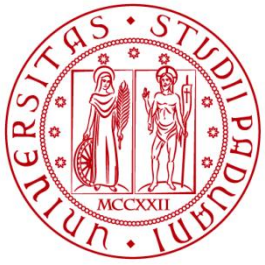

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Improvement of milk coagulation properties in dairy chain

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CONTENTS

SUMMARY

In many countries it has been found that as a result of cattle breeding there has been an increase in milk production, but the milk coagulation properties (MCP) have decreased, and the number of cows in the population producing non-coagulated milk has increased. The general aim of this thesis was to gain further knowledge about improving of milk coagulation properties in Italian dairy industries. The specific aims were: to propose a method for the transformation of the values of MCP traits analyzed using different methodologies; assess the influence of chemical and technological quality of milk on cheese yield; quantify the contribution of composite β- and κ-CN genotypes on additive genetic variance of MCP traits; develop a method for calculating economic values of milk coagulation properties traits; estimate the annual genetic response of MCP in Italian Holstein Friesian. The main results were that the transformation of MCP traits analyzed with different methodologies is feasible but was more precise for rennet coagulation time (RCT) than for curd firmness (a_{30}). In field condition for Grana Padano cheese production the milk characterized by high values of a_{30} resulted in higher cheese yield than milk with low values of a_{30}. In animal phenotypic recording, heritability of RCT was still appreciable after adjustment for composite β- and κ-casein genotypes, suggesting that the recording of this trait cannot be replaced by genotyping of animals for milk protein variants. Accounting for the effect of MCP on cheese yield, the weight for MCP in a possible sub-index for milk production and quality traits ranged from 2.1 to 8.2 %. Current selection index for Italian Holstein Friesian seems not affecting significantly MCP traits. Selection criteria with the implementation of Mid-Infrared spectroscopy prediction of MCP in the current recording systems allow to reach higher genetic response for MCP traits compare direct measure of MCP. Moreover including MCP traits in the selection index can increase casein:protein ratio and could be an indirect way for decrease SCS.

RIASSUNTO

In molti paesi si è riscontrato che, a seguito della selezione genetica animale si è registrato un aumento della produzione di latte, ma un graduale peggioramento delle proprietà di coagulazione del latte, e la percentuale di campioni non coagulati è aumentata. L'obiettivo generale di questa tesi è stato quello di acquisire ulteriori conoscenze sul miglioramento delle proprietà di coagulazione del latte nella filiera lattiero-casearia italiana. Gli obiettivi specifici erano quelli di proporre un metodo per la trasformazione dei dati di attitudine casearia analizzati usando differenti metodologie; valutare l'influenza dei contenuti e della qualità tecnologica del latte sulla resa in formaggio; quantificare il contributo del genotipo composto β- e κ-caseina sulla varianza genetica additiva dei caratteri di attitudine casearia, sviluppare un metodo per calcolare i valori economici dei caratteri di attitudine casearia; stimare la risposta genetica alla selezione per i caratteri di attitudine casearia in Frisona Italiana. I principali risultati sono stati che la trasformazione dei dati di attitudine casearia analizzata con metodologie diverse è fattibile, ma è più precisa per tempo di coagulazione (RCT) che per consistenza del coagulo (a_{30}). In condizioni di campo per la produzione di Grana Padano il latte caratterizzato da alti valori di a_{30} aveva una maggior resa casearia del latte con bassi valori di a_{30}. Per quanto riguarda i fenotipici di singolo animale, l'ereditabilità stimata per RCT era ancora apprezzabile dopo l'aggiustamento per il genotipo composto β-e κ-caseina, suggerendo che la raccolta fenotipica di questo carattere non può essere sostituita dalla genotipizzazione e selezione per le varianti proteiche del latte. Tenendo conto dell'effetto dell'attitudine casearia sulla resa in formaggio, il peso di questa in un eventuale sub-indice di selezione per la produzione e caratteristiche di qualità del latte variava dal 2,1-8,2%. L'attuale indice di selezione per la Frisona italiana non sembra influenzare significativamente l'attitudine casearia. L'implementazione nei controlli funzionali dell'analisi dell'attitudine casearia attraverso la predizione con il medio infrarosso permetterebbe di raggiungere una più elevata risposta selettiva per l'attitudine casearia rispetto all'analisi diretta. Inoltre l'inserimento dell'attitudine casearia nell'indice selezione può portare all'aumento del rapporto caseina:proteina totale e alla selezione indiretta per la diminuzione delle cellule somatiche.

General Introduction

Dairy market

According to the Food and Agricultural Policy Research Institute (FAPRI, 2008), world cheese production is expected to grow 22.3 % over ten years, with the US and the EU accounting for over 64 %. The expected trends in milk and cheese production in Europe from 2010 to 2017 will be stationary for milk but +10.8 % for cheese production. Within the same period, the consumption of milk is expected to decrease (-0.3 %) whereas the consumption of cheese is expected to have a marked increase (+12 %).

Besides Italy is one of the country with the largest number of locally-made cheeses. Dairy sector accounts for 13 % of the food industry incomes and exerts a key role for the Italian food industry on an international level, involving more than 2,000 dairy implants and transformation facilities. Italy accounts 9 % of the European milk production (11 million tons, 94 % cow milk), but 15 % of the cheese production. At the present 70 % of available milk in Italy is used in the cheese manufacture, 50 % of which is used for PDO (Protected Designation of Origin) products. The internal consumption decreased to -1.4 % from 2005 to 2009 but export of Italian cheeses has a positive trend with an increase to +9.5 % for the same period (Pieri, 2010).

The quota system impose a limit of production and the amount of quota limit is around the 70 % of the self-supply of milk for Italian dairy industries. Nevertheless future market liberalization is expected in the UE: recent policy developments including reductions of intervention prices and an increase of quotas by 1% annually from 2009 to 2013 and consequently expiring of the quota system in 2015 (Kempen et al., 2011). In this situation the dairy industry is expected to maintain its economic importance for Italian agriculture sector and the production of milk suitable for cheese processing becoming more and more important for increase efficiency of dairy chain.

Milk coagulation properties

Milk coagulation properties (MCP) in general can be define as the feature of the milk to react with a clotting enzyme and form a curd with a suitable firmness in a reasonable time. Milk coagulation process and cheese-making enzymatic coagulation of milk is a process of three overlapping steps, which can be described with a diagram produced by a milk-coagulation meter

(Figure 1). During the primary, enzymatic phase (RCT in Figure 1), chymosin, which is the clotting enzyme usually extracted from calf abomasum, splits k-casein at the Phe_{105}-Met_{106} bond into para-k-casein and a macropeptide. Because of this splitting of k-casein, casein begins to aggregate. This second, non-enzymatic phase of milk coagulation begins before all of the k-casein has been split. During the third step of milk coagulation, aggregated casein micelles form a more or less firm gel structure. Curd-firming time, K_{20}, describes the time needed until the curd is firm enough to be cut (the width of the diagram (Figure 1) is 20 mm), and curd firmness, a_{30}, describes the firmness of the curd 30 min after addition of the clotting enzyme. These MCP are measured for 30 min or more, because in cheese-making for most cheese types, the curd is cut about 30 min after addition of the clotting enzyme to the milk (Ikonen, 2000).

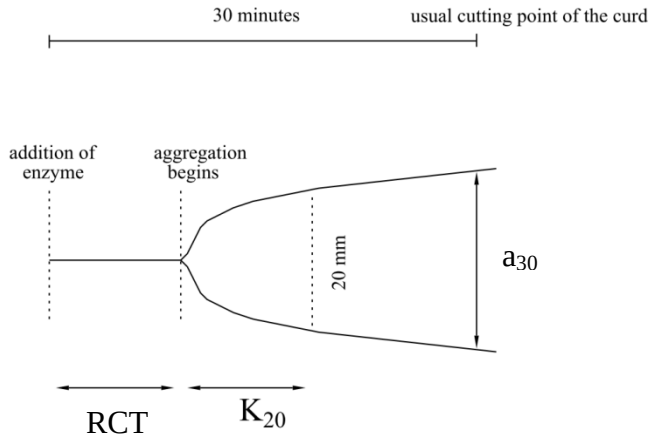

Figure 1: Diagram produced by a milk-coagulation meter, and the three milk coagulation property traits calculated from the diagram: RCT, rennet coagulation time; K_{20}, curd-firming time; a_{30} , curd firmness 30 min after addition of the clotting enzyme (Ikonen, 2000).

The MCP and composition of milk have a rather clear effect on cheese-making properties. Milk that begins to aggregate soon after addition of the enzyme, and forms a firm curd within a reasonable time is expected to produce higher dry-matter cheese yields than does milk with unfavourable coagulation properties (Ng-Kwai-Hang et al., 1989; Martin et al., 1997; Wedholm et al., 2006). This occurs because milk that coagulates quickly is able to entrap more casein and fat into the coagulum before it is cut than does slowly coagulating milk. Casein and fat constitute about 90% of the solids in cheese, so the amount of casein and fat lost in the cheese whey has a substantial effect on the efficiency of cheese-making (Johnson, 1988, Politis and Ng-Kwai-Hang

1988a, 1988b; Lawrence et al., 1993). Because the possibility to vary the cutting point is limited in commercial large scale cheese production, it is important that the curds are firm enough to allow cutting at the usual cutting time.

The MCP traits can be recorded by direct or indirect way. For the direct way exist alternative systems based on optical, thermal, mechanical, and vibrational methods, which have been comprehensively reviewed by O'Callaghan et al. (2002) and Lucey (2002). In addition to the measurement principle, the direct MCP analysis can be different because of the final coagulant activity, type of coagulant, temperature of analysis. Differences in analyses methodology probably exist since there are no standard methods for MCP analyses, in contrast to existing standard methods established for instance for the determination of total milk-clotting activity of bovine rennets (ISO/IDF, 2007). The direct way is expensive and time consuming and for this difficult to record in all the population. A feasible indirect way for the implementation of MCP assessment in the phenotypic recoding system has been proposed recently using mid-infrared spectroscopy (MIRS) (De Marchi et al., 2009; Cecchinato et al., 2009). This technology is based on the determination of a calibration equation predicted from spectral data and a reference methods.

Factors affecting MCP

A general worsening of MCP has been observed in several countries. For example although there are no published data on variation of MCP over the past decades in Finland, according to observations made in Finnish dairies, the average coagulation ability of milk has been deteriorating during the past 20 to 30 years, and the ratio of cows producing non-coagulating (NC) milk has increased (Ikonen et al., 1999). Unfavorable trends over years on MCP, at the phenotypic level, have been evidenced by some authors (Mariani et al., 1992; Cassandro and Marusi, 1999; Sandri et al., 2001) on milk yielded in dairy herds located in traditional areas for cheese production in Italy. Problem of non-coagulated samples has been reported around 10% in Italian Holstein-Friesian population (Cassandro et al., 2008) and a decrease on percentage of cheese wheels labelled as first quality has been reported for some Italian dairy products (Bittante et al., 2011).

Variations in milk composition are the major influencing factors in the rennet coagulation properties of milk. Strong influencing factors in the rennet coagulation properties of milk are: pH

(Okigbo et al., 1985; Hooydonk et al., 1986; Ostersen et al., 1997; Ikonen et al., 2004); calcium content (Ostersen et al., 1997); protein content, including the influence of caseins (Ostersen et al., 1997; Guinee et al., 2001; Auldist et al., 2002); and casein number of milk (Wedholm et al., 2006). Other influencing factors of milk coagulation properties that are related to composition and genetic factors of milk are age of animals (Schaar, 1984; Tyrisevä et al., 2003), stage of lactation (Okigbo et al., 1985; Davoli et al., 1990; Ostersen et al., 1997; Tyrisevä et al., 2004; Vallas et al., 2010), composition of feeding rations (Macheboeuf et al., 1993; O'Brien et al., 1999; Guinee et al., 2001), season (Okigbo et al., 1985; O'Brien et al., 1999), and breed (Grandison, 1986; Auldist et al., 2002; De Marchi et al., 2007).

Within breed MCP can be improved by genetic selection because of heritability and repeatability values are showed to be moderate. Heritability has been estimated with a range from 0.25 to 0.28 for RCT and from 0.15 to 0.41 for a_{30} (Ikonen et al., 2004; Cassandro et al., 2008; Vallas et al., 2010). Genetic variants of milk proteins have been shown to be associated with the protein composition and thereby with the technological properties of milk (Buchberger and Dovč, 2000). The best alleles for MCP traits is reported to be allele B for β-casein, B for k-casein and B for β-lactoglobulin (Bittante, 2011). Moreover Tyrisevä et al. (2008) found candidate genes for non-coagulation of milk. As a consequence, DNA information could be utilized to improve milk MCP trough marker assisted selection at early age both for cows and bulls.

Traits in a genetic improvement program

Animal breeding aims for the next generation of animals to produce more efficiently, under the future farm economic and social circumstances, than under the present generation. To ensure maximal benefit from genetic improvement, selection index should be for an appropriate breeding goal (Wolfová et al., 2007). Definition of a breeding goal is the first step in designing an animal breeding program for the exploitation and enhancement of genetic resources. The direction of the improvement is formalized in the breeding objective. Given a goal, the breeding objective can then be formally developed. This involves two somewhat discrete steps. First, identify the list of traits that influence the goal; second, the relative emphasis of each of the traits in the list (Lopez-Villalobos and Garrick, 2005). In the selection-index theory, the aggregate genotype is usually defined as a linear function of traits to be improved, each multiplied by its

economic value (Groen, 1989a; Dekkers, 1991). The economic value of a trait can be defined as the change in profit of the farm expressed per lactating cow per year, as a consequence of one unit of change in the genetic merit of the trait considered, keeping all other traits in the aggregate genotype constant (Hazel, 1943, Groen, 1989b). The economic values for each of the traits considered in the breeding objective are derived from a farm model that includes incomes of the animal products and all the costs associated with them.

The following step in designing a breeding program is determination of the selection criteria. It can be defined as those traits that can be measured easily and in cheap way on the animals and can be used as predictors of the traits include in the breeding objective.

During the past decades in Italian dairy cattle population the focus of milk production has been kg of milk protein, but total milk protein content is a poor indicator of MCP. In a study conducted by Ikonen et al. (2004), none of traits usually recorded in recording systems were strongly related with MCP traits. Neither protein nor casein content of milk was found to be suitable for implementing an indirect selection aimed to improve MCP. The genetic correlation between them was almost one, indicating that the protein content reflects the casein content well. Genetic correlations between MCP and protein and casein content of milk were, however, almost zero.

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AIMS

The general aim of this thesis was to gain further knowledge about improving of milk coagulation properties in Italian dairy industries. The coagulation properties of milk are of great importance because they influence cheese yield and quality. In many countries it has been found that as a result of cattle breeding there has been an increase in milk production, but the coagulation properties of milk have decreased, and the number of cows in the population producing non-coagulated milk has increased. Several aspects about measure, evaluate the effect on dairy industries and genetically improve of MCP have been focused through five scientific papers. Specific aims for each paper were:

- propose a method for the transformation of the values of MCP traits and a method to predict non-coagulation probability of milk samples, analyzed using different methodologies (Chapter 1);

- assess the influence of chemical and technological quality of vat milk on cheese yield, whey fat and whey protein content in a commercial Grana Padano dairy plant (Chapter 2);

- quantify the contribution of composite β- and κ-CN genotypes on additive genetic variance of rennet coagulation time, curd firmness, milk yield, and milk quality traits in Italian Holstein Friesian cows (Chapter 3);

- develop a method for calculating economic values of milk coagulation properties traits and estimate economic value for milk production, SCS and milk coagulation properties in Italian Holstein-Friesian dairy cattle for destination of milk into two cheese manufactures (Chapter 4);

- estimate the annual genetic response of milk yield, milk components, somatic cell score, milk coagulation properties and milk acidity in Italian Holstein Friesian under current selection index and alternative selection indexes and selection criteria (Chapter 5).

Chapter 1

Relationships between milk coagulation property traits analyzed with different methodologies

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ABSTRACT

Milk Coagulation Properties (MCP) analysis is performed using a wide range of methodologies in different countries and laboratories, using different instruments, coagulant activity in the milk and type of coagulant. This makes it difficult to compare results and data from different research. The aims of this study were to propose a method for the transformation of values of rennet coagulation time (RCT) and curd firmness (a_{30}) and to predict the non-coagulation (NC) probability of milk samples analyzed using different methodologies. Individual milk samples were collected during the morning milking in October 2010 from each of 165 Holstein Friesian dairy cows in two free-stall barns in Italy, and sent to three laboratories for MCP analysis. For each laboratory MCP analysis was performed using a different methodology: A - with a Computerized Renneting Meter instrument using 0.051 IMCU/mL of coagulant activity; B - with a Lattodinamografo using 0.051 IMCU/mL of coagulant activity; C - with an Optigraph using 0.120 IMCU/mL of coagulant activity. The relationships between MCP traits were analyzed with correlation and regression analyses for each pair of methodologies. For each MCP trait two regression models were applied: model 1 was a single regression model, where the dependent and independent variables were the same MCP trait determined by two different methodologies; in model 2 both a_{30} and RCT were included as independent variables. The NC probabilities for laboratories with the highest number of NC samples were predicted based on the RCT and a_{30} values measured in the laboratories with lower number of NC samples using logistic regression and receiver operating characteristic analysis. The percentages of NC samples were 4.2%, 11.5% and 0.6% for A, B and C, respectively. The transformation of MCP traits was more precise with model 1 for RCT (R^2 0.77-0.82) than for a_{30} (R^2 0.28-0.63). The application of model 2 was needed when the C measurements were transformed into the other scales. The analyses of NC probabilities of milk samples showed that NC samples from one methodology were well distinguishable (with an accuracy of 0.972-0.996) based on the rennet coagulation time measured with the other methodology. A standard definition for MCP traits analysis is needed to enable reliable comparisons between MCP traits recorded in different laboratories, and in different animal populations and breeds.

