.95	3.39	14.0	14.4	1.04	20.8	21.6	781	928	70.7	2.98
218-259 d	3.85	4.45	4.93	3.42	14.2	14.6	1.05	19.3	20.1	737	859	65.6	2.78
260-301 d	3.95	4.81	4.88	3.58	15.0	15.4	1.11	17.9	20.0	709	868	64.3	2.77
≥302 d	4.12	4.84	4.87	3.62	15.4	15.7	1.14	15.5	17.6	628	749	55.6	2.43
DIM contrasts (F-value):													
Linear	671.8***	105.6***	110.7***	183.7***	232.1***	232.6***	232.6***	552.1***	129.9***	172.0***	90.0***	204.3***	129.9***
Quadratic	12.5***	7.0*	7.0*	6.4*	7.4**	6.8**	6.8**	8.7**	6.1*	14.2**	1.9	6.8**	6.1*
Cubic	0.0	2.4	12.2**	2.1	1.4	1.5	1.5	0.6	0.5	0.0	1.6	0.4	0.5
Interactions (F-value):													
Herd prod. × parity	1.9	0.7	1.5	0.8	0.6	0.6	0.6	3.7*	2.9	5.3	2.3	3.6*	2.9
Herd prod. × DIM	2.9*	0.9	1.0	1.0	1.1	1.1	1.1	1.2	1.0	1.4	0.7	0.9	1.0
Breed × parity	1.1	0.5	0.5	0.4	0.4	0.4	0.4	1.5	2.4**	2.4	1.8	2.3	2.4**
RMSE	0.3	0.9	0.3	0.4	2.08	2.11	0.153	5.0	5.8	200	306	18.8	0.80

[1]: The index expresses the relative value of milk for cheese production respect to average milk (a cheese-corrected milk).

[2]: ChCM = Cheese corrected milk obtained multiplying actual dMY time the cheese-making relative index.

Table 4. Milk productivity ratios obtained dividing a measure of daily output of individual cows (milk weight, milk energy or fresh cheese weight) for a scaling unit to take into account different animal size and feeding costs (BW, body weight; MW, metabolic weight; PW, estimated body protein weight).

Output:	Milk yield			Energy yield			Cheese yield		
Scaling unit:	BW	MW	PW	BW	MW	PW	BW	MW	PW
	g/kg	g/kg$^{0.75}$	g/kg	kJ/kg	kJ/kg$^{0.75}$	kJ/kg	g/kg	g/kg$^{0.75}$	g/kg
Herd productivity:									
High, (LSM)	42.7	210	301	145	716	1024	6.15	30.2	43.2
Low, (LSM)	31.5	151	218	105	502	728	4.42	21.1	30.6
F-value	38.6***	43.7***	41.6***	42.2***	47.5***	45.2***	42.0***	47.3***	45.0***
Herd variance (% of total)	29.0	29.7	30.9	26.4	27.2	27.2	26.6	27.6	27.8
Breed LSM:									
Holstein Friesian (HF)	39.8	201	273	125	632	860	4.87	24.5	33.4
Brown Swiss (BS)	35.2	177	245	117	588	814	5.03	25.4	35.2
Jersey (Je)	44.9	197	308	177	776	1215	7.80	34.3	53.7
Simmental (Si)	34.4	175	244	113	571	798	4.67	23.7	33.1
Rendena (Re)	36.0	178	256	112	554	792	4.68	23.2	33.2
Alpine Grey (AG)	32.5	159	232	109	530	774	4.65	22.7	33.2
Breed contrasts (F-value):									
HF+BS+Je *vs* Si+Re+AG	34.1***	19.8***	23.1***	66.2***	43.1***	53.0***	66.4***	42.6***	53.5***
HF+BS *vs* Je	17.2***	0.9	16.2***	73.4***	27.0***	72.0***	103.5***	45.7***	102.0***
HF *vs* BS	50.4***	53.4***	38.6***	13.4***	14.2***	7.9**	2.6	2.8	6.4*
Si *vs* Re+AG	0.0	1.1	0.0	0.3	1.9	0.3	0.0	0.7	0.0
Re *vs* AG	4.0*	5.1*	3.7	0.3	0.6	0.2	0.1	0.1	0.0
Parity LSM:									
1st	36.0	175	253	122	589	858	5.31	25.6	37.4
2nd	37.4	182	261	127	618	888	5.28	25.6	36.8
≥3rd	38.0	186	264	127	619	881	5.26	25.7	36.6