Key words: dairy cattle, milk coagulation property, different method, conversion method

INTRODUCTION

Milk Coagulation Properties (**MCP)** are considered to have an important role in cheese production mainly because of their relationships with cheese yield (Aleandri et al., 1989; Martin et al., 1997; Wedholm et al., 2006) and cheese quality (Ng-Kwai-Hang et al., 1989; Johnson et al., 2001). MCP have been widely studied in recent years and have been proposed as technological trait for increasing dairy industry efficiency (Ikonen et al., 2004; Jõudu, 2008a). The MCP have been found to have an exploitable additive genetic variation in dairy cattle population, and an estimated heritability range from 15 to 41% (Ikonen et al., 2004; Cassandro et al., 2008; Vallas et al., 2010). These studies showed that it is possible to improve MCP genetically. There are also proposals to include these traits in payment systems of milk used for cheese production (De Marchi et al., 2008; Pretto and Cassandro, 2010). Commonly, the main MCP traits studied are milk rennet coagulation time (**RCT**, min), which is the time from the addition of coagulant to milk until the beginning of coagulation, and curd firmness at 30 minutes after coagulant addition ($\mathbf{a_{30}}$, mm). These traits are recorded using alternative systems based on optical, thermal, mechanical, and vibrational methods, which have been comprehensively reviewed by O'Callaghan et al. (2002) and Lucey (2002). There are different instruments that are available commercially and currently widely used in research institutes in order to record MCP traits for genetic studies. The Computerized Renneting Meter (Polo Trade, Monselice, Italy) (**CRM**) was used for example in the work of Ikonen et al. (2004) and Cassandro et al. (2008); the Optigraph (Ysebaert, Frepillon, France) (**OPT**) was used in the work of Vallas et al. (2010) and the Lattodinamografo (Foss-Italia, Padova, Italy) (**LAT**), which replaced worldwide the now unavailable Formagraph (Foss Electric, Hillerød, Denmark) used for example by Ikonen et al. (1999), Jõudu (2008a) and Jõudu et al. (2008b). These instruments measured the same traits but with different principles. The principle of the CRM and the LAT is classified as a mechanical system or rheological method (O'Callaghan et al., 2002) and it is based on the recording of oscillation, which is driven by an electromagnetic field created by the swinging of a small, stainless steel, loop pendulum immersed in the samples of coagulating milk. A survey system measures differences in the electromagnetic field caused by milk coagulation: the greater the extent of coagulation, the smaller the swings of the pendulum. This analysis produces a diagram, as reported by Dal Zotto et al. (2008). On the other hand, measurements made with the OPT are not based on a rheological method but on an optical signal in the near-infrared wavelength. During a coagulation test, the light emitted through the milk gradually weakens, because of

changes in the micellar structure of casein. The OPT calculates the coagulation parameters (coagulation time, curd firmness, speed of aggregation) by means of particular feature points extracted from the optical information acquired in real time (Optigraph User's Manual). Because of the different scales used by the OPT, the value of curd firmness from the optical signal (volts) is transformed into values for a_{30} (mm) using a calibration equation (Kübarsepp et al., 2005a) to give comparable data with the same units. All these instruments use 10 mL of milk for each sample. In addition to the measurement principle, the MCP analysis can be different because of the final coagulant activity in the milk used to induce the coagulation of samples. The coagulant activity is expressed as International Milk Clotting Units (IMCU) per mL of milk. Since rennet is the key enzyme for the enzyme-induced coagulation process of milk, its activity in the milk can affect MCP as found in several studies which showed that RCT is linearly related with the inverse of the coagulant activity (Brown and Collinge, 1986; Karlsson et al., 2007). Coagulant activity in the milk for MCP analysis has a wide variability reported in the literature. It is in the range of 0.050-0.060 IMCU/mL of milk in Italian research (Zannoni and Annibaldi, 1981; Cassandro et al., 2008; Cecchinato et al., 2009), in the range of 0.110-0.150 IMCU/mL of milk in Estonian and Finnish studies (Ikonen et al., 2004; Kübarsepp et al., 2005b; Vallas et al., 2010) and in the range of 0.330-0.580 IMCU/mL in Swedish studies (Hallén et al., 2007; Hallén et al., 2010). In addition, the Swedish works differ from the previous examples in using defatted milk, and during the analysis the samples were kept at 30°C instead of at 35°C. Furthermore, different types of coagulant were used: calf rennet (Zannoni and Annibaldi, 1981; Cassandro et al., 2008; Cecchinato et al., 2009) or microbial coagulant (Kübarsepp et al., 2005a; Kübarsepp et al., 2005b; Vallas et al., 2010). Each country has used a different coagulant activity in the milk, according to their methodology, for MCP analysis. This could be related to the differences in the manufacturing processes and cheese types of national dairy industries. For instance, in the manufacturing process of some of the main Italian Protected Designation of Origin cheeses, such as Grana Padano, Asiago and Piave, milk coagulant activity in the range 0.035-0.045 IMCU/mL is usually used, while in the manufacturing processes of some of the main North European cheeses, such as Edam and Gouda, it is in the order of 0.080 IMCU/mL (Cheese Dairy Plant Managers: M. Dalla Riva, M. Centeleghe, G. Zambon, L. Maroso, and G. Toniolo for Italian cheeses, and U. Saks and T. Tupasela for North European Gouda and Edam cheeses, personal communication, 2010).

Differences in analyses methodology probably exist since there are no standard methods for MCP analyses, in contrast to existing standard methods established for instance for the

determination of total milk-clotting activity of bovine rennets (ISO/IDF, 2007). This situation makes it difficult to compare results from different research that uses different methodologies for the analysis of MCP traits in individual animal samples and bulk milk samples. This could be a technical problem for both the future international genetic evaluation for MCP traits and the application of milk payment systems.

Recently, mid-infrared spectroscopy (MIRS) technology has been proposed as a cheaper method to predict MCP routinely, and for large-scale recording (De Marchi et al., 2009). However, MIRS technology is based on the determination of a calibration equation predicted from spectral data and a reference methods and therefore, the existence of different methodologies for MCP analysis without a method for converting the data could cause further complication.

Some studies have found that comparison between different methodologies for MCP analysis is possible due to strong correlations between MCPs measured with these methodologies (Laporte et al., 1998; Kübarsepp et al., 2005a; Klandar et al. 2007). Nevertheless, a critical feature of MCP data is the presence of non-coagulated milk (**NC**) records, that is when milk does not coagulate at all within a standard 30 min testing time (Tyrisevä et al., 2008). Usually these samples are discarded from statistical analysis and, to our knowledge, no research has been done to compare the probability of NC milk samples from MCP analyses using different methods.

The objectives of this study were to propose 1) a method for the transformation of the values of MCP traits and 2) a method to predict non-coagulation probability of milk samples, analyzed using different methodologies.

MATERIALS AND METHODS

Milk Sample Collection

Individual milk samples (4 subsamples per cow) were collected during the morning milking of a test day in October 2010 from 165 Holstein Friesian dairy cows fed *at libitum* with TMR in two free-stall barns in Italy. The samples were processed according to International Committee for Animal Recording procedures (ICAR, 2009) and combined with preservative (Bronopol, Knoll Pharmaceuticals, Nottingham, UK). After collection, milk samples were stored in portable refrigerators (at 4°C) and transferred to the Milk Laboratory of the Veneto region breeders association (ARAV; Padova, Italy) (**Lab1**). For MCP analysis, one random

subsample pack was sent to the milk quality laboratory of Veneto Agricoltura Institute (Thiene, Italy) (**Lab2**); one random subsample pack was sent by international express shipping to the Laboratory of Milk Quality (Institute of Veterinary Medicine and Animal Sciences, Department of Nutrition and Animal Products Quality) of the Estonian University of Life Sciences (Tartu, Estonia) (**Lab3**); and the remaining two random subsample packs were kept in Lab1 for MCP analysis and the determination of milk fat and protein content (Milkoscan FT 6000, Foss Electric, Hillerød, Denmark), somatic cell count (Fossomatic 5000, Foss Electric, Hillerød, Denmark) and pH (pH-Burette 24, Crison, Barcelona, Spain). The temperature of the samples was maintained at 4°C throughout transport and storage. The subsample for Lab3 was sent in an insulated box with cooling bodies to maintain constant temperature and when it arrived, the temperature inside the box was checked to ensure that it was at 4°C. Because MCP analysis and shipping to the Estonian Lab3 were time-consuming, MCP analysis was performed in two parts: two days (50% of samples) and three days (50% of samples) after collection. The analyses of the 4 subsamples of a same cow were performed in the same day in all three laboratories. The sampled cows were at different stages of lactation (5-703 DIM) and parity (1-7), as shown in Table 1. Milk yield, milk composition and their variability were representative of Holstein Friesian performance in the Veneto region (Cassandro et al., 2008; A.I.A., 2009).

Table 1. Parity, days in milk, milk yield and milk composition of sampled cows (n = 165).

Trait	Mean	CV, %	Min	Max
Parity	2.5	57.7	1	7
Days in milk	181	85.8	5	703
Milk yield, kg day^{-1}	32.2	27.8	7.9	56.4
Fat, %	3.93	20.0	2.04	6.91
Protein, %	3.50	14.4	2.46	5.34
SCC[1], 10^3 cells mL^{-1}	200.9	179.6	8	2945
SCS[2], units	2.80	63.5	-0.64	7.88
pH	6.68	1.0	6.45	6.88

[1] SCC = somatic cell count.

[2] SCS = somatic cell score $[3 + \log_2 (SCC/10^5)]$.

Milk Coagulation Properties

As the aim of the study was to find the relationship of MCP data in field conditions, using routine recording by different methodologies, each laboratory used their own standard protocol for MCP analysis. The methodologies used in this work are suitable for every single

piece of equipment suggested by the producer and already tested in each laboratory over the years.

Three methodologies of analysis were indentified **A**, **B**, and **C**. In method A CRM and standard rennet were used (Hansen standard 160 IMCU/mL, with 80% chymosin and 20% pepsin, Pacovis Amrein AG, Bern, Switzerland) which was diluted in distilled water (1.6:100 vol/vol). In method B the same coagulant with the same dilution as in method A was used, and measurement was made using an LAT. In method C the instrument was the OPT and a microbial coagulant (Milase MRS 600 IMCU/mL, CSK Food Enrichment B.V., Netherlands) was diluted in distilled water (1:100 vol/vol). Fresh coagulant solution was prepared every 3 h. In each laboratory, milk samples for MCP were removed from the fridge 15 min before analysis and heated in a water bath to 35°C. Once 35 °C was reached, 200 μL of coagulant solution was added to 10 mL of milk and the analysis began within 15 s. According to these protocols, final coagulant activities in the milk were: 0.051 IMCU/mL, 0.051 IMCU/mL and 0.120 IMCU/mL, for the A, B and C methodologies, respectively.

The MCP was determined at 35°C and completed within 30 min after the addition of the coagulating enzyme to samples. An attempt to use uniform analysis protocol was made with 60 random samples where the coagulant solution was prepared in order to produce equal coagulant activity in the milk and using the same coagulant. These 60 samples were analyzed by CRM, LAT and OPT by using 0.051 IMCU/mL coagulant activity for all equipment and rennet Hansen standard 160 (methodology **C***).

Statistical Analysis

Two MCP traits were measured: RCT and a_{30}. The OPT signal for a_{30} (in volts) was transformed into values for a_{30} (mm) using the calibration equation proposed by Kübarsepp et al. (2005a). Samples that did not coagulate within 30 min were classified as non-coagulated (**NC**).

Only samples that coagulated (**CO**) with all methodologies were used in order to compare the mean values and variances of MCP traits, and to estimate the relationships between MCP traits. The proportions of NC samples, mean values and variances of MCP traits with different methodologies were compared with McNemar's test for matched pairs, the paired samples t-test and the F-test. The relationships between MCP traits were analyzed with correlation and regression analyses for each pair of methodologies. Two regression models were applied for each MCP trait: model 1 was a single regression model where the dependent and independent variables were the same MCP trait in two different methodologies, while in model 2 both a_{30}

and RCT were included as independent variables. Additionally, the effects of sample age, farm, parity of cows and DIM on the parameters of regression analysis were tested.

The NC probabilities for laboratories with the highest number of NC samples were predicted based on the RCT and a_{30} values measured in the laboratories with lower number of NC samples using logistic regression and receiver operating characteristic (**ROC**) analysis. The ROC analysis was used to find the optimal RCT and a_{30} values to discriminate the NC and CO samples. Moreover, the corresponding non-coagulation probabilities were estimated and the percentage of correctly classified NC samples (sensitivity), correctly classified CO samples (specificity) and the overall probability of concordance (area under the ROC curve, AUC) were calculated.

A 0.05 level of significance was used. All statistical analyses were performed using SAS software (version 9.2, SAS Institute Inc., Cary, NC) and figures were drawn from R software (version 2.10.1).

RESULTS AND DISCUSSION

Descriptive Statistics

The number and percentage of CO and NC samples, and descriptive statistics of MCP traits measured by different methodologies, are presented in Table 2. The number (and percentage) of NC samples were 7 (4.2%), 19 (11.5%) and 1 (0.6%) for the methodologies A, B and C, respectively. All NC samples with A did not coagulate at the same time as B, while the only NC sample with C coagulated both with A and B. This could be related to the different principles of analysis for OPT from the CRM and LAT instruments and, as will be discussed later, differences in the context of the different coagulant activity used and type of coagulant in the different methodologies. The percentages of NC samples in this study for A and B were comparable with those found in Cassandro et al. (2008) and Ikonen et al. (2004) who used CRM and found NC percentages of 9.7% and 13.2%, respectively. The C results were similar to Vallas et al. (2010) where, using the same equipment (OPT), 0.3% of samples did not coagulate within 30 minutes after the addition of coagulant.

The means of RCT measured on the CO samples differed significantly between the three methodologies (Table 2). On average, the RCT values measured with C were much lower than those measured with both A and B (8.0 min compared to 15.6 and 18.2 min). This large difference is probably due to the higher concentration of coagulant in C compared to both A

and B. The variation coefficients for RCT were 23%, 24% and 25% in A, B and C, respectively, and the standard deviation was significantly lower for the analyses of C compared to both A and B.

Table 2. Description of methods used for determination of MCP, number of non-coagulated samples and descriptive statistics for milk rennet coagulation time (RCT, min) and curd firmness (a_{30}, mm).

Methodology		A	B	C	C*[1]
Instrument		Computerized Renneting Meter	Lattodinamografo	Optigraph	Optigraph
Coagulant		Hansen standard 160[2]	Hansen standard 160[2]	Milase MRS 600[3]	Hansen standard 160[2]
Coagulant activity, IMCU mL^{-1} of milk		0.051	0.051	0.120	0.051
n[4]	CO	158	146	164	30
	NC	7 (4.2%)^{b}	19 (11.5%)^{a}	1 (0.6%)^{b}	30 (50.0%)
RCT[5], min	Mean	15.6^{b}	18.2^{a}	8.0^{c}	28.2
	SD	4.0^{a}	4.5^{a}	1.8^{b}	
a_{30}[5], mm	Mean	36.2^{a}	29.6^{b}	29.7^{b}	5.1
	SD	9.9^{b}	12.9^{a}	8.5^{b}	

[1] 60 random samples were analysed with this method.
[2] produced by Pacovis Amrein AG, Bern, Switzerland.
[3] produced by CSK Food Enrichment B.V.,Netherlands.
[4] CO = coagulated samples; NC = non-coagulated samples.
[5] Only coagulated samples with A, B and C methodologies (n = 145).
[a-c] Proportion of non-coagulated samples, means and SD within a row for A, B and C methodology with different superscripts differ ($P < 0.05$).

The means of a_{30} were similar for both B and C, 29.6 and 29.7 mm, respectively, while for A the mean of a_{30} was significantly higher, at 36.2 mm. The standard deviation of a_{30} was significantly higher for the analyses of B compared to both C and A.

In general, RCT and a_{30} values of the samples analyzed with A were similar to those reported by Cassandro et al. (2008) for Italian Holsteins, and for C to the values reported by Vallas et al. (2010) in the Estonian Holstein population.

These results confirm that the MCP measured with different methodologies, with different instruments and coagulant activity, may give considerably different values, and to use them in joint genetic evaluation some transformation into one common scale is needed.

Correlation and Regression Analysis

The RCT and a_{30} were strongly and negatively correlated in the analyses with A and B (r = -0.79 and r = -0.86, respectively; Table 3). The correlation between RCT and a_{30} measured with C was significantly lower but still negative (r = -0.23). These results are logical as the time for instrumental assessment of MCP through current milk coagulation meters is restricted to 30 min from the time of coagulant addition, and a later start of curd firmness leaves less time for the firmness process, which results in a weaker curd (Dal Zotto et al., 2008). Similar results have been presented in other studies (Kübarsepp et al., 2005b; Cassandro et al., 2008; Vallas et al., 2010).

Table 3. Pearson correlation coefficients for milk rennet coagulation time (RCT, min) and curd firmness (a_{30}, mm) measured with the three different methodologies (A, B and C subscripts) for the coagulated samples with all methodologies (n = 145; $P < 0.05$ for all correlations).

Trait	RCT_B	RCT_C	$a_{30\ A}$	$a_{30\ B}$	$a_{30\ C}$
RCT_A	0.906	0.902	-0.787	-0.735	-0.297
RCT_B		0.879	-0.742	-0.862	-0.375
RCT_C			-0.627	-0.683	-0.233
$a_{30\ A}$				0.793	0.533
$a_{30\ B}$					0.657

Strong positive linear relationships were found between RCT values measured with the different methodologies (correlation coefficients ranged from 0.88 to 0.91; Table 3). The a_{30} values measured with different methodologies are less related than the RCT. This is in agreement with less favorable values of analytical repeatability and reproducibility for a_{30} compare to RCT reported by Dal Zotto et al. (2008) for measures of MCP traits obtained using CRM. The strongest correlation was found between A and B measured a_{30} values (r = 0.79; Table 3), while the correlation of a_{30} measured with C and those measured with A and B were moderate (r = 0.53 and r = 0.66, respectively).

Figure 1 and Figure 2 present the results of regression analysis with model 1 for RCT and a_{30}, respectively. The high goodness of fit values of the regression between RCT data (R^2 ranged from 0.77 to 0.82) allows the reliable conversion of RCT values between laboratories and instruments. Additionally, including the a_{30} as an independent variable (model 2) did not

increase the R^2 value by more than 0.01-0.03. The transformation of a_{30} values between pairs of methodologies was most reliable between A and B ($R^2 = 0.63$; Figure 2). Applying the models with both a_{30} and RCT as independent variables (model 2) the reliability of predictions for conversion of the data into C using both a_{30} and RCT traits (model 2) increased by almost double ($R^2 = 0.55$ and $R^2 = 0.73$ the predicting of a_{30} measured with A and B, respectively). The transformation of curd firmness values in these cases should include both a_{30} and RCT data (equations not showed in figure: $a_{30\ A} = 44.74 + 0.473\ a_{30\ C} - 2.839\ RCT_{\ C}$; $a_{30\ B} = 37.09 + 0.797\ a_{30\ C} - 3.921\ RCT_{\ C}$).

Figure 1. Distribution, regression parameters and regression line (solid line) shown with 95% confidence interval (dashed lines) of milk rennet coagulation time (RCT, min) measured with 3 different methodologies (A, B, and C subscripts) for the coagulated samples with all methodologies (n = 145; $P < 0.05$ for all models). Methods: A: computerized renneting meter (Polo Trade, Monselice, Italy) and 0.051 IMCU/mL of milk of Hansen standard (160 IMCU/mL, with 80% chymosin and 20% pepsin; Pacovis Amrein AG, Bern, Switzerland); B: Lattodinamografo (Foss-Italia, Padova, Italy) and 0.051 IMCU/mL of milk of Hansen standard (160 IMCU/mL, with 80% chymosin and 20% pepsin; Pacovis Amrein AG); C: Optigraph (Ysebaert, Frépillon, France) and 0.120 IMCU/mL of milk of Milase de Man, Rogosa, and Sharpe (MRS; 600 IMCU/mL; CSK Food Enrichment B.V., Leeuwarden, the Netherlands). RMSE = root mean square error.

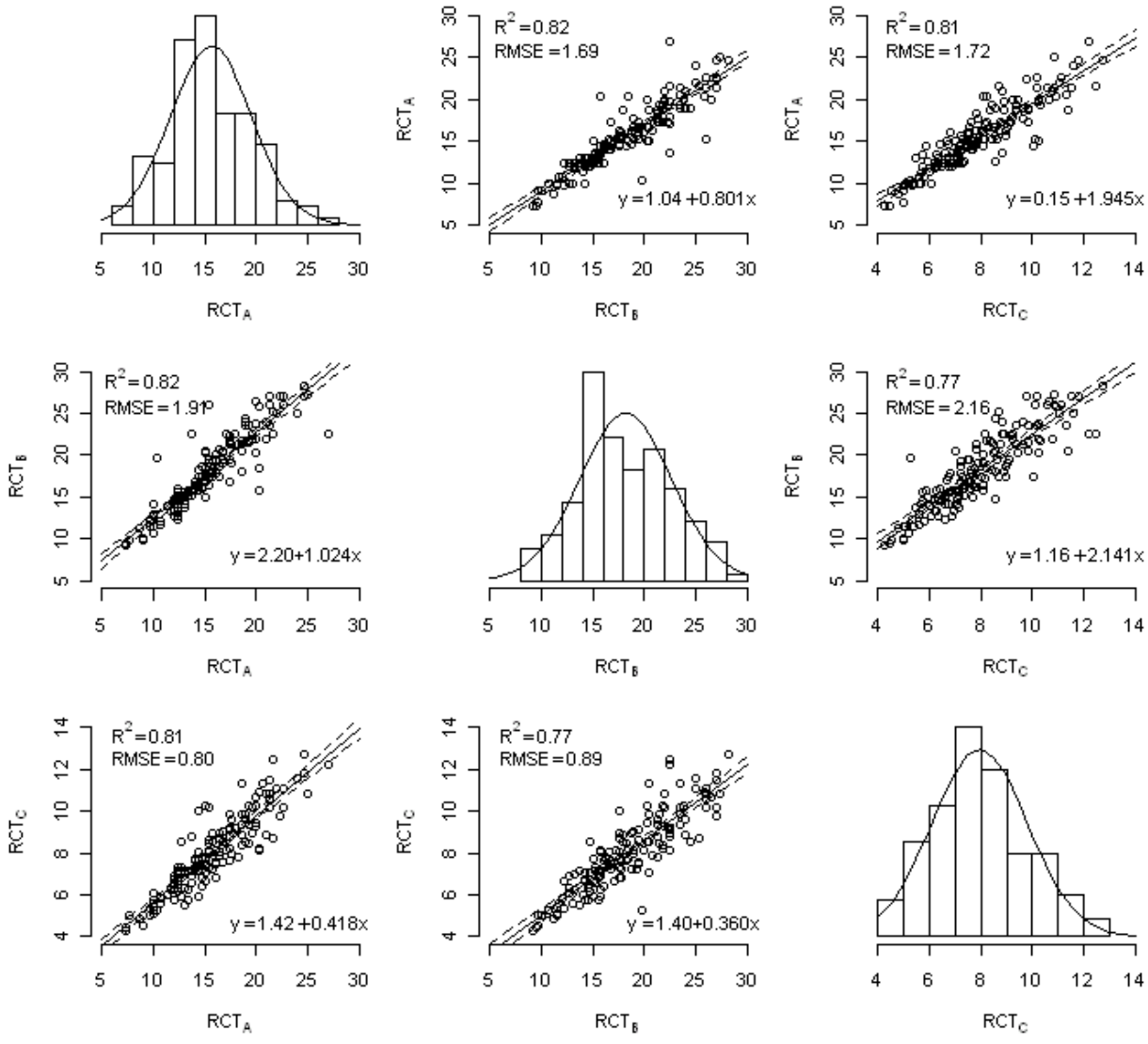
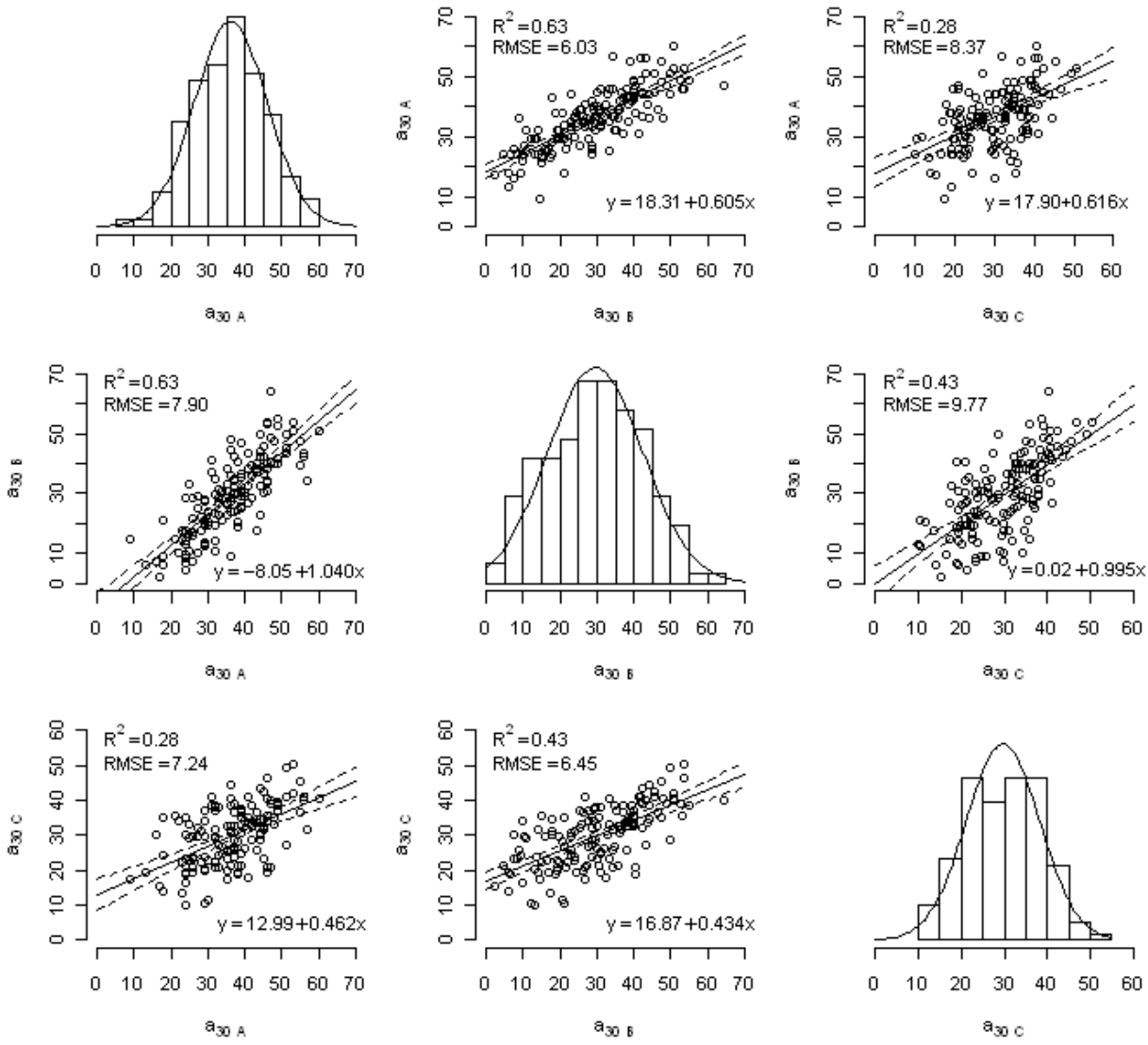


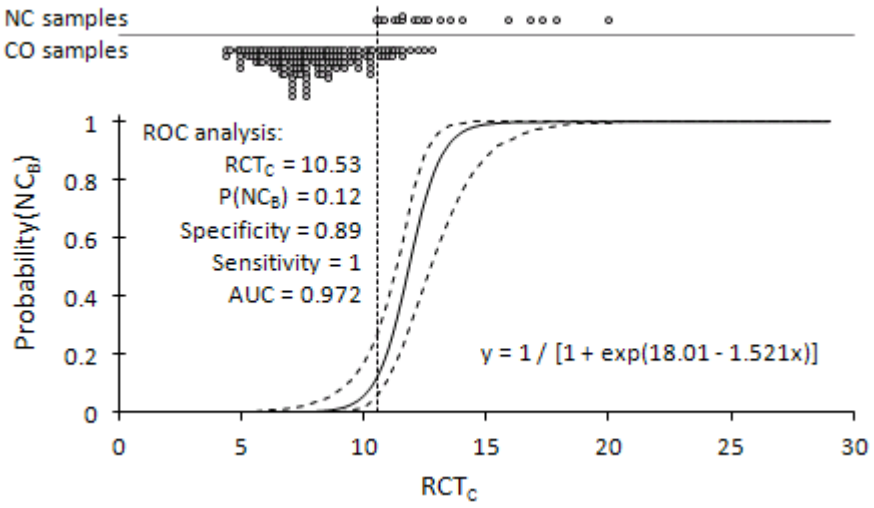
Figure 2. Distribution, regression parameters and regression line (solid line) shown with 95% confidence interval (dashed lines) of curd firmness (a 30 , min) measured with 3 different methodologies (A, B, and C subscripts) for the coagulated samples with all methodologies (n = 145; P < 0.05 for all models). Methods: A: computerized renneting meter (Polo Trade, Monselice, Italy) and 0.051 IMCU/mL of milk of Hansen standard (160 IMCU/mL, with 80% chymosin and 20% pepsin; Pacovis Amrein AG, Bern, Switzerland); B: Lattodinamografo (Foss-Italia, Padova, Italy) and 0.051 IMCU/mL of milk of Hansen standard (160 IMCU/mL, with 80% chymosin and 20% pepsin; Pacovis Amrein AG); C: Optigraph (Ysebaert, Frépillon, France) and 0.120 IMCU/mL of milk of Milase de Man, Rogosa, and Sharpe (MRS; 600 IMCU/mL; CSK Food Enrichment B.V., Leeuwarden, the Netherlands). RMSE = root mean square error.



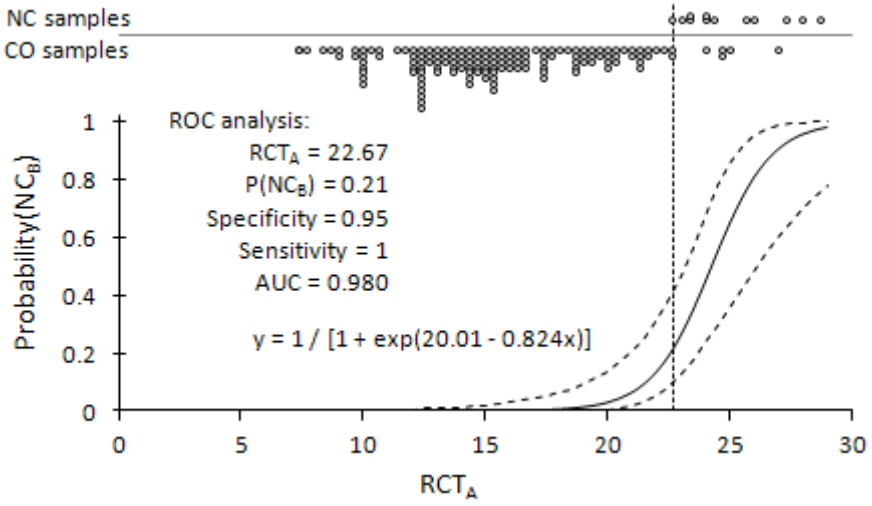
Prediction of Non-coagulation Probability

The logistic regression and ROC analysis results for RCT in three pair combinations of methodologies are presented in Figure 3. The probabilities of NC samples with B were highly predictable based on the milk rennet coagulation time measured in either C or A. Also, the probabilities of NC samples in A were highly predictable based on the milk rennet coagulation time measured in C. The optimal RCT values to distinguish NC and CO samples resulted in the probabilities of concordance with empirical data of between 0.972 and 0.996, whereby the NC samples were determined with a probability of 1.000 and coagulated samples with probabilities of 0.89-0.98 (Figure 3). Furthermore, the prediction accuracy did not increase by taking into account the curd firmness values. Predicting the NC probability based on the curd firmness only resulted in less accurate predictions.

Figure 3. Results of logistic regression analyses predicting (a) the probability of noncoagulated (NC) samples in method B when the analyses for milk rennet coagulation time (RCT, min) are performed by method C, (b) the probability of NC samples in method B when the analyses for RCT are performed by method A, and (c) the probability of NC samples in method A when the analyses for RCT are performed by method C. The distribution of NC and coagulated (CO) samples, and the logistic regression curve (solid line) with 95% confidence interval (dashed lines) are shown. The results of the receiver operating characteristic (ROC) analyses presented are the optimal RCT value to distinguish NC and CO samples (vertical dotted line), corresponding noncoagulation probability [p(NC)], specificity and sensitivity, and area under the ROC curve (AUC). Methods: A: computerized renneting meter (Polo Trade, Monselice, Italy) and 0.051 IMCU/mL of milk of Hansen standard (160 IMCU/mL, with 80% chymosin and 20% pepsin; Pacovis Amrein AG, Bern, Switzerland); B: Lattodinamografo (Foss-Italia, Padova, Italy) and 0.051 IMCU/mL of milk of Hansen standard (160 IMCU/mL, with 80% chymosin and 20% pepsin; Pacovis Amrein AG); C: Optigraph (Ysebaert, Frépillon, France) and 0.120 IMCU/mL of milk of Milase de Man, Rogosa, and Sharpe (MRS; 600 IMCU/mL; CSK Food Enrichment B.V., Leeuwarden, the Netherlands).



a)



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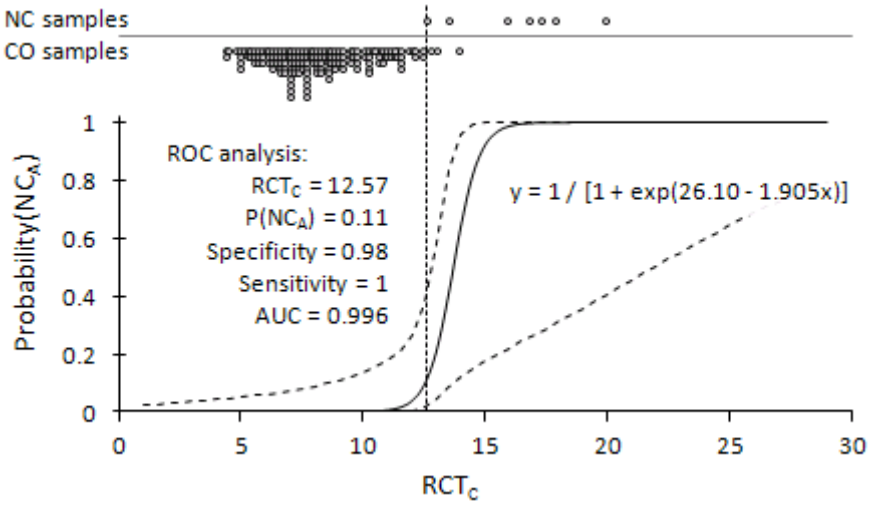

c)

Uniform Coagulant Activity

Additionally analyses were made with 60 randomly chosen samples (from 165 initial samples) using OPT with the same coagulant activity and the same coagulant as was used in the A and B methodologies (Hansen standard 160, 0.051 IMCU/mL) (**C*;** Table 2). The milk yield and composition values of the 60 selected samples did not differ significantly from the corresponding values of the whole dataset. As a result we recorded 30 NC samples; the percentage of NC samples with C* increased from an initial 0.6% in C to 50% (Table 2). An explanation for this increase of NC could be that coagulant activity and/or type of coagulant (calf rennet vs microbial coagulant) used in A and B are unsuitable for the Optigraph instrument.

The random sample contained six NC samples from B and five NC samples from the A analyses, and of all these samples none also coagulated with C*.

The average RCT of CO samples for C* was 21.0 min, which is significantly higher compared with the initial RCT measured with C (8.0 min). The average a_{30} of the CO samples was 5.9 mm, which is significantly lower compared with the initial a_{30} measured with C (29.7 mm).

Furthermore we tried to distinguish the CO and NC samples of C* based on the MCP of the same samples measured with either A or B. The ROC analyses showed that more than 95% of the samples were correctly classifiable based on the RCT, and more than 82% based on the a_{30}. The probability of non-coagulated samples seems to be quite well predictable irrespective of the instrument used, especially based on the RCT.

Possible Causes of Difference for MCP Traits

There are certainly three main reasons that could cause a difference in the values of the MCP traits: the first is the different equipment used in each laboratory; the second the difference in coagulant activity in the milk to induce coagulation and the third the type of coagulant used.

In this study it was difficult to distinguish the impact of instrument and coagulant effects on the MCP for all the methodologies. The instrument effect is clear in the A (CRM) and B (LAT) comparison since, in these analyses, the same coagulant activity and type of coagulant was used. The CRM had different sensitivity compared to the LAT, since in the CRM analyses the number of NC samples was smaller, the milk rennet coagulation time was shorter and the curd firmness was thicker. Nevertheless, the working principles of the CRM and the LAT are similar enough to allow reliable transformation of MCP onto one scale. The differences in the OPT working principles, in comparison with the two other equipment investigated, could be the cause for the weak correlation of C with the other two methodologies, and a low prediction ability in the conversion of a_{30} in this methodology. Related to this, the type of coagulant used could also affect the comparison among methodologies. For instance Jacob et al. (2010) found that a lower concentration of calf rennet was necessary compared to that of a microbial coagulant to ensure a specified curd cutting time. Even in the case of a coagulant effect there should be a strong relationship between the same MCP traits with different instruments, assuming that the instruments measure the same aspects of the coagulation process. The RCT indeed showed a strong relationship between measurements from different laboratories, even when different methodologies were used. On the contrary, the moderate correlations between a_{30} measured with LAT or CRM and OPT are

probably due to the different principles of the instruments, since the LAT and the CRM are based on the direct measurement of viscoelasticity, whereas the OPT measures are indirect and based on the optical signal. O'Callaghan et al. (2002) reported that while the characteristic of optical changes accompany coagulation, the correlation between optical signal and curd tension is to some extent confounded by the rate of reaction, indicating that changes in optical properties are not exclusively related to curd firming. This was also found by Vallas et al. (2010) where genetic and phenotypic correlations between milk coagulation time and curd firmness measured by OPT were different from those reported in previous studies using other equipment, indicating that coagulation aspects measured by OPT, compared to mechanical systems, may be different.