Parity contrasts (F-value):									
1st vs ≥2nd	5.2^{*}	6.7^{*}	3.2	3.6	4.8^{*}	2.0	0.1	0.0	0.7
2nd vs ≥3rd	0.3	0.6	0.2	0.1	0.0	0.1	0.1	0.1	0.1
DIM LSM:									
8-49 d	44.6	217	310	144	699	1000	5.94	28.8	41.3
50-91 d	44.4	216	308	141	685	977	5.82	28.2	40.3
92-133 d	41.8	204	290	136	661	947	5.69	27.6	39.6
134-175 d	39.9	195	277	131	638	913	5.52	26.8	38.4
176-217 d	36.1	176	253	124	604	870	5.26	25.6	36.9
218-259 d	32.9	161	231	114	557	802	4.87	23.7	34.2
260-301 d	30.9	151	219	113	549	797	4.88	23.7	34.4
≥302 d	26.4	128	189	98	476	700	4.29	20.8	30.6
DIM contrasts (F-value):									
Linear	621.7^{***}	607.9^{***}	589.2^{***}	264.4^{***}	254.7^{***}	239.5^{***}	179.1^{***}	170.3^{***}	157.7^{***}
Quadratic	12.6^{***}	13.0^{***}	10.4^{*}	6.2^{*}	6.5^{*}	4.8^{*}	5.6^{*}	5.9^{*}	4.3^{*}
Cubic	1.6	1.3	1.4	0.1	0.1	0.1	0.1	0.1	0.1
Interactions (F-value):									
Herd prod. × parity	2.1	2.4	1.8	2.3	2.6	2.0	1.8	2.1	1.5
Herd prod. × DIM	1.2	1.2	1.2	0.9	0.9	0.9	0.9	0.9	1.0
Breed × parity	1.8	1.9^{*}	2.0^{*}	2.4^{*}	2.4^{*}	2.6^{**}	2.8^{**}	2.7^{**}	3.0^{**}
RMSE	8.4	41.7	57.5	30.8	152	211	1.32	6.5	9.1

Table 5. Estimated energy requirements of lactating cows, income over feed costs (IOFC) and energetic and economic efficiency of milk production

	Energy requirements (MJ/d):					IOFC	Efficiency (%):	
	Maintenance	Activity	Lactation	Pregnancy	Total	(€/d)	Energy[1]	Costs[2]
Herd productivity:								
High, (LSM)	37.0	3.4	86.9	3.0	129.9	4.00	63.9	196
Low, (LSM)	34.9	1.6	55.5	2.8	94.5	2.17	56.3	170
F-value	58.2***	12.4**	64.1***	62.3***	77.0***	56.4***	24.2***	25.6***
Herd variance (% of total)	11.2	98.4	27.1	12.3	28.7	27.2	28.4	30.2
Breed LSM:								
Holstein Friesian (HF)	39.0	2.7	81.8	3.3	126.3	3.06	62.2	175
Brown Swiss (BS)	38.8	2.7	75.6	3.2	119.9	3.46	60.8	190
Jersey (Je)	26.6	1.7	63.6	1.9	93.9	3.29	63.2	200
Simmental (Si)	40.1	2.8	75.4	3.4	121.1	3.12	59.7	180
Rendena (Re)	36.5	2.6	68.4	3.0	109.8	2.85	57.9	177
Alpine Grey (AG)	34.6	2.5	62.5	2.8	102.1	2.74	56.7	176
Breed contrasts (F-value):								
HF+BS+Je *vs* Si+Re+AG	98.8***	134.7***	5.0*	100.4***	1.1	7.2*	22.8***	16.8***
HF+BS *vs* Je	880.8***	478.4***	13.8***	903.6***	52.5***	0.0	1.2	12.8**
HF *vs* BS	2.6	13.7**	18.1***	2.1	19.4***	19.3**	6.3*	66.9***
Si *vs* Re+AG	278.8***	45.5***	13.3***	296.9***	31.7***	3.6	5.2*	1.0
Re *vs* AG	23.1***	3.4	2.2	23.7***	3.8	0.2	0.7	0.1
Parity LSM:								
1st	35.5	2.5	68.0	2.9	108.3	3.10	60.3	190
2nd	35.9	2.5	72.0	2.9	112.9	3.03	59.9	179
≥3rd	36.5	2.6	73.6	3.0	115.4	3.13	60.0	180
Parity contrasts (F-value):								
1st vs ≥2nd	12.3**	17.1***	7.8*	13.7***	11.5***	0.1	0.3	23.1***