It is supposed from the results of samples analyzed with uniform coagulant activity (methodology C*) that, by decreasing the coagulant activity in the OPT analyses, it should be possible to achieve the same RCT as with A or B, but concurrently the a_{30} values will decrease considerably. Moreover, the relationships between MCP measured with C* and other methodologies decreased compared with C, especially for a_{30}. From these analyses it can be concluded that more coagulant is needed for the OPT than the LAT and the CRM, since a higher activity of coagulant in the milk is needed to cause differences in the optical signal. In addition, higher coagulant activity improves the coagulation process, and the variability of the MCP decreases. This can be deduced from the smaller variability of the MCP, and the smaller number of NC samples in the initial OPT analyses where higher coagulant activity was used.

CONCLUSIONS

The method proposed provides the opportunity to convert MCP data obtained by different methodologies into comparable datasets across different methodologies. The results of this study have shown that MCP traits analyzed with different methodology have significantly different values due to the diversity of the instruments and the coagulant activity. The type of coagulant could have a further effect, since two different coagulants were used, calf rennet that contain two milk-clotting enzyme and one of microbial origin which contained a single milk-clotting enzyme; but more investigations are need to clarify this effect. The transformation of the other methodologies is more precise for RCT (R^2 0.77-0.82) than for a_{30} (R^2 0.28-0.63). The a_{30} was transformable, with moderate accuracy, between A and B while

the C measurements could be transformed into the other scales with a moderate accuracy only when both curd firmness and rennet coagulation time were included in the model. The method proposed for harmonization of non-coagulation probability of milk samples showed that NC samples from one methodology were highly predictable based on the rennet coagulation time measured with another methodology.

This work pointed out the problem that a standard definition for MCP traits analysis is needed to enable reliable comparisons between MCP traits recorded in different laboratories, and in different animal populations and breeds.

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Chapter 2

Effect of milk composition and coagulation traits on Grana Padano cheese yield under field conditions

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ABSTRACT

The aim of this study was to assess the effect of chemical composition, coagulation properties, and acidity (pH and titratable acidity) of vat milk on cheese yield (CY) and whey content in Grana Padano under field conditions. Milk coagulation properties (MCP) have been suggested as a tool for monitoring cheese making efficiency. Twelve cheese-making sessions were carried out from February to December 2009 in a dairy cooperative of Grana Padano consortium, for a total of 96 vats of milk processed. For each vat, samples of raw milk and whey were collected. Whey was analysed for fat (WF) and protein (WP) content, whereas CY was expressed as kilograms of product per 100 kg of milk transformed, and was measured after 2 days of drainage. Fat, protein, and casein contents were positively and strongly correlated with CY (coefficients of correlation, r = 0.72, 0.88, and 0.84, respectively), whereas somatic cell score (SCS) was negatively and moderately correlated with CY (r = -0.59). Coagulation properties were significantly related to CY, WF, and WP: milk that coagulated earlier and had stronger curd firmness after 30 min from coagulant addition (a_{30}, mm) was associated to higher CY and WP, but lower WF. Cheese yield was analyzed with a model that accounted for fixed effects of cheese-making day, fat and protein content, titratable acidity, and a_{30}. Significance was found for all the effects. Milk characterized by high values of a_{30} resulted in higher CY than milk with low values of a_{30}. Findings from our study indicate that MCP could be used as indicators of cheese making efficiency and proposed as traits to be included in multiple component milk pricing systems.

Keywords: cheese yield, Grana Padano, milk coagulation property, whey loss

INTRODUCTION

Cheese yield (**CY**) is an important indicator of profit for the dairy industry, as it reflects the amount of cheese obtained from a given amount of milk (Marziali & Ng-Kwai-Hang, 1986; Lucey & Kelly, 1994; De Marchi *et al.* 2008). Improving CY is a main objective in countries where milk is predominantly processed into cheese. This is the case of Italy that uses 70% of available milk to manufacture typical cheeses, in particular Protected Designation of Origin (**PDO**) products (European Commission, 2006). The most popular PDO cheese in Italy, with 158,326 ton produced and 16% of total milk processed, is the Grana Padano (Pieri, 2010). It is a hard-textured, cooked, and long ripened cheese (at least 9 and even beyond 20 months of ripening, depending on product category) obtained from partially skimmed raw milk produced in a restricted area of North Italy (Battistotti & Corradini, 1993; European Commission, 2009).

Besides the cheese-making conditions such as the type of coagulant, cheese vat and milk treatments, CY is determined by the protein (or casein) and fat content of milk (Lucey & Kelly, 1994), and the importance of these constituents is demonstrated by their use to predict CY as reviewed by Emmons *et al.* (1990). Other milk quality parameters such as acidity, somatic cell count (**SCC**) and milk coagulation properties (**MCP**) may have a quantitatively marginal but significant effect on CY (Verdier-Metz *et al.* 2001).

Milk coagulation properties have been widely studied in recent years and their potential role as technological traits to improve the efficiency of dairy industry has been discussed (Ikonen *et al.* 2004; Cassandro *et al.* 2008; Jõudu, 2008). Milk that coagulates rapidly and has high curd firmness (**a_{30}**, mm) is expected to result in more cheese with more desirable composition and to lose less milk components in the whey compared with milk that coagulates late and has low a_{30} (Aleandri *et al.* 1989; Ng-Kwai-Hang *et al.* 1989; Lucey & Kelly, 1994; Martin *et al.* 1997; Wedholm *et al.* 2006).

Recently, there are proposals to include MCP traits predicted by mid-infrared spectroscopy (De Marchi *et al.* 2009) in payment systems of milk destined to cheese production (De Marchi *et al.* 2008; Pretto & Cassandro, 2010). Also, MCP are heritable traits (Cassandro *et al.* 2008; Vallas *et al.* 2010) and thus there is opportunity to genetically improve them through selection.

To date, most trials have been conducted in laboratory or pilot-scale plants using milk from individual cows (Ng-Kwai-Hang *et al.* 1989; Wedholm *et al.* 2006) or little amount of bulk milk (Grandison & Ford, 1986; Martin *et al.* 1997; Verdier-Metz *et al.* 2001). Only few

studies dealt with milk collected in several herds and processed in commercial dairy plants (Aleandri *et al.* 1989; Ikonen *et al.* 1999), and none was conducted on Grana Padano cheese. Carrying out a trial in field conditions may better reflect the true variation of MCP of bulk milk supplied to commercial dairy factories and better evaluate the economic impact of this variation.Therefore, the aim of this study was to assess the influence of chemical and technological quality of vat milk on CY, whey fat (**WF**) and whey protein (**WP**) content in a commercial Grana Padano dairy plant.

MATERIALS AND METHODS

Cheese-making

Twelve cheese-making days (8 vats for each day) were carried out in 2009 in one dairy cooperative of North Italy producing Grana Padano cheese; 4 sessions took place in February, 4 in July and 4 in December. The production of Grana Padano cheese followed the official protocol approved by the Consortium (European Commission, 2009; Grana Padano Consortium, 2011). The day before cheese-making the milk from morning and evening milkings was collected from farms and delivered to the dairy factory. Milk was stored at 8°C in shallow settling tanks for 12 h so that the fat could naturally rise to the surface (natural creaming). Following fat separation, the partially skimmed raw milk was drained from the bottom of the settling tanks and transferred to the copper bell-shaped vats that contain 1,000 kg of milk.

Before the beginning of cheese-making, a sample of milk was collected directly from the vat, stored in portable refrigerator (4°C), transferred to the milk quality laboratory of Veneto Agricoltura (Thiene, Italy), and analyzed within 6 h for fat, protein, and casein contents, SCC, pH, titratable acidity (**TA**), and MCP.

Cheese-making started with the addition of the natural whey starter (32 L per vat) obtained from the spontaneous acidification of whey from previous day's cheese-making. Milk was heated to 33°C and 56 g of powder calf rennet (strength, defined as the number of volumes of milk clotted by 1 volume of rennet in 40 min at 35°C, was 1:100,000; 95% chymosin and 5% pepsin) per vat were added. The coagulation process was manually checked and the curd was broken up into small granules after 10 min from the rennet addition. The curd was then cooked by increasing the temperature from 33 to 53.5°C in 10 min and it was agitated continuously. The broken-up curds aggregated and blended together during the so-called resting period, and deposited at the bottom of the vat. During this step the temperature was

maintained at 53.5°C and the process lasted 45 min. A sample of whey was then collected from each vat, stored in portable refrigerator (4°C), transferred to the milk quality laboratory of Veneto Agricoltura (Thiene, Italy), and analyzed for WF and WP.

The cheese mass was cut in two wheels with a specific tool, removed from the vat and placed in Grana Padano cheese moulds. The wheels were weighted (precision: ± 0.01 kg) after 2 d of drainage and placed into brine. Cheese yield was expressed as kilograms of product per 100 kg of milk processed. A total of 192 wheels was produced and 96 records for CY were available.

Laboratory analyses

Fat, protein, and casein contents, and SCC (Combi Foss 6000 FC, Foss Electric A/S, Hillerød, Denmark), pH, and TA expressed in Soxhlet-Henkel degrees [°SH 50 = mL of NaOH (0.25 mol/1,000 mL)/ 50 mL of milk; Crison Compact D, Crison Instruments SA, Alella, Spain] of herd bulk and vat milk samples were determined at the milk quality laboratory of Veneto Agricoltura (Thiene, Italy). Values of SCC were log-transformed to SCS [SCS = 3 + $\log_2$(SCC/100,000)], and casein number was calculated as the ratio of casein to protein content. Fat and protein contents of whey were also determined (Combi Foss 6000 FC, Foss Electric A/S, Hillerød, Denmark).

Measures of MCP of vat milk samples were obtained from the same laboratory by using a computerized renneting meter (CRM, Polo Trade, Monselice, Italy) following Dal Zotto *et al.* (2008).

Statistical analysis

Sources of variation of CY, WF, and WP were investigated using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Several fixed effects models were tested and the final one was chosen on the basis of the significance of the factors included and the amount of variability explained by each of them:

$$y_{ijklmn} = \mu + CMD_i + FAT_j + PROT_k + TA_l + CF_m + e_{ijklmn},$$

where y_{ijklmn} is the observed trait (CY, WF or WP); μ is the overall intercept of the model; CMD_i is the fixed effect of the *i*th cheese-making day (i = 1 to 12); FAT_j is the fixed effect of the *j*th class of milk fat content (j = 1 to 3); $PROT_k$ is the fixed effect of the *k*th class of milk protein content (k = 1 to 3); TA_l is the fixed effect of the *l*th class of milk titratable acidity (l = 1 to 3); CF_m is the fixed effect of the *m*th class of curd firmness after 30 min from coagulant addition (m = 1 to 3); and e_{ijklmn} is the random residual N ~ (0, σ^2_e). Except for cheese-making

day, classes of explanatory variables were defined according to mean ± 0.5 SD so that class 1: ≤ -0.5 SD, class 2: from -0.5 SD to 0.5 SD, and class 3: ≥ 0.5 SD.

A multiple comparison of means was performed for the class effects of milk fat and protein content, titratable acidity, and curd firmness using Bonferroni's test ($P < 0.05$).

RESULTS AND DISCUSSION

Protein and casein contents averaged 3.49 and 2.67%, respectively, in agreement with findings from Cassandro *et al.* (2008), whereas the value for SCS (2.38 units) was lower (Table 1). Fat content (2.55%) after the process of natural creaming was optimal to produce Grana Padano cheese, as the fat to casein ratio should be comprised between 0.80 and 1.05 (Battistotti and Corradini, 1993; European Commission, 2009).

Table 1. Descriptive statistics of vat milk quality traits, cheese yield (CY), whey fat (WF), whey protein (WP) and Pearson correlations (r) between vat milk quality traits and CY, WF and WP.

Trait[1]	n	Mean	CV, %	Minimum	Maximum	r		
						CY	WF	WP
Fat, %	96	2.55	6.0	2.28	2.87	0.72***	0.01	0.03
Protein, %	96	3.49	4.0	3.13	3.72	0.88***	-0.47***	0.48***
Casein, %	96	2.67	4.8	2.33	2.92	0.84***	-0.42***	0.29**
Casein number	96	0.765	1.8	0.729	0.787	0.26*	-0.06	-0.27**
SCC, n/mL	96	71,063	39.4	20,000	140,000	-0.58***	0.66***	-0.25*
SCS, units	96	2.38	26.1	0.68	3.49	-0.59***	0.67***	-0.27**
RCT, min	96	19.27	9.8	15.17	23.67	-0.45***	0.38***	-0.25*
k_{20}, min	83	7.88	26.2	4.20	18.40	-0.49***	0.50***	-0.12
a_{30}, mm	96	25.73	19.1	15.00	36.00	0.51***	-0.54***	0.40***
TA, SH°/50 mL	96	3.31	6.9	2.35	3.71	0.58***	-0.31**	0.28**
pH	96	6.57	1.0	6.45	6.73	-0.06	-0.06	0.58***
CY, %	96	8.59	5.1	7.70	9.40			
WF, %	96	0.56	17.4	0.34	0.80			
WP, %	96	0.94	8.4	0.76	1.08			

[1]SCC = somatic cell count; SCS = somatic cell score; RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness after 30 min from coagulant addition; TA = titratable acidity; WS = fat plus protein content in the whey; CY = cheese yield after 2 d of drainage.

†$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Rennet coagulation time and a_{30} averaged 19.27 min and 25.73 mm, respectively; overall, these values are worse than those previously suggested as optimal for cheese-making (Zannoni and Annibaldi, 1981; De Marchi *et al.*, 2007; Cassandro *et al.*, 2008), and confirm the worsening of technological properties of milk in the last years (Formaggioni *et al.*, 2005). The coefficient of variation of quality traits were much lower than those reported earlier (Wedholm *et al.*, 2006; Cassandro *et al.*, 2008), mainly because we used vat samples whereas in the previous studies samples were from individual cows.

Cheese yield after 2 d of drainage averaged 8.59% and showed relatively low variation (CV = 5.1%; Table 1). The mean value from our work is consistent with those reported for Grana Padano, Grana Trentino, and Parmigiano Reggiano cheeses (Malacarne *et al.*, 2006; Mordenti *et al.*, 2007; De Marchi *et al.*, 2008), but the variability is wider.

As expected, fat, protein, and casein contents were positively and strongly correlated with CY (r = 0.72, 0.88, and 0.84, respectively; Table 1), while correlations between SCS and CY was moderate and negative (r = -0.59). Rennet coagulation time and curd-firming time (**k_{20}**, min), defined as the minutes required to achieve 20 mm of firmness, were negatively associated to CY (r = -0.45 and -0.49, respectively), whereas a_{30} was positively correlated (r = 0.51). These estimates are in agreement with previous studies; milk with shorter RCT, faster k_{20}, and higher a_{30} results in higher CY (Aleandri *et al.*, 1989; Ng-Kwai-Hang *et al.*, 1989; Martin *et al.*, 1997). In the present work, all samples coagulated within 30 min and exhibited a value of a_{30}, but approximately 14% of samples did not have information on k_{20}. Titratable acidity was positively correlated with CY (r = 0.58), whereas no association was found between pH and CY.

Lower values of WF were associated with better MCP (r = 0.38 and –0.54 with RCT and a_{30}, respectively): milk that coagulates early and has high a_{30} incorporates the maximum amount of fat into cheese curd (Ng-Kwai-Hang *et al.*, 1989; Formaggioni *et al.*, 2005). However, the relationship between WP and MCP was opposite (r = -0.25 with RCT and 0.40 with a_{30}, respectively).

Results from ANOVA of CY are reported in Table 2. All effects included in the model were significant in explaining the variability of the trait ($P < 0.05$). Cheese-making day accounted for the largest proportion of variation followed by fat and protein contents; this was expected as seasonal variation of milk composition exists. Also, the Grana Padano production is not totally standardized and some steps of the process are still artisanal. Although MCP traits are highly correlated to each other (Ikonen *et al.*, 2004; Cassandro *et al.*, 2008; Pretto *et al.*, 2011), only a_{30} resulted significant in explaining the variability of CY.

Table 2. Results from ANOVA for cheese yield (R^2 = 0.905, RMSE = 0.153), whey fat (R^2 = 0.690, RMSE = 0.061) and whey protein (R^2 = 0.973, RMSE = 0.015).

		Cheese yield		Whey fat		Whey protein	
Effect[1]	df	SS	*P-value*	SS	*P-value*	SS	*P-value*
Cheese-making day	11	1.246	<0.0001	0.257	<0.0001	0.356	<0.0001
Milk fat, %	2	0.525	<0.0001	0.054	0.0012	0.007	<0.0001
Milk protein, %	2	0.446	0.0002	0.021	0.0632	0.003	0.0006
TA, SH°/50 mL	2	0.383	0.0006	0.003	0.6713	0.001	0.1617
a_{30}, mm	2	0.175	0.0277	0.008	0.3381	0.000	0.5791
Residual	76	1.770		0.279		0.016	

[1]TA = titratable acidity; a_{30} = curd firmness after 30 min from coagulant addition.

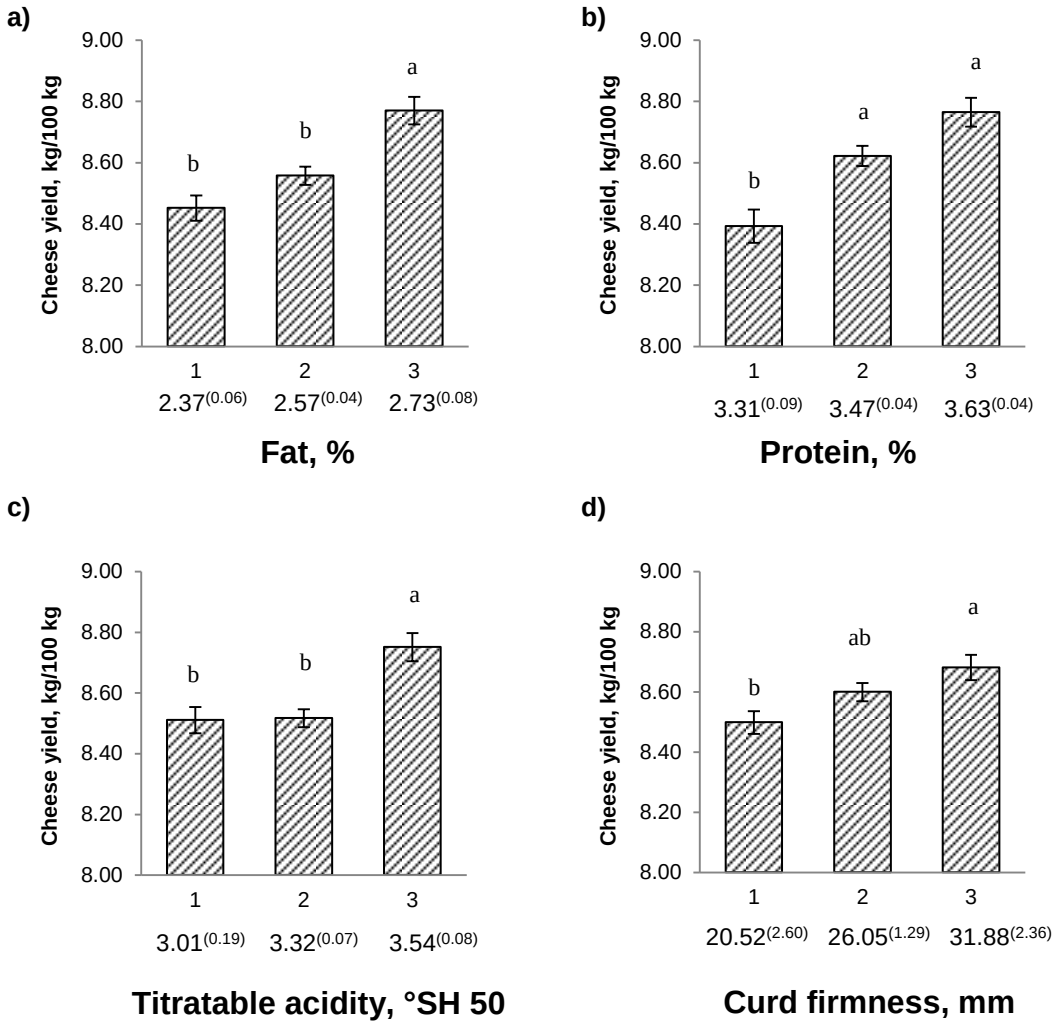
Most milk components and quality traits are related to each other; for example, high correlations are often reported between protein or casein and fat contents, or between MCP traits and milk acidity (Ikonen *et al.*, 2004; Cassandro *et al.*, 2008). Hence, in some cases it is not possible to determine whether a correlation is causal or arises by association with other traits.

Least squares means of CY across classes of fat and protein contents, TA, and a_{30} are depicted in Figure 1. Class 1 was always significantly lower than class 3. This is true for fat and protein contents, and TA, and it is particularly interesting in the case of a_{30}: results showed that the class of a_{30} averaging 31.88 mm resulted in 2.12% higher CY than the class averaging 20.52 mm.

Auldist *et al.* (1996) showed that somatic cells had deleterious effects on the yield and quality of cheese. This was not the case of our study as in a preliminary analysis, we did not detect significant effects of somatic cells on CY and thus this factor was not included in the final model; reasons for the lack of significance may be probably related to the low level of SCC in the vat milk (mean of 71,063 cells/mL; Table 1). As reported by Lucey and Kelly (1994), the decrease of CY seems to occur when SCC in milk is larger than 100,000 cells/mL.

Concerning WF and WP, results from ANOVA showed that, besides cheese-making day and milk fat, milk protein were significant ($P < 0.05$) in explaining the variability of WP (Table 2), whereas a30 and TA did not affect significantly whey content. Previous studies reported stronger association between MCP traits and losses of fat and protein in the whey (Ng-Kwai-Hang *et al.*, 1989; Formaggioni *et al.*, 2005). The weaker relationship between MCP and milk contents in the whey of our study may be the consequence of conducting the trial under field conditions and using bulk milk, which exhibits small variation of milk quality parameters.

Figure 1. Least square means (with SE) of cheese yield after 2 d of drainage across classes of a) milk fat content, b) milk protein content, c) titratable acidity, and d) curd firmness after 30 minutes from coagulant addition. For each class the mean and SD are given. Levels with different letters are significantly different (P < 0.05, Bonferroni's test).



CONCLUSIONS

Findings from the present study demonstrated that a_{30} is associated to CY in the Grana Padano dairy industry. As expected, fat and protein contents are the key drivers of CY, followed by TA. Milk coagulation properties have a positive impact on CY and could be used as indicators of cheese making efficiency in the dairy industry. The possibility to routinely record MCP traits using mid-infrared spectroscopy, as recently demonstrated, allows the genetic improvement of technological aspects, the practical use in cheese making process and the inclusion in the milk payment systems.

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Chapter 3

Short Communication: Influence of composite casein genotypes on additive genetic variation of milk production traits and coagulation properties in Holstein Friesian cows

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ABSTRACT

The aim of the study was to quantify the effects of composite *β*- and *κ*-casein (CN) genotypes on genetic variation of milk coagulation properties (MCP), milk yield (kg/d), fat, protein and CN contents (%), somatic cell score (SCS), pH, and titratable acidity (TA, SH°/50 mL) in 1,042 Italian Holstein Friesian cows. Milk coagulation properties were defined as rennet coagulation time (RCT, min) and curd firmness (a_{30}, mm). Variance components were estimated using two animal models: Model 1 included herd, days in milk, and parity as fixed effects, and animal and residual as random; Model 2 was Model 1 with the addition of composite *β*- and *κ*-CN genotype as a fixed effect. Genetic correlations between MCP, and between them and milk production traits were obtained with bi-variate analyses, based on the same models. The inclusion of casein genotypes led to a decrease of 47, 68, 18, and 23% in the genetic variance for RCT, a_{30}, pH, and TA, respectively, and less than 6% for other traits. Heritability of RCT and a_{30} decreased from 0.248 to 0.143, and from 0.123 to 0.043, respectively. A moderate reduction was found for pH and TA, while negligible changes were detected for other milk traits. Estimates of genetic correlations were comparable between the two models. Results show that composite *β*- and *κ*-CN genotypes are important for RCT and a_{30}, but can not replace the recording of MCP themselves.

Key words: additive variance, casein genotype, coagulation properties, Holstein breed

INTRODUCTION

The improvement of milk coagulation properties (**MCP**) is a hot topic in dairy cattle genetics, mainly because the amount of milk used for manufacturing cheese is growing worldwide (Schmit and Kaiser, 2006) and several studies have confirmed the role of MCP in cheese-making (Aleandri et al., 1989; Wedholm et al., 2006; De Marchi et al., 2008). Exploitable additive genetic variation exists for coagulation ability of milk, and heritability estimates range from 15 to 40% (Ikonen et al., 1999a; Ikonen et al., 2004; Cassandro et al., 2008). Recently, De Marchi et al. (2009) proposed the use of mid-infrared spectroscopy to routinely record MCP on individual milk samples, and Cecchinato et al. (2009) suggested that the application of this technique on a large-scale for the genetic improvement of MCP is feasible.

Casein polymorphisms have been extensively investigated in the past along with their associations with milk production traits (Ng-Kwai-Hang et al., 1986; Aleandri et al., 1990) and technological properties of milk (Okigbo et al., 1985; Marziali and Ng-Kwai-Hang, 1986). In these studies, attention was focused on the effect of individual milk CN loci separately, rather than the effect of composite genotypes on yield, composition, and cheese-making properties of milk. Because of the close linkage between the CN loci on the bovine chromosome 6 (Ferretti et al., 1990; Threadgill and Womack, 1990), the alleles of different CN are in linkage disequilibrium, leading to biases in estimation of genotype effects when individual CN are simultaneously included in a model (Mayer et al., 1997; Ojala et al., 1997; Ikonen et al., 1999b). Consequently, the use of CN genotype combinations or haplotypes is a more appropriate approach to estimate the effects of these loci on milk production traits (Ikonen et al., 1999b, 2001; Boettcher et al., 2004; Caroli et al., 2004) and MCP (Hallén et al., 2007; Comin et al., 2008).

Only Ikonen et al. (1999a) investigated changes in additive genetic variance of MCP when records were adjusted for CN genotype effects using an animal model. That study was mainly based on information from Finnish Ayrshire cows, and no data are currently available on the effects of aggregate CN genotypes on the genetic variation of MCP in Holstein populations. Hence, the objective of the present study was to quantify the contribution of composite β- and κ-CN genotypes on additive genetic variance of rennet coagulation time (**RCT**, min), curd firmness ($\mathbf{a_{30}}$, mm), milk yield, and milk quality traits in Italian Holstein Friesian cows.

MATERIALS AND METHODS

Data from a previous research study were used (for details see Cassandro et al., 2008). Briefly, a total of 1,042 multiparous Holstein cows, progeny of 54 AI sires, were sampled once in 34 herds located across the provinces of Padova, Treviso, and Venezia (northeast Italy) from January to July 2004. The average number of daughters per sire was 19 (range 3 to 86), and the average number of sampled cows per herd was 31 (range 8 to 107). Milk samples were collected during the morning milking, concurrently with the monthly test-day milk recording, and were analyzed within three hours from collection for RCT, a_{30}, milk yield, fat, protein and CN contents, SCC, pH, and titratable acidity (**TA**, SH°/50 mL). Values of SCC were transformed to SCS by means of a logarithmic function. Milk coagulation properties were measured through a computerized renneting meter (Polo Trade, Monselice, Italy) for 31 min after the addition of rennet. Samples not forming a curd within this testing time were classified as non-coagulating (9.7% of the total), and were excluded from the subsequent statistical analysis of RCT and a_{30}. Isoelectric focusing analysis (Erhardt, 1989) was used to determine the CN genetic polymorphisms at β- and κ-CN loci. Descriptive statistics of all the variables are summarized in Cassandro et al. (2008), and observed frequencies of composite β- and κ-CN genotypes are reported in Comin et al. (2008).

Heritability for the studied traits was estimated using single-trait animal models, while genetic correlations between MCP and milk production traits were assessed using 14 sequential bi-variate analyses in which a milk coagulation characteristic was analyzed simultaneously with milk yield, fat content, protein content, CN content, SCS, pH, or TA. The genetic correlation between RCT and a_{30} was also estimated in these analyses. All models accounted for herd (34 levels), DIM (14 levels: 10 monthly classes up to 300 d, 3 bimonthly classes up to 480 d, and 1 open class beyond 480 d), and parity (3 levels: first, second, and third and later lactations) as fixed effects, and additive genetic animal and residual as random effects. In order to investigate the contribution of the composite β-and κ-CN genotypes on additive genetic variance of the traits, the aforementioned analyses were repeated including the composite genotypes grouped into 16 classes as described in Comin et al. (2008).

Variance and covariance components for the random factors were obtained with the VCE software package (Neumaier and Groeneveld, 1998), which uses REML procedures. The number of animals in the additive relationship matrix was 7,387, and included all cows with phenotypic

record and their ancestors up to 8 generations back. Pedigree information was supplied by the Italian Holstein Friesian Cattle Breeders Association (ANAFI, Cremona, Italy).

RESULTS AND DISCUSSION

The inclusion of casein genotypes led to a decrease of 47 and 68% in the genetic variance for RCT and a_{30}, respectively (Table 1), revealing the strong contribution of genotypes to MCP variation, and suggesting that β- and κ-CN are major genes for RCT and a_{30} in Italian Holstein Friesian cows. Results agree with the relevant effect of the genotypes on MCP in this population (Comin et al., 2008). Only few research studies have reported the impact of milk protein genotypes on additive genetic variation of MCP (Oloffs et al., 1992; Ikonen et al., 1999a). In particular, Ikonen et al. (1999a) adjusted MCP records of Finnish dairy cows for composite *β*- and *κ*-CN and *β*-LG genotype effects, and found a much less pronounced influence of them on estimates of genetic variance of RCT and a_{30} (20 and 24%, respectively) compared to the present study. On the other hand, Oloffs et al. (1992) reported an increase in additive genetic variation of MCP as milk protein genotypes were included in the model; this disagrees with the notable effect of protein loci on technological properties of milk. However, the authors did not use an animal model, and this could have influenced the outcome of their analysis.

The contribution of composite *β*- and *κ*-CN genotypes to additive genetic variance for milk yield, fat, protein and CN contents, and SCS was negligible (Table 1), in accordance with findings from Ikonen et al. (1999a). Ojala et al. (1997) reported that composite *κ*-*β*-α_{s1}-CN genotypes explained approximately 15 and 7% of the additive genetic variance for milk production and fat content, respectively, in Holstein Friesian cows in California. The genetic variance of pH and TA was reduced by 18 and 23%, respectively, when records where adjusted for composite *β*- and *κ*-CN genotypes (Table 1). Ikonen et al. (1999a) reported a negligible effect of composite genotypes on pH, while no studies quantifying the effect on TA are available in scientific literature that the authors are aware of.

As a consequence of the relevant changes in additive genetic variation, heritability estimate for RCT decreased from 0.248 to 0.143, and approached zero for a_{30}, changing from 0.123 to 0.043 (Table 1). For pH and TA, moderate changes were detected (0.216 to 0.181, and 0.195 to 0.154, respectively), while changes were negligible for other milk traits.

Table 1. Variance components[1] and heritability of coagulation properties, milk yield, and milk quality traits obtained with two linear models[2].

Trait[3]	σ^2_e		σ^2_a		$\Delta\sigma^2_a$, %	$h^2 \pm$ SE		$h^2_{\beta\text{-}\kappa\text{-CN}}$
	Model 1	Model 2	Model 1	Model 2	Model 2 – 1	Model 1	Model 2	Model 1 - 2
RCT, min	13.3423	13.9202	4.3992	2.3289	-47	0.248 ± 0.065	0.143 ± 0.063	0.105
a_{30}, mm	92.7888	92.6136	13.0255	4.1247	-68	0.123 ± 0.049	0.043 ± 0.042	0.080
Milk yield, kg/d	41.3220	40.8556	3.1168	3.0046	-4	0.070 ± 0.040	0.069 ± 0.041	0.001
Fat, %	0.2649	0.2648	0.1790	0.1809	+1	0.403 ± 0.079	0.406 ± 0.074	-0.003
Protein, %	0.0647	0.0656	0.0293	0.0278	-5	0.312 ± 0.073	0.298 ± 0.072	0.014
CN, %	0.0408	0.0412	0.0214	0.0206	-4	0.344 ± 0.072	0.333 ± 0.072	0.011
SCS	3.0613	3.0675	0.1436	0.1423	-1	0.045 ± 0.030	0.044 ± 0.030	0.001
pH	0.00599	0.00616	0.00165	0.00136	-18	0.216 ± 0.060	0.181 ± 0.064	0.035
TA, SH°/50 mL	0.1081	0.1119	0.0262	0.0203	-23	0.195 ± 0.060	0.154 ± 0.062	0.041

[1]The term σ^2_e is the residual variance, σ^2_a is the additive genetic variance, h^2 is the heritability, and $h^2_{\beta\text{-}\kappa\text{-CN}}$ is the contribution of the composite *β*- and *κ*-CN genotypes to h^2 from Model 1.

[2]Model 2 is Model 1 with the inclusion of composite *β*- and *κ*-CN genotypes.

[3]RCT = rennet coagulation time; a_{30} = curd firmness; TA = titratable acidity (Soxhlet-Henkel degrees); SCS = somatic cell score.

Differences were not always significantly greater than zero ($P > 0.05$) but heritability estimates consistently decreased after including the genotype effects in the model (except for fat content). Furthermore, changes in RCT and a_{30} were considerable, suggesting that these loci may account for a substantial proportion of heritability. Ojala et al. (1997) observed that removing milk protein genotypes from the model increased heritability estimates of milk production and fat content by 0.05 and 0.03 units, respectively.

Genetic correlations between RCT and a_{30}, and between them and milk production traits obtained by including composite *β*- and *κ*-CN information (Model 2) were almost comparable with those obtained using Model 1, but with higher standard errors of estimates (Table 2).

Table 2. Genetic correlations (±SE) between coagulation properties and milk yield and milk quality traits obtained with two linear models[1].

Trait[2]	RCT		a_{30}	
	Model 1	Model 2	Model 1	Model 2
RCT, min	-	-	-0.974 ± 0.050	-0.960 ± 0.108
Milk yield, kg/d	-0.173 ± 0.263	-0.355 ± 0.343	0.186 ± 0.314	0.272 ± 0.476
Fat, %	0.030 ± 0.160	0.096 ± 0.195	0.036 ± 0.197	-0.033 ± 0.278
Protein, %	-0.037 ± 0.175	-0.009 ± 0.222	0.435 ± 0.211	0.484 ± 0.377
CN, %	-0.154 ± 0.169	-0.128 ± 0.214	0.559 ± 0.194	0.667 ± 0.444
SCS	0.168 ± 0.321	0.538 ± 0.348	-0.312 ± 0.345	-0.700 ± 0.447
pH	0.851 ± 0.093	0.862 ± 0.126	-0.908 ± 0.117	ne[3]
TA, SH°/50 mL	-0.502 ± 0.167	-0.326 ± 0.256	0.727 ± 0.104	0.841 ± 0.394

[1]Model 2 is Model 1 with the inclusion of composite *β*- and *κ*-CN genotypes.

[2]RCT = rennet coagulation time; a_{30} = curd firmness; TA = titratable acidity (Soxhlet-Henkel degrees); SCS = somatic cell score.

[3]ne = not estimable.

No reason for the increase of standard errors was immediately obvious. Only the genetic correlation between SCS and MCP increased notably (more than doubled) in absolute value, albeit not-significantly ($P > 0.05$) when composite genotypes were included in the analysis. No estimates of the amount of changes in genetic correlations of MCP with production and quality traits after adjustment for composite genotypes are available in literature and further research is needed on a larger dataset to confirm these results.

In conclusion, this study reported the notable influence of composite *β*- and *κ*-CN genotypes on MCP, and the moderate to negligible impact on milk production traits. Heritability of RCT was still appreciable after adjustment for composite *β*- and *κ*-CN genotypes, suggesting that the

recording of this trait can not be replaced by genotyping of animals for milk protein variants. However, because MCP are affected by CN loci, and cheap and powerful techniques for simultaneous detection of the genetic variants are available, it can be argued that combining RCT data and CN information could provide a tool to better address future selection decisions aiming to improve MCP, particularly in countries such as Italy where the majority of milk is destined to cheese production.

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Chapter 4

Estimation of economic values for milk production, somatic cell score and milk coagulation properties in Italian Holstein-Friesian dairy cattle

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INTRODUCTION

To ensure maximal benefit from genetic improvement, selection index should be for an appropriate breeding goal. Definition of breeding goal, not only genetic parameters, but also economic values (EV) for traits are required (Wolfová et al., 2007a).

In the selection-index theory, the aggregate genotype is usually defined as a linear function of traits to be improved, each multiplied by its EV (Groen, 1989a; Dekkers, 1991). The EV of a trait can be defined as the change in profit of the farm expressed per lactating cow per year, as a consequence of one unit of change in the genetic merit of the trait considered, keeping all other traits in the aggregate genotype constant (Hazel, 1943, Groen, 1989b).

When traits are expressed at different ages or in different groups of animals, the cumulative number of discounted expressions of each trait should be included in the economic value of a trait (Brascamp et al., 1985). When the breeding goal contains milk yield traits only, the cumulative discounted expression of traits are equal for a given selection path and consequently do not influence the relative importance of each trait (Pieters et al., 1997).

The EV of cattle production traits are sensitive to circumstances that change over the time like presence of quota system limitations and milk pricing systems (PS) (Pieters et al., 1997; Wolfová et al., 2007a): for example EV for fat can turn negative when herd milk and fat output were restricted (Pieters et al., 1997).