2^{nd} vs $\geq 3^{rd}$	6.4*	4.0*	0.5	6.8**	1.3	0.5	0.0	0.1
DIM LSM:								
8-49 d	35.3	2.5	81.4	2.9	121.5	3.54	63.2	188
50-91 d	35.1	2.5	80.0	2.8	120.0	3.45	63.2	188
92-133 d	35.3	2.5	77.1	2.9	117.1	3.38	62.6	188
134-175 d	35.6	2.5	74.7	2.9	115.1	3.27	61.8	187
176-217 d	36.1	2.5	70.9	2.9	112.3	3.10	60.3	184
218-259 d	36.4	2.5	65.8	3.0	107.5	2.82	57.8	178
260-301 d	36.5	2.6	64.3	3.0	106.3	2.82	58.1	181
≥302 d	37.0	2.6	55.5	3.0	98.3	2.32	53.6	169
DIM contrasts (F-value):								
Linear	112.6***	54.4***	210.7***	125.4***	163.3***	111.9***	191.1***	68.8***
Quadratic	3.8	1.9	7.2*	4.0*	5.3*	6.4*	18.4***	13.4***
Cubic	2.4	0.5	0.2	2.2	0.7	0.4	0.2	0.3
Interactions (F-value):								
Herd prod. × parity	0.0	1.1	3.4*	0.0	3.5*	2.6	0.6	0.5
Herd prod. × DIM	0.6	1.0	0.9	0.7	1.0	1.0	2.3*	1.9
Breed × parity	1.4	1.8	2.2*	1.5	2.3*	2.5**	3.5***	3.9***
RMSE	2.0	0.2	19.2	0.2	19.0	1.19	7.3	23.3

[1]: Energy of milk produced daily / Sums of estimated energy lactation (in MJ/d NE_L) requirements for maintenance, activity, lactation and pregnancy.

[2]: Income from the selling of daily milk produced by each cow (price = 0.30 €/kg × ratio between actual/average theoretical %CY) / costs of predicted daily feed intake (feed cost = 0.03 €/Mj NE_L × sum of predicted energy requirements).

7 General conclusions

To the best of our knowledge, this is first large scale study comparing the performance of six different dairy cattle breeds on novel technological and nutritional properties of milk. The study included a considerable large number of cows, reared in many multi-breed herds, classified in low- and high-yielding groups. The experimental design allowed to distinguish the effect of breeds from the effect of herd (and herd productivity), a point of major concern in previous studies.

Breed dominated on milk quality, intended as traditional composition, mineral profile (excluding the majority of environmental minerals), renneting aptitude and cheese-making efficiency. On the other side herd acted on daily milk and cheese production, on milk pH and on those minerals strictly related to the location of sampling (i.e., Boron, Silicon, Tin and Strontium, among environmental micro-minerals).

Large differences were observed across breeds. Within herds, Holstein-Friesian cows produced the highest daily milk and cheese production. However, this was accompanied with the lowest milk protein content as well as with the poorest mineral profile (low content for the majority of macro-minerals and few among micro essential minerals) compared to the other breeds. Moreover, the Holstein-Friesian's milk had the worst coagulation time, in terms of delayed curd-firming time and the weakest curd firmness. In addition, the curd-firming and syneresis rates were the slowest. In fact, the time to reach a maximum consistence of the curd had the highest values, among all breeds. All the recovery of nutrients in the curd had the lowest values, including the fresh cheese and water retention. Both the cheese-making efficiencies were the lowest.