The Italian Holstein-Friesian (**IHF**) is the main Italian dairy cattle breed (76 % of total dairy cows reared; 85 % of total cows recorded in Italy) with 1.114 million of recorded cows in 13,164 herds. The IHF selection index, milk production and quality traits account for 59% of total index: the traits are weighted as 8, 36, 2, 3 and 10 % for fat yield, protein yield, fat percentage, protein percentage and somatic cell, respectively (ANAFI, 2011).

At the present 70 % of available milk in Italy is used in the cheese manufacture, 50 % of which is used for PDO products. Schmit and Kaiser (2006) estimated that cheese demand is expected to grow during the years and in case of Italy the internal consumption decreased to -1.4 % from 2005 to 2009 but export of Italian cheeses has a positive trend with an increase to +9.5 % for the same period (Pieri, 2010).

The quality of milk used for cheese production is very important, mainly the composition, level of somatic cell count and milk coagulation properties (MCP) (Lucey & Kelly, 1994). The MCP have been widely studied in recent years and have been proposed as technological traits for increasing dairy industry efficiency (Ikonen et al., 2004; Cassandro et al., 2008; Jõudu, 2008). Milk that have a shorter rennet coagulation time (RCT) and has a stronger curd

firmness (a_{30}) is expected to produce higher cheese yield with more desirable composition and lose less milk components in the whey than milk with unfavourable properties (Aleandri et al., 1989; Ng-Kwai-Hang et al., 1989; Martin et al., 1997; Wedholm et al., 2006). Moreover MCP have a exploitable additive genetic variation, and a heritability estimates range from 15 to 40% (Ikonen et al., 2004; Cassandro et al., 2008; Vallas et al., 2010).

Few studies estimated how much is the effect of MCP on the cheese yield: the difference in cheese yield from a milk with good to undesirable MCP can range from around 2 % to 10 % (Pretto et al., 2011 unpublished results, Aleandri et al., 1989, respectively).

Due to its importance in Italy there are more interests to include the MCP in milk payment systems and in breeding selection index. The EV are necessary to determine the relative importance of the traits in the breeding objective but no studies until now estimated them for MCP traits.

The objective of this study was to develop a method for calculating economic values of milk coagulation properties traits and estimate economic value for milk production, SCS and milk coagulation properties in Italian Holstein-Friesian dairy cattle for destination of milk into two cheese manufactures.

MATERIAL AND METHODS

Model description

For derivation of the economic values, a profit function that describe the production system was used, based on the work of Pieters *et al*. (1997). The total annual profitability of a dairy cow (T) can be defined by the following equation:

$$T=R-C-\frac{c_f}{N} \quad (1)$$

Where N= number of lactating cows, R= average revenues during a lactation per cow, C= average costs during a lactation per cow and c_f= fixed costs of total enterprise. Fixed costs are all those costs which cannot be expressed directly per cow for example: building, machinery and milking parlour. The economic value (EV) of trait x was obtained as the partial derivative of the profit function with respect to that trait, evaluated at the current population mean:

$$EV_x=\frac{\partial T}{\partial x}=\frac{\partial R}{\partial x}-\frac{\partial C}{\partial x} \quad (2)$$

Up to now the dairy market in Italy has been restricted by quota system introduced in the EU since 1980s that consider the milk yield and as well as the actual fat content of the milk. In this situation a reformulation of profit equation should be done with the addition of a scaling factor that consider the total amount of output constant and any increase in milk or fat yield per cow is then reflected by a decrease in the number of cows (Pieters et al., 1997; Wolfová et al., 2007b). Future market liberalization is expected in the UE: recent policy developments including reductions of intervention prices and an increase of quotas by 1% annually from 2009 to 2013 and consequently expiring of the quota system in 2015 (Kempen et al., 2011). Moreover in Italy the self-supply of dairy chain is around 70 % and the exportation of processed dairy products is increasing (Peri, 2009). For these reasons economic value were calculated in situation of free market, without quota restriction and in a not-saturated market.
The variable R and C in equation (1) can be distinguished in costs and revenues related to milk production level, cost for maintenance, revenue for meat production derived from sold calves per year and carcass value at the end of productive life, heifer rearing, labour, artificial insemination. Some of these are related to as called functional traits, for example: fertility traits and longevity. Since purpose of this study was to estimate EV for milk production and quality traits in the profit equation (1) were considered variable only parameters related to the milk production level and milk quality, while were assumed constant for all other traits. The costs (C_{milk}) and revenues (R_{milk}) for milk production were estimated using the following equations:

$$R_{milk}=p_mM+p_fF+p_pP+ p_{scs}*(M+F+P) + p_{RCT}RCT*(M+F+P) + p_{a30}a_{30}*(M+F+P) \quad (3)$$

$$C_{milk}= c_mM + c_fF + c_pP \quad (4)$$

Where:

M, F, P: annual yield level of milk carrier, fat and protein (kg);

RCT, a_{30}: average rennet coagulation time (min) and curd firmness (mm) in the population;

p_m, p_f, p_p: price of milk carrier, fat and protein (€/kg);

p_{scs} = amount of premium or penalty for SCS (€/kg),

p_{RCT} = price for RCT (€/min);

p_{a30} = price for a_{30} (€/mm);

c_m, c_f, c_p: feed costs for milk carrier, fat and protein (€/kg).

Production factors

The parameters in the profit equation reflect the Italian dairy industry situation where usually pure breed system is adopted and Italian Holstein-Friesian cow is the main dairy cattle breed reared. In 2010, the cows produced an average of 8961 kg of milk with 3.65 % of fat and 3.30 % of protein (Table 1). Casein was 2.57 % and was derived from protein assuming a casein/protein ratio of 0.78 while lactose content was set to 4.80 %. Milk carrier yield was defined as milk yield minus protein and fat yield. Population mean for SCS and MCP traits were assumed the value reported by Cassandro et al. (2008) for IHF.

For estimation of c_m, c_f, c_p in the equation (4) a simple ration with 4 feeds was used (Table 2) for derived the average cost per MJ of net energy. The energy requirement to produce an extra kg of carrier, fat and protein were assumed to be 0.948, 37.6 and 20.9 MJ of NE, respectively (AFCR, 1993).

Table 1 Production parameters in the basic situation

Production factors	
Milk yield (kg/305d)	8961[a]
Fat (%)	3.65[a]
Protein (%)	3.30[a]
Casein (%)	2.57[b]
Lactose (%)	4.80[c]
Milk carrier yield (kg/305 d)	8338[d]
Fat yield (kg/305 d)	327
Protein yield (kg/305 d)	296
Lactose yield (kg/305 d)	430
SCS (point)	3.08[e]
R (min)	16.90[e]
A30 (mm)	32.00[e]
Energy requirements	
Milk carrier (MJ NE/kg)	0.948[f]
Milk fat (MJ NE/kg)	37.6[f]
Milk protein (MJ NE/kg)	20.9[f]

SCS = somatic cell score.
[a] AIA (2010).
[b] assuming a protein/casein ratio 0.78 (Norman et al., 1991)
[c] Pieters et al. (1997).
[d] Milk yield – (Fat yield + Protein yield).
[e] Cassandro et al. (2008).
[f] AFCR (1993).

Table 2 Feed price and energy content of the ration assumed for dairy cows.

Feed	Price[a] (€/ton)	DM[b] (%)	ME[b] (MJ/kg DM)	NE[b] (MJ/kg DM)	% DM in the ration
Alfalfa hay	124.4	87	8.5	5.5	25
Ryegrass	133.7	85	9.2	5.9	25
Mais	193.2	86	13.8	8.9	30
Soybean	359.4	89	15.3	9.8	20

DM= dry matter; ME= metabolized energy; NE= net energy.
[a] average of 3 years (2009 to 2011) (ISMEA, 2011)
[b] AFCR (1993).

Estimation of price for milk quality traits

In order to assign a value to milk components, payment systems (PS) for two destination of milk were considered: 1) Asiago cheese production and 2) Grana Padano cheese production. The first represent soft cheeses type where whole milk without standardization for fat is used, while the second represent very hard cheeses type from partly skimmed milk.

Product yields and values of milk component were estimated separately for the two cheeses type based on milk quality parameters in the basic situation (Table 1) and proper cheese yield formulas.

For Asiago cheese yield (Y_{Asiago}) was used equation [12] reported by Emmons et al. (1990):

$$Y_{Asiago}\ (kg/100\ kg\ of\ milk) = \frac{(0.93*Fat+Casein-0.01)*1.09}{100-\ cheese\ moisture}*100 \quad (5)$$

Parameters for Asiago cheese production were set to 20 days of ripening and 39.50 % of moisture (Table 3) based on legal limits by specification rules for production (Consorzio Tutela Formaggio Asiago, 2011). Total profit per kg of milk (T_{Asiago}) was calculated as:

$$T_{Asiago} = \frac{Y_{Asiago}*(p_{Asiago} - c_{Asiago})}{100} \quad (6)$$

Where p_{Asiago} is the cheese price and c_{Asiago} is the production cost per kg of cheese (Table 3). Production cost was estimated from the Y_{Asiago} in the basic situation and milk price (p_{milk}):

$$c_{Asiago} = \frac{Y_{Asiago}*p_{Asiago}-(p_{milk}*100)}{Y_{Asiago}} \quad (7)$$

For Grana Padano cheese amount of cream required to be removed from milk (Cream yield = Y_{cream}) to obtain a "cheese milk" with the desired fat to casein ratio (F:C) was estimated by the following equation:

$$Y_{cream}\ (kg/100\ kg\ of\ milk) = 100 - \left(\frac{Fat - Casein * F:C}{Fat_{cream}}\right) \quad (8)$$

Fat cream was assumed 25 % and F:C=1.00 as suggested by specification rules for production (Consorzio per la tutela del Formaggio Grana Padano, 2011).

Table 3 Dairy industries parameters in the basic situation

Asiago cheese	
Type of cheese	soft
Type of milk used	whole
Ripening (days)	20
Cheese moisture (%)	39.50
Cheese yield (kg/100 kg of milk)	10.57
Price (€/kg)	4.35[a]
Production cost (€/kg of cheese)	0.72
Grana Padano cheese	
Type of cheese	very hard
Type of milk used	partly skimmed
Ripening (months)	9
Fat to casein ratio for defatted milk	1.00
Fat of cream (%)	25
Fat of butter (%)	82
Cheese yield for defatted milk (kg/100 kg of milk)	7.62
Cream yield (kg/100 kg of milk)	4.30
Butter yield (kg/100 kg of milk)	1.31
Price (€/kg)	6.84[a]
Production cost (€/kg of cheese)	2.16
Other prices	
Milk price (€/kg)	0.384[a]
Lactose price (€/kg)	0.57[b]
Butter price (€/kg)	3.23[a]

[a] average of 3 years (2009 to 2011) (ISMEA, 2011)
[b] average of 3 years (2009 to 2011) (CLAL, 2011)

Cheese yield for defatted milk (Y_{grana}) was estimated based on equation reported by Aleandri et al. (1989):

$$Y_{Grana}\ (kg/100\ kg\ of\ milk) = 2.83329 + 0.9877*Fat + 0.179*(Protein)^2 \quad (9)$$

Total profit per kg of milk (T_{Grana}) was calculated as:

$$T_{Grana} = \frac{\left(1 - \frac{Y_{cream}}{100}\right)(Y_{Grana}*(p_{Grana} - c_{Grana}) + Y_{butter}*p_{butter}}{100} \quad (10)$$

Where Y_{butter} is the amount of butter produced from the cream removed assuming a final fat content of 82 %, p_{butter} is the butter price, p_{Grana} is the cheese price and c_{Grana} is the production cost per kg of cheese (Table 3). Production cost was estimated from the Y_{Grana} in the basic situation and milk price (p_{milk}) assumed negligible production cost for butter:

$$c_{Grana} = \frac{\left(1 - \frac{Y_{cream}}{100}\right)* Y_{Grana}*p_{Grana} - (p_{milk}*100)}{Y_{Grana}} \quad (11)$$

For both milk destinations, fat and protein prices were obtained as the partial derivative of the profit equations (6 and 10) with respect to fat or protein, evaluated at the basic situation and assumed fix the production costs for cheese.

The estimations of p_{RCT}, p_{a30} (equation 3) were based on simulated payment systems for MCP: $PS_{MCP2.5\%}$, $PS_{MCP5\%}$ and $PS_{MCP10\%}$. The payment systems assumed that the maximum difference in cheese yield between a milk with the worst to the best MCP were 2.5 %, 5% and 10%, respectively. For each situation p_{RCT} and p_{a30} were estimated based on the following equations:

$$p_{RCT}\ (€/min\ per\ kg) = -\frac{\frac{Y_x*(p_x - c_x)}{100}*effect_{MCP}}{max_{RCT} - min_{RCT}}*0.50 \quad (12)$$

$$p_{a30}\ (€/mm\ per\ kg) = \frac{\frac{Y_x*(p_x - c_x)}{100}*effect_{MCP}}{max_{a30} - min_{max_{a30}}}*0.50 \quad (13)$$

Where Y_x, p_x, c_x are yield, price and production cost of cheese x, respectively; $effect_{MCP}$ is the maximum difference in cheese yield between a milk with the worst to the best MCP (2.5 %, 5% or 10%), max and min is the maximum and minimum value for RCT or a_{30} in the population. These values were set to 7.20 and 29.40 minutes for RCT and 4.00 and 60.00 mm for a_{30} (Cassandro et al., 2008). The number 0.50 in equations (12) and (13) is for split the effect of MCP 50% for RCT and 50% for $a_{30,}$ while negative sign was in equation (12) because the best MCP is assumed to have shorter RCT.

Carrier value was derived from the lactose price, assuming 4.80 % the lactose content.

Table 4 Milk price premium or penalty for class of somatic cell.

SCC class	SCS class	Frequency[a]	premium/penalty (€/kg)
<200.000	4.00	68.2	0.003
200.000-400.000	4.00-5.00	15.7	0.000
400.000-500.000	5.00-5.32	3.7	-0.012
500.000-600.000	5.32-5.58	2.6	-0.018
>600.000	>5.58	9.8	-0.024

SCC= somatic cell count (n/mL); SCS somatic cell score (point).

[a] calculated from normal distribution for SCS with mean= 3.08 and s.d.= 1.94 (Cassandro et al., 2008)

The p_{scs} in equation (3) is derived from the premium/penalties of a common payment system applied in Italy (Table 4). Usually the payment systems provide a discontinuous penalty to the price of milk for classes of somatic cell count (SCC) level. Table 4 gives classes range for SCC and SCS, frequencies for each SCS class assuming normal distribution (mean= 3.08 and s.d.= 1.94 from Cassandro et al., 2008) and the associated price premium/penalties. The method used to calculate p_{scs} and computed partial differentiation (a_{SCS}) respect to the population mean of SCS is based to the Meijering method. The Meijering method is a threshold model as described by Meijering (1986) to derive economic values of dystocia and has been applied to SCS assuming price penalties (Charfeddine, et al., 1996; Wolfova et al., 2007b; Sadeghi-Sefidmazgi et al., 2010). The equation for derive a_{SCS} was:

$$a_{SCS} = -\frac{1}{\sigma} * \sum_{n=1}^{x-1} \left[\left(P_{n+1} - P_n \right) * \theta * \left(\frac{t_n - \mu}{\sigma} \right) \right] \qquad (14)$$

Where:

$\theta(t)$ = probability density function of the normal distribution

t_n = threshold limit of class n in units of SCS scale

P_n = premium/penalty associated of class n

x = total number of classes in the payment system

μ= mean of SCS in base population

σ = standard deviation of SCS

All prices for feeds and dairy products were an average of last 3 years (2009 to 2011). Sensitivity analysis of the economic values were carried out for different prices for costs or revenues.

A sensitivity analysis was carried out to analyze the robustness of the profit equation and consequently of the EV's by changing the prices of costs or revenues. Changes of ±20% with respect to the price of dairy products or feed. The changes were performed one at time, keeping all other parameters constant.

RESULTS

In the basic situation, using the feed ration in Table 2, the cost of net energy was 0.023 €/MJ and the resulting cost for produce 1 extra kilograms of carrier, fat and protein was 0.028, 1.102 and 0.613 €. From the calculation the resulting price for fat and protein was 6.080 and 5.099 €/kg, respectively in Asiago cheese destination, while 3.326 and 6.791 €/kg, respectively in Grana Padano destination. The price of milk carrier was 0.027 €/kg while for somatic cell in the basic situation is expected an increasing of 0.012 €/kg of the average penalty when SCS increase 1 point for both cheese destinations.

Table 5 Absolute economic value for milk production and quality traits for the four simulated payment systems and destination of milk for Asiago and Grana Padano cheese.

	Carrier (€/kg)	**Fat** (€/kg)	**Protein** (€/kg)	**SCS** (€/point)	**RCT** (€/min)	**a_{30}** (€/mm)
Asiago cheese destination						
$PS_{MCP0\%}$	-0.001	4.98	4.49	-105.22	-	-
$PS_{MCP2.5\%}$	-0.001	4.98	4.49	-105.22	-1.94	0.77
$PS_{MCP5\%}$	-0.001	4.98	4.49	-105.22	-3.87	1.53
$PS_{MCP10\%}$	-0.001	4.98	4.49	-105.22	-7.74	3.07
Grana Padano cheese destination						
$PS_{MCP0\%}$	-0.001	2.22	6.18	-105.22	-	-
$PS_{MCP2.5\%}$	-0.001	2.22	6.18	-105.22	-1.72	0.68
$PS_{MCP5\%}$	-0.001	2.22	6.18	-105.22	-3.44	1.37
$PS_{MCP10\%}$	-0.001	2.22	6.18	-105.22	-6.89	2.73

In table 5 is reported economic value for milk production and quality traits for the two destination. The EV for carrier was slightly negative (-0.001 €/kg) while the EV for fat was slightly higher than protein in Asiago cheese destination (4.98 and 4.49 €/kg, respectively) while in Grana Padano cheese destination lower EV for fat and higher for protein were estimated (2.22 for fat and 6.18 €/kg for protein). The increase of 1 point of SCS in basic situation decrease of 105.22 €/year the profit per cow.

In Asiago cheese destination EVs for MCP traits ranged from -1.94 to 7.74 €/min for RCT and from 0.77 to 3.07 €/mm for a_{30} from $PS_{MCP2.5\%}$ to $PS_{MCP10\%}$, respectively. In Grana Padano cheese EV ranged from -1.72 to 6.89 €/min for RCT and from 0.68 to 2.73 €/mm for $a_{30,}$ from $PS_{MCP2.5\%}$ to $PS_{MCP10\%}$, respectively. The EV for RCT was negative because an increase of the time for coagulation is expected to decrease cheese yield.

Table 6 Relative economic value standardized by genetic standard deviation (σ_a) for milk production and quality traits for the four simulated payment systems and destination of milk for Asiago and Grana Padano cheese.