Brown Swiss cows within herds, were classified third based on daily milk and cheese productions, after the Holstein-Friesian and Simmental cows. This breed had the highest content of SCS, that may explain the highest concentration of K in milk. Other elements, such as Fe, B and Si at most present in this breed, compared to the other breeds. The renneting aptitude of Brown Swiss milk was similar to those of Simmental and Alpine Grey breeds, albeit at shorter curd-firming time, better syneresis and higher curd firmness over time. Moreover, the recovery of milk fat in the curd was similar to Alpine Grey, and the worst after Holstein-Friesian, while cheese yield traits and efficiencies were more similar to Simmental breed.

In our study, Jersey breed, within herds, produced the lowest quantity of milk and cheese per day, compensated by the highest protein and fat contents in milk, the highest concentrations of Ca, Mg and S among macro-minerals, and Cu, Mn Se and Zn among essentials micro-minerals. Jersey presented the shortest rennet coagulation and curd-firming time, the highest curd firmness values and the highest curd-firming and syneresis rates. In addition, this breed presented the highest cheese yield traits, due to the highest recovery of all nutrients in the curd. However cheese yield efficiencies were similar to those of Brown Swiss and Simmental breeds.

Simmental milk and cheese production per day was close to Holstein-Friesian. Protein and fat contents in milk were intermediate between Brown Swiss and Jersey breeds. The SCS values were the lowest, as typically showed by this breed, and this could explain why Na had the lowest concentration in milk of these cows. The mineral profile and the renneting aptitude was similar to the local Rendena and Alpine Grey breeds. However, the cheese yield characteristics of this breed were more similar to Brown Swiss, and better than the two local breeds.

Concerning the local Rendena breed, it presented modest daily milk and cheese productions, with a mineral profile being intermediate of the Brown Swiss and Alpine Grey cows. This breed had, the shortest rennet and curd-firming time, and the highest curd-firming and syneresis rates, after Jersey. The cheese yield traits were found similar to Holstein-

Friesian. However, it should be noted that Rendena cows had the highest cheese-making efficiencies compare to the other breeds.

Alpine Grey cows had a daily milk production between Jersey and Rendena cows, but also the lowest daily cheese yield production. Renneting and cheese-making aptitude, including the recovery of nutrients in the curd, cheese yield traits and efficiencies were comparable to those of Brown Swiss.

Our results showed that the breed, and not milk composition, was the predominant effect, with large differences between specialized and dual-purpose breeds, but also within the two groups. These findings indicate that the differences among breeds are mainly due to the genetic makeup of the animals, and not only to the chemical composition of milk.

An important part of the productive differences among dairy and dual-purpose cattle breeds is due to differences in dairy systems and herd characteristics. When the comparison among different breeds is carried out within dairy system and within individual herds, the differences in productivity traits are much reduced, and depends a lot on the differences in body size of animals. Scaling production traits on the basis of body size indicators allow to obtain a more correct indication of breeds' productivity (instead of production). Among different scaling factors, metabolic weight could be criticized when used to compare breeds of very different size but similar body composition (like Holstein and Jersey), while the use of body protein mass weight allows for a more correct comparison, also when breeds are characterized by different body condition and composition.

High and low herd productivity had lower impact compared to breed effect. However, this effect in general influenced daily milk and cheese productions, and milk quality, intended as protein, fat, casein, solids contents, and also as mineral composition (i.e., Ca, Mg, S, Cu and Zn); rennet coagulation time and curd consistence for coagulative skills, and theoretical cheese yields (since they are calculated from casein and solids in milk) for cheese-making. So, while increasing herd productivity increases milk yield and quality, percentage cheese yields, and daily cheese production per cow, it has only a slight effect on nutrient recovery and a negative effect on overall cheese-making efficiency.

The knowledge and correct quantification of the importance of productive, qualitative and technological properties of different breeds offers interesting new insights for modifying selection indices within dairy and dual purpose breeds and for projecting crossbreeding plans across breeds.