	Carrier	**Fat**	**Protein**	**SCS**	**RCT**	**a_{30}**
σ_a	875.40[a]	33.74[a]	27.14[a]	0.47[b]	2.22[b]	4.06[b]
Asiago cheese destination						
$PS_{MCP0\%}$	-0.2%	49.4%	35.8%	-14.6%	0.0%	0.0%
$PS_{MCP2.5\%}$	-0.2%	48.4%	35.1%	-14.2%	-1.2%	0.9%
$PS_{MCP5\%}$	-0.2%	47.4%	34.3%	-14.0%	-2.4%	1.8%
$PS_{MCP10\%}$	-0.2%	45.5%	33.0%	-13.4%	-4.7%	3.4%
Grana Padano cheese destination						
$PS_{MCP0\%}$	-0.2%	25.6%	57.3%	-16.9%	0.0%	0.0%
$PS_{MCP2.5\%}$	-0.2%	25.1%	56.0%	-16.5%	-1.3%	0.9%
$PS_{MCP5\%}$	-0.2%	24.5%	54.8%	-16.2%	-2.5%	1.8%
$PS_{MCP10\%}$	-0.2%	23.5%	52.5%	-15.5%	-4.8%	3.5%

[a] Interbull (2011)

[b] Cassandro et al. (2008)

For a better comparison of the relative importance of different traits in the PS considered, the EV were standardized by multiplying them by the genetic standard deviation of each respective trait and expressed as percentage of the total (Table 6).

In Asiago cheese destination with $PS_{MCP0\%}$ the relative weights for milk carrier, fat yield, protein yield and SCS were -0.2, 49.4, 35.8 and -14.6 %, respectively. In Grana Padano cheese with $PS_{MCP0\%}$ the relative weights for milk fat yield was 25.6% while for protein yield was 57.3%. The relative EV for carrier, fat, protein and SCS slightly decrease from $PS_{MCP0\%}$ to $PS_{MCP10\%}$ due to the increase of importance of MCP traits.

Table 6 Relative economic value standardized by genetic standard deviation (σ_a) for milk production and quality traits for the four simulated payment systems and destination of milk for Asiago and Grana Padano cheese.

	Carrier	**Fat**	**Protein**	**SCS**	**RCT**	**a_{30}**
σ_a	875.40[a]	33.74[a]	27.14[a]	0.47[b]	2.22[b]	4.06[b]
Asiago cheese destination						
$PS_{MCP0\%}$	-0.2%	49.4%	35.8%	-14.6%	0.0%	0.0%
$PS_{MCP2.5\%}$	-0.2%	48.4%	35.1%	-14.2%	-1.2%	0.9%
$PS_{MCP5\%}$	-0.2%	47.4%	34.3%	-14.0%	-2.4%	1.8%
$PS_{MCP10\%}$	-0.2%	45.5%	33.0%	-13.4%	-4.7%	3.4%
Grana Padano cheese destination						
$PS_{MCP0\%}$	-0.2%	25.6%	57.3%	-16.9%	0.0%	0.0%
$PS_{MCP2.5\%}$	-0.2%	25.1%	56.0%	-16.5%	-1.3%	0.9%
$PS_{MCP5\%}$	-0.2%	24.5%	54.8%	-16.2%	-2.5%	1.8%
$PS_{MCP10\%}$	-0.2%	23.5%	52.5%	-15.5%	-4.8%	3.5%

[a] Interbull (2011)

[b] Cassandro et al. (2008)

As expected, sensitivity analysis for EV of carrier, fat and protein was affected by selling price of dairy products (Table 7). Economic value for carrier ranged from -0.006 to 0.005 €/kg with change of dairy product prices. Wide change was expected for EV for fat, protein and MCP traits. The most variation was found in Grana Padano cheese destination where EV ranged from 1.26 to 3.18 €/kg for fat, from 4.18 to 8.17 €/kg for protein, from -4.87 to -8.90 €/min for RCT, from 1.93 to 3.53 €/mm for a_{30}, with -20% to +20% of dairy product prices, respectively. Sensitivity analysis for feed price affect EV for carrier on the same magnitude of change in dairy product prices. For fat and protein the effect is much lower than change in dairy product prices, while no effects there were for MCP traits since in this model no feed costs were related to MCP traits. With sensitivity analysis no variations were observed for SCS economic value.

DISCUSSION

In this study, we obtained the economic values for yield traits and determined their robustness to changes in price and production circumstances. The main purpose of this study was to support an eventual implementation of a new selection index for the IHF breed and contribute to decision-making on diversification of the breeding goal in Italy to meet the requirements of the different markets better.

Differences in production models, definitions of traits, and assumptions about management system, effects on genetic improvement of particular traits make a direct comparison of

economic values among different countries very difficult. The relative economic importance of traits calculated on the basis of standardized economic values depends very strongly on the genetic standard deviations of the traits, which may differ considerably among population (Wolfova et al., 2007b). In spite of the limitations cited above, some general statements about economic values can be derived from the literature and from the present study.

Economic values for milk yield reported in the literature are normally negative (Groen, 1989a; Bekman and Van Arendonk, 1993). In these studies fat and protein yield was paid for and the base price for milk was negative. In the present study, in the basic situation EV for milk carrier was negative but very close to zero. Only feed costs were accounted for produce lactose in the carrier and in this case EV is underestimated because cost for transportation could be also added.

The EV ratio protein:fat was 0.9 in Asiago cheese destination while 2.8 for Grana Padano cheese destination. This was because in the cheese yield prediction equations weight for protein is higher for Grana Padano than for Asiago cheese. Moreover fat EV for Grana Padano cheese was lower than for Asiago Cheese because extra fat in the milk, higher that the limit suggested for Grana Padano cheese (fat:casein ratio ~1.00; Consorzio per la tutela del Formaggio Grana Padano, 2011), is removed from the milk and used for butter production. In according with current butter price, the use of fat in the milk for produce butter is less profitable than produce cheese.

The premium/penalty using in this work for SCS (Table 4) is an average payment system for somatic cell in Italy, but very large variability exist across the country and dairy factories for somatic cell payment (Pretto and Cassandro, 2011).

The EV for MCP traits in Asiago cheese is slightly higher than in Grana Padano because: 1) the method for estimate EV for MCP traits is based on the maximum difference in cheese yield between a milk with the worst to the best MCP expressed in percentage, so for Asiago cheese the absolute range of yield is higher than Grana Padano cheese since the average yield in basic situation is higher (10.57 and 7.62 % for Asiago and Grana Padano cheese, respectively); 2) a portion of milk for Grana Padano cheese is used for butter production where the MCP doesn't affect the yield.

The weight for MCP ranged from 2.1 to 8.1 % (sum of relative weight for RCT and $_{a30}$) in Asiago cheese destination while 2.2 to 8.3 % for Grana Padano cheese destination. These range of economic value could be used for simulate selection indexes for improve MCP traits and estimate the response of selection for all the traits. The EV for a given milk destination

were found to be very robust to changes in feed price but very wide variation was found for change in dairy product price (Table 7).

CONCLUSION

Results obtained in this study provide important information about economic values for milk production, quality traits and gives the first estimation for MCP traits.

The EV found for milk production traits indicate that negative value should be given to milk yield. Large variation was observed for fat and protein EV with different destination of milk. This model account only two kind of cheeses and a further analysis should be done accounting for all the main dairy products processed in the country. The current payment system used in Italy usually a higher price for protein compared to fat is applied as suggested from this simulation in case of Grana Padano destination but no or small payment is applied for MCP traits. The implementation of MCP in milk payment systems could stimulate the farmers to improve this technological trait. Difference in dairy products in the country could justify a diversification of the breeding goal in Italy even if in this case the selection intensity for each segment of the market will be lower. This loss in selection intensity needs to be balanced against the increase in selection response due to the use of appropriate economic value.

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Chapter 5

Genetic response for milk yield, milk components, somatic cell score, milk coagulation properties and milk acidity in Italian Holstein-Friesian population under current and alternative selection indexes and selection criteria

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ABSTRACT

The aims of this work were to estimate the annual genetic response of milk yield (MY), milk components, somatic cell score (SCS), milk coagulation properties (MCP) and milk acidity in Italian Holstein Friesian (IHF) under current selection index and alternative selection indexes and selection criteria. The selection index analysed included protein and fat yield, protein and fat percentage and SCS. Genetic response per year (GR_y), for all traits in selection index and correlated response for MCP, milk yield and milk acidity was estimated using selection index theory. Genetic parameters for all traits were assumed from different sources and validated for positive definite matrix. Alternative selection indexes were simulated with inclusion of MCP traits and three alternative selection criteria were considered: "today", nowadays phenotypic recording systems; "MCPd", traits in "today" plus MCP analysed in the milk by direct instrument; "MCPp", traits in "today" plus indicator traits for MCP measured by mid-infrared spectroscopy. Current selection index seems not affecting significantly MCP traits. Selection criteria with the implementation of MIRS prediction of MCP in the current recording systems was the option that allow to reach higher genetic response for MCP traits. An emphasis slightly more than 12.5% with MCPp selection criterion should reach an improvement of about 0.10 genetic standard deviation per year for both rennet coagulation time and curd firmness without lose GR_y for MY, FY and PY. Including MCP traits in the selection index can increase casein:protein ratio and could be an indirect way for decrease SCS.

Keywords: Holstein-Friesian dairy cow, genetic response, selection index, milk coagulation property

INTRODUCTION

The Italian Holstein-Friesian (**IHF**) is the main Italian dairy cattle breed (76 % of total dairy cows reared; 85 % of total cows recorded in Italy) with 1.114 million of recorded cows in 13,164 herds. The herd book managed by the ANAFI (National Association of IHF Breeders) has been created in 1959 and since 1985 started to formalize and expand a breeding scheme based on progeny testing and at the same time defined a selection index with greater emphasis on fat and protein yields (Burnside et al., 1992). In 2001 a new selection index, called PFT was introduced where relative emphasis for production traits decreased to 59%, somatic cell score (SCS) was included with relative emphasis of 8%, as well morphological and functional traits with relative emphasis of 33%.

Under this selection index has been reported an actual genetic response for cow population of +66 kg/year for milk yield, +0.002 %/year for protein content, but not change in the SCS (average of 2003-2007). Last up-to-date of selection index was in 2009 when the sum of emphasis for production traits was set to 49 % and 10 % for SCS (ANAFI, 2011).

A selection objective should be defined for the genetic improvement of a cow population such that the future cows will produce the desired products more efficiently under expected future economic production environment (Lopez-Villalobos and Garrick, 2005). Italy has a peculiar and specialized structure of dairy industry producing many high value typical and traditional cheeses, in particular Protected Designation of Origin (PDO) products like the Grana Padano, Parmigiano Reggiano, Gorgonzola and Asiago in order of importance (Pieri, 2010). At the present 70 % of available milk in Italy is used in the cheese manufacture, 50 % of which is used for PDO products. Schmit and Kaiser (2006) estimated that cheese demand is expected to grow during the years and in case of Italy the internal consumption decreased to -1.4 % from 2005 to 2009 but export of Italian cheeses has a positive trend with an increase to +9.5 % for the same period (Pieri, 2010).

The quality of milk used for cheese production is very important, mainly the composition, level of somatic cell count and milk coagulation properties (MCP) (Lucey & Kelly, 1994). The MCP have been widely studied in recent years and have been proposed as technological traits for increasing dairy industry efficiency (Cassandro et al., 2008; Ikonen et al., 2004; Jõudu, 2008). Milk that have a shorter rennet coagulation time (RCT) and has a stronger curd firmness (a_{30}) is expected to produce higher cheese yield with more desirable composition and lose less milk components in the whey than milk with unfavourable properties (Aleandri et al., 1989; Martin et al., 1997; Ng-Kwai-Hang et al., 1989; Wedholm et al., 2006). Problem of

non-coagulated samples has been reported around 10% in IHF population (Cassandro et al., 2008) and same studies noticed a worsening of MCP of milk used for cheese processing over the years in Italy (Sandri et al., 2001; Cassandro and Marusi, 2001; Formaggioni et al., 2005) or change on percentage of cheese wheels labelled as first quality (Bittante et al., 2011).

Milk coagulation properties are different among cattle breeds (De Marchi et al., 2007; De Marchi et al., 2008) and within breed MCP can be improved by genetic selection because of heritability and repeatability values are showed to be moderate (Ikonen et al., 2004; Cassandro et al., 2008; Vallas et al., 2010). Milk coagulation properties are affected by β- and κ-casein genotypes (Comin et al. 2008) but Penasa et al. (2010) showed that composite β- and κ-CN genotypes cannot replace the recording of MCP themselves. Milk coagulation properties can be analysed directly by different instruments and methodologies (O'Challagan et al., 2002; Pretto et al., 2011a) but it is expensive, time consuming and for this difficult to record in all the population. A feasible way for the implementation of MCP assessment in the phenotypic recoding system has been proposed recently using mid-infrared spectroscopy (MIRS) (De Marchi et al., 2009; Cecchinato et al., 2009).

Although the technological properties of milk are important to the dairy industry, genetic response under current selection index have not been reported. Moreover, no work has estimated genetic response of MCP traits and correlated response of other traits with a selection index to improve MCP.

Aims of this work were to estimate the annual genetic response of milk yield, milk components, somatic cell score, milk coagulation properties and milk acidity in Italian Holstein Friesian under current selection index and alternative selection indexes and selection criteria.

MATERIALS AND METHODS

Selection indexes

Different selection indexes (I) were considered using estimated breeding values (EBV) for the different traits with each trait having a relative economic importance (a) as described in selection index theory (Hazel, 1943):

$$I = \sum_{j=1}^{n} a_j EBV_j$$

Traits considered for current selection index (PFT) in IHF were fat yield (FY), protein yield (PY), fat content (F%), protein content (P%) and SCS (table 1) with weights in the index of +4.0, +22.4, 108.8, 350.0 and -143.9, respectively. This selection index accounted for those traits related to production and quality of milk. It was a simplified form of that used by the IHF accounting for 59 % of total index, since the functional traits (longevity and fertility) and morphological traits were not included in this simulation because of genetic and phenotypic parameter estimates for all traits were unavailable. For a better comparison of the relative importance of different traits, the weights in the index were standardized by multiplying them by the genetic standard deviation (σ_a) of each respective trait and expressed as relative emphasis (table 1).

Table 1 Index weights of current selection index in Italian Holstein-Friesian (PFT) and relative emphasis for traits in the simulated selection indexes.

Selection index [a]	Trait in selection objective [b]						
	FY	PY	F%	P%	SCS	RCT	a_{30}
	Index weight						
PFT	4.0	22.4	108.8	350.0	-143.9		
	Relative emphasis, % [c]						
PFT	13.6	61.0	3.4	5.1	-16.9		
$MCP_{5\%}$	12.9	58.0	3.2	4.8	-16.1	-2.5	2.5
$MCP_{12.5\%}$	11.9	53.4	3.0	4.4	-14.8	-6.3	6.3
$MCP_{25\%}$	10.2	45.8	2.5	3.8	-12.7	-12.5	12.5
$MCP_{50\%}$	6.8	30.5	1.7	2.5	-8.5	-25.0	25.0
$MCP_{75\%}$	3.4	15.3	0.8	1.3	-4.2	-37.5	37.5
$MCP_{100\%}$	0.0	0.0	0.0	0.0	0.0	-50.0	50.0

[a] PFT= current selection index in Italian Holstein-Friesian (ANAFI, 2011); $MCP_{5\%}$-$MCP_{100\%}$= alternative selection indexes with relative emphasis for milk coagulation properties from 5 to 100 % split between rennet coagulation time and curd firmness.

[b] FY= fat yield (kg/305 d); PY= protein yield (kg/305 d); F% = fat (%); P% = protein (%); SCS = somatic cell score; RCT= rennet coagulation time (min); a_{30} = curd firmness after 30 min from coagulant addition (mm).

[c] Relative emphasis in Italian Holstein-Friesian simulated index for milk yield and milk quality (59 % of total merit index).

Alternative selection indexes were simulated with inclusion of MCP traits. Since for cheese processing is better to have shorter RCT and higher a_{30}, these two traits has been included with a negative weight for RCT and positive for a30. Six alternative selection indexes were formulated with an increase of relative emphasis for MCP traits of 5, 12.5, 25, 50, 75, 100%. The weights in the index was recalculated in order to have a proportionally decreasing of

relative emphasis for all the other traits and the relative emphasis for MCP split half for RCT and half for a_{30}.

Selection criteria

Selection criteria are the list of those traits that can be measured on the animals and can be used as predictors of the traits include in the aggregate genotype. Three selection criteria were simulated (table 2): "today" included those traits currently recorded in official phenotypic milk recording system in Italy that are milk yield (MY), FY, PY, F%, P% and SCS; "MCPd", traits in "today" plus RCT and a_{30} analysed in the milk by direct instrument; "MCPp", traits in "today" plus indicator traits for MCP measured by mid-infrared spectroscopy for RCT (pRCT) and a_{30} (pa_{30}).

Table 2 Traits included in the tree alternative selection criteria.

	Trait in selection criterion [b]									
Selection criterion [a]	MY	FY	PY	F%	P%	SCS	RCT	a_{30}	pRCT	pa_{30}
Today	✓	✓	✓	✓	✓	✓				
MCPd	✓	✓	✓	✓	✓	✓	✓	✓		
MCPp	✓	✓	✓	✓	✓	✓			✓	✓

[a] today= current recording system in Italy; MCPd= recording of milk coagulation properties (MCP) by direct analysis in the milk; MCPp= recording of MCP by mid-infrared spectroscopy prediction.

[b] FY= fat yield (kg/305 d); PY= protein yield (kg/305 d); F% = fat (%); P% = protein (%); SCS = somatic cell score; RCT= rennet coagulation time (min); a_{30} = curd firmness after 30 min from coagulant addition (mm); RCT predicted by mid-infrared spectroscopy (MIRS); pa_{30} = a_{30} predicted by MIRS.

Breeding scheme

Four pathways of selection as described by Rendel and Robertson (1950) were considered (table 3), namely, cows to breed cows (CC), cows to breed bulls (CB), bulls to breed cows (BC), and bulls to breed bulls (BB). Selection intensity (i) and generation interval in years (GI) for the respective pathways were in according with current breeding scheme for IHF (ANAFI, 2011). Selection on the CC was considered to be negligible with just i = 0.049 accounting for the high replacement rate in IHF. Elite cows for CB were the top 2 % of IHF dairy cows population and mated with the top 1% of bulls (BB). About 400 young bulls entered in progeny testing every year and the top 5 % were eligible to be proven bulls (BC). Generation interval and average number of own phenotypic records were set to 1 lactation for CC and daughters in progeny testing while information from 2 lactations for CB. Generation

intervals were estimate in 4 and 5 years to complete 1 and 2 lactation respectively, while number of records for each lactation was one for MY, FY and PY traits and in average 8 for F%, P% and SCS, in according with monthly milk recording system.

Table 3 Assumed parameters for the four pathways of selection in the breeding scheme for Italian Holstein-Friesian and average number of phenotypic records available for genetic evaluation.

	Parameter [d]			Number of records per animal for each trait [e]									
Pathway [a]	p	i	GI	MY	FY	PY	F%	P%	SCS	RCT	a_{30}	pRCT	pa_{30}
CC [b]		0.049	4	1	1	1	8	8	8	1	1	8	8
CB [b]		2.421	5	2	2	2	16	16	16	1	1	16	16
BC [c]	100	2.063	6	1	1	1	8	8	8	1	1	8	8
BB [c]	100	2.665	8	1	1	1	8	8	8	1	1	8	8

[a] CC= cows to breed cows; CB = cows to breed bulls; BC = bulls to breed bulls; BB = bulls to breed cows.

[b] Own performance.

[c] performance per each daughter in progeny testing.

[d] p= number of progeny; i= selection intensity; GI= generation interval in years.

[e] FY= fat yield (kg/305 d); PY= protein yield (kg/305 d); F% = fat (%); P% = protein (%); SCS = somatic cell score; RCT= rennet coagulation time (min); a_{30} = curd firmness after 30 min from coagulant addition (mm); RCT predicted by mid-infrared spectroscopy (MIRS); pa_{30} = a_{30} predicted by MIRS.

Generation interval for BC and BB were set to 6 and 8 years, respectively, in according to the time need to complete a conventional progeny testing and each bull was evaluated on at least 100 daughters. In the case of MCPd selection criterion, for difficulties related to large-scale recording of individual MCP phenotypes was assumed to have a single measure per cow of RCT and a_{30} during the first lactation. In alternative in the case of MCPp selection criterion where MIRS technology was used to predict all milk quality traits from milk recording samples, the number of records available per cow for MCP traits (pRCT and pa_{30}) were the same of F%, P% and SCS (8 per lactation).

Calculation of genetic response

Genetic response (GR) to selection per trait from each combination of selection index and criterion was calculated with a deterministic procedure (Cameron, 1997) applying the equation:

$$GR_j = \frac{\mathbf{b}'\mathbf{G}_j}{\sqrt{\mathbf{b}'\mathbf{P}\mathbf{b}}}$$

Where GR_j is the GR for generation for trait j; $\mathbf{G}_j$ is j^{th} column of the matrix **G**; **G** is the genetic covariance matrix between traits in the selection index and the traits in selection criterion; **P** is the phenotypic variance-covariance matrix of traits in the selection criterion; vector **b** was derived from the equation:

$$\mathbf{b} = \mathbf{P}^{-1}\mathbf{G}\mathbf{a}$$

Where **a** are the elements of vector of index weights. Estimated correlated GR of those traits that are not in the selection index (e.g., milk acidy) were computed by expanding matrix G with columns that contain covariances between traits in the index and the additional traits.

Matrixes **P** and **G** were derived from the parameters assumed in Appendix A. Genetic and phenotypic correlation were taken from different sources. For MCP traits and milk acidity the parameters were taken from the work of Cassandro et al., 2008 while the correlation between MCP traits and MCP traits predicted by MIRS were taken from the work of Cecchinato et al., 2009 that well estimated this parameters. As assumptions the correlation between pRCT and pa_{30} with other traits were assumed to be the same of RCT and a_{30}, while when no parameters were available correlation was set to be neutral.

The resulting matrixes of all traits was checked if were positive definitive and if not, bending procedure with the method propose for correlation matrix by Jorjani et al., 2003 was applied. All variances and co-variances were formulated separately for the four pathways because of the differences in their sources of information using equation reported in Appendix B.

Annual genetic response to selection for trait j ($GR_{y\,j}$) were calculated by summing the GR for the four pathways of selection and dividing by the sum of the four generation intervals:

$$GR_{y\,j} = \frac{GR_{CC\,j} * i_{CC} + GR_{CB\,j} * i_{CB} + GR_{BC\,j} * i_{BC} + GR_{BB\,j} * i_{BB}}{GI_{CC} + GI_{CB} + GI_{BC} + GI_{BB}}$$

The estimation of accuracy of the alternative selection indexes for each pathway of selection was also calculated. The calculation was implemented with proc iml of SAS (version 9.2; SAS Institute Inc., Cary, NC).

RESULTS

Genetic response with current selection index

Table 4 shows the GR_y estimated that resulted in different milk production and quality traits from current selection index (PFT) and current selection criterion in IHF. There is high selection pressure for FY and PY with a genetic increase of +6.74 and +6.66 kg per lactation,

or +0.20 and +0.25 σ_a per year, respectively. Milk yield even if it isn't as direct trait in selection index, is expected to increase 128.73 kg per lactation or 0.15 σ_a per year.

Table 4 Genetic response for current Italian Holstein-Friesian selection index (PFT) and nowadays selection criterion.

Selection Index	Trait [a]										
	MY	FY	PY	F%	P%	C%	SCS	pH	TA	RCT	a_{30}
PFT	128.73	6.74	6.66	0.010	0.013	0.012	-0.008	-0.001	0.004	-0.018	0.161
	in genetic standard deviation										
PFT	0.15	0.20	0.25	0.03	0.09	0.08	-0.01	-0.03	0.03	-0.01	0.04

[a] MY= milk yield (kg/305 d); FY= fat yield (kg/305 d); PY= protein yield (kg/305 d); F% = fat (%); P% = protein (%); C% = casein (%); SCS = somatic cell score; TA = titratable acidity (°SH/50 mL); RCT= rennet coagulation time (min); a_{30} = curd firmness after 30 min from coagulant addition (mm).

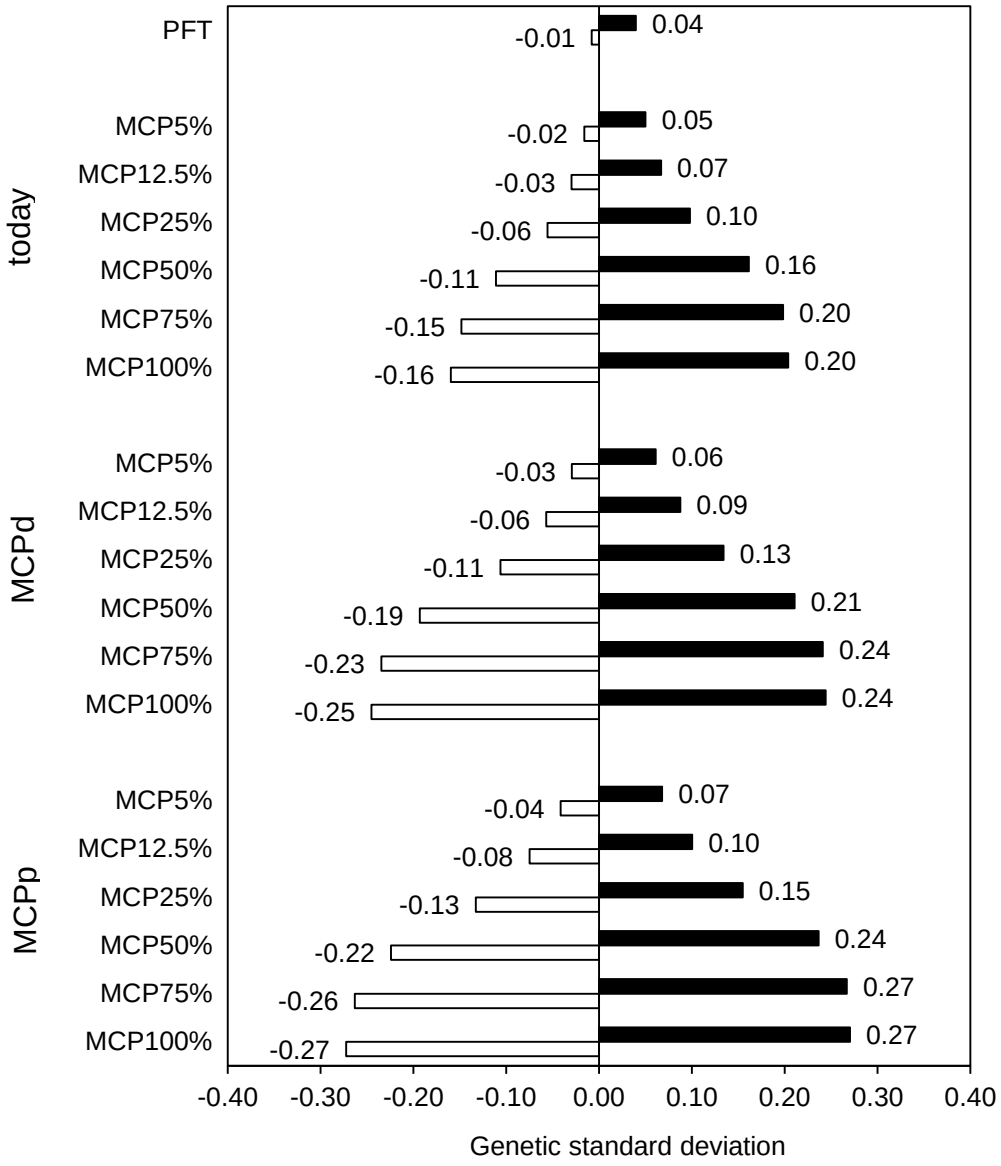
This is due because high genetic correlation exist between MY and FY or PY. Genetic responses per year expressed in genetic standard deviations are expected to be +0.03, +0.09 and +0.08 σ_a for F%, P% and C%, respectively.. The GR_y for SCS is expected to be very low (-0.01 σ_a per year) even when SCS is included in the current selection index with a high relative economic importance of 16.9 %.

Expected genetic changes for MCP traits were -0.018 min for RCT and +0.161 mm for a_{30} per year. These correlated responses under the current selection index showed a negligible genetic change for MCP over the year with -0.01 and +0.04 σ_a for RCT and a_{30}, respectively. Slight GR_y was observed for milk acidy too.

Genetic response with alternative selection index and criteria

The comparison of GR_y for MCP in all the simulations are outlined in figure 1. The index $MCP_{100\%}$ represents the genetic gain that can be obtained when MCP traits alone are included in the selection index. The GR_y with $MCP_{100\%}$ can be -0.16 and +0.20 σ_a for RCT and a_{30} respectively with nowadays selection criteria. In this case MCP traits aren't recorded phenotypically from the population and for their improvement is used the other traits as indicator traits thanks to their correlated genetic relationship with MCP traits. When for MCP traits is available a single measure per cows with direct laboratory analysis (MCPd selection criteria), GR_y with $MCP_{100\%}$ is estimated to -0.25 and +0.24 σ_a for RCT and a_{30}, respectively. Phenotypic correlation between RCT and pRCT has been estimated around +0.67 and +0.51 between a_{30} and pa_{30} (Cecchinato et al., 2009).

Figure 1. Genetic response for rennet coagulation time (unshaded bars) and curd firmness (shaded bars) for current (PFT) and alternative selection indexes ($MCP_{5\%}$-$MCP_{100\%}$) for Italian Holstein-Friesian with the tree selection criteria (today, MCPd, MCPp).



Genetic correlation is higher (+0.93 for RCT and pRCT; +0.77 a_{30} and pa_{30}) but anyway less than 1. However in the case of $MCP_{100\%}$ selection index with selection criterion MCPp, GR_y for MCP traits is higher than MCPd with -0.27 and +0.27 σ_a for RCT and a_{30}, respectively. In table 5 are reported GR_y for all the traits, except for MCP traits, for the alternative selection indexes and MCPp selection criterion. Genetic response for MY show a negligible correlated effects in terms of GR_y compare to current PFT, with a slightly improvement with selection indexes from $MCP_{5\%}$ up to $MCP_{25\%}$.

Table 5 Genetic response for alternative selection index in Italian Holstein-Friesian population with selection criterion where recording of milk coagulation properties was by mid-infrared spectroscopy prediction (MCPp).

Selection Index	Trait [a]								
	MY	FY	PY	F%	P%	C%	SCS	pH	TA
	in genetic standard deviation								
$MCP_{5\%}$	0.15	0.20	0.24	0.03	0.09	0.09	-0.02	-0.06	0.05
$MCP_{12.5\%}$	0.16	0.19	0.23	0.04	0.10	0.10	-0.03	-0.09	0.07
$MCP_{25\%}$	0.16	0.17	0.21	0.04	0.11	0.12	-0.05	-0.14	0.10
$MCP_{50\%}$	0.13	0.11	0.13	0.04	0.11	0.13	-0.09	-0.22	0.16
$MCP_{75\%}$	0.09	0.04	0.05	0.04	0.09	0.12	-0.10	-0.25	0.18
$MCP_{100\%}$	0.07	0.00	0.00	0.03	0.08	0.11	-0.10	-0.26	0.18

[a] MY= milk yield (kg/305 d); FY= fat yield (kg/305 d); PY= protein yield (kg/305 d); F% = fat (%); P% = protein (%); C% = casein (%); SCS = somatic cell score; TA = titratable acidity (°SH/50 mL).

With the increase of emphasis for MCP more than 25% is estimate a progressive decrease of the GR_y for MY down to less than halving with $MCP_{100\%}$. Fat yield and PY show a decrease of +0.03 and +0.04 σ_a from current PFT, respectively when emphasis for MCP increase up to 25 %. All alternative selection indexes don't affect GR_y for F%. With the increase of emphasis for MCP up to 25 %, increase the GR_y +0.02 and +0.04 σ_a for P% and C%, respectively, compare to current PFT. Somatic cell score showed a progressive improvement of this traits with the increasing of emphasis for MCP with up to -0.09 σ_a GR_y for $MCP_{100\%}$ compare to current PFT. Increasing emphasis for MCP traits in the index showed a correlated strong improvement for milk acidity, up to -0.26 and +0.18 σ_a for pH and TA, respectively with $MCP_{100\%}$.

DISCUSSION

Before setting the G and P matrixes a bending procedure was applied to the phenotypic (P_r) and genotypic (G_r) correlation matrixes in order to convert them to positive definitive matrixes. This was need because 2 and 3 negative eigenvalues for P_r and G_r, respectively were detected. This was probably due because subset of parameters were taken from different sources (Jorjani et al., 2003) and probably because linear dependency of one variable on another exist (Searle, 1982). Comparing bended matrixes and original matrixes, for P_r the procedure adjusted correlation parameters with a magnitude less than 0.03 and not change occurred in sign. For G_r, 12 correlation parameters changed more than 0.05 in magnitude with bending procedure comparing to the original matrix, with a maximum of 0.108 for correlation

between MY and PY, although not change in sign occurred for all parameters. Eight times of 12, occurred in correlation with MY. Linear dependency exist probably because both milk components yield (FY, PY) and milk components as percentage (F% and P%) are included at the same time in IHF selection index. Other counties like Switzerland, Germany, Spain and France adopted an index where components yield and components percentage are both in the index, but commonly only milk components yield plus MY usually with negative weight is formulated (Miglior et al., 2005). This last option is preferred for those traits expressed as ratio like fat and protein percentage for avoid linear dependency among traits and for optimization the improvement of ratio (Gunsett, 1984).

Current selection index in IHF can slightly improve MCP traits. This calculation is deterministic, so it is influenced by the parameters used. Pretto et al. (2011b) showed that using parameters estimated by Vallas et al. (2010) the selection could worsen slightly RCT. The correlation set chosen in this work should be although more representative to the IHF population.

The inclusion of MCP traits in the index with current selection criteria, although showed a fairly GR_y for RCT and a_{30}, it is difficult to apply because there are not phenotypic information of MCP traits and the accuracy of selection is reduced in all pathways of selection (data not reported). The improvement on MCP traits is due to the genetic relationship with other traits in the index, however none of the traits in the current selection criteria can be a strong indicator of MCP traits.

Selection criteria MCPd could be a strategy that might be used for recording MCP directly only in a random sample of available daughters per sire, but need changing in the organization of recording system. Selection criteria MCPp seems the best way for phenotypic recording of MCP traits in the whole population since is more easily to implement with prediction of these traits by MIRS technology in the same sample of recording system used for other traits, without changing the organization of recording system. Moreover with MCPp the improvement for MCP traits is higher than MCPd since repeated information for each cow are available during lactation with consequent increasing the accuracy of genetic prediction.

A relative emphasis slightly more than 12.5% with MCPp selection criterion seems to be optimal for an improvement of about 0.10 σ_a per year for both RCT and a_{30} without lose GR_y for MY, FY and PY.

Including MCP traits in the selection index can increase the GR_y for both P% and C% but with more pressure for casein resulting in an increase of casein:protein ratio.

An interesting result is that including MCP traits in the selection index could be an indirect way for decrease SCS. The current selection index shows a slight improvement for this traits and somatic cell score is important traits both for the health status of the animal as an indirect measure to identify clinical and sub-clinical mastitis (Schutz, 1994) as well as for define the suitability and value of milk for cheese production (Auldist et al., 1996; Lucey and Kelly, 1994) but this trait have a low heritability and repeatability .

The present study considered only some traits from current selection index in IHF because the lack of genetic parameters with remaining traits like morphological, longevity and fertility traits. A recent work reported an negligible genetic relationships between MCP traits and some fertility traits like cow's conception rate at first insemination and calving interval (Kaart et al., 2010) but further investigations are need.

This study was conducted holding steady the current breeding scheme based on conventional progeny testing. Calculation of genetic response that take into account genomic selection could be considered with decreasing of GI in BB and BC pathways.

CONCLUSIONS

This work is one of the first studies providing estimation of genetic response for MCP in dairy cattle population and simulation of alternative selection index with inclusion of these traits.

Current selection index in IHF is not affecting significantly MCP traits. Alternative options were considered in both the selection index and the selection criteria. Selection criteria with the implementation of MIRS prediction for MCP in the current recording systems was the option that allow to reach higher genetic response for RCT and a_{30}. The optimum relative emphasis for MCP traits should be chosen for the option that optimize the overall economic response of breeding program. However is need an up-to-date estimation of economic value for the milk production and quality traits that take into account the future economic situation for Italian dairy industries with removal of quota system limits and the increase of pressure for the dairy process optimisation.

Appendix A

Assumed phenotypic correlations (above diagonal) and genetic correlations (below diagonal) for Italian Holstein-Friesian dairy cows.

Trait [a]	σ_a	h^2	r	MY	FY	PY	F%	P%	C%	SCS	pH	TA	RCT	a_{30}	pRCT	pa_{30}
Milk yield (kg/305 d)	875.40 [b]	0.31 [b]	0.48 [f]		0.49 [g]	0.83 [g]	-0.50 [g]	-0.43 [g]	-0.44 [c]	0.22 [g]	-0.19 [c]	0.19 [c]	-0.24 [c]	0.22 [c]	-0.24 [h]	0.22 [h]
Fat yield (kg/305 d)	33.74 [b]	0.29 [b]	0.40 [f]	0.74 [g]		0.66 [g]	0.51 [g]	0.20 [g]	0.20 [h]	0.07 [g]	0.00 [i]	0.00 [i]	0.00 [i]	0.00 [i]	0.00 [i]	0.00 [i]
Protein yield (kg/305 d)	27.14 [b]	0.30 [b]	0.46 [f]	0.92 [g]	0.81 [g]		-0.16 [g]	0.15 [g]	0.15 [h]	0.22 [g]	0.00 [i]	0.00 [i]	0.00 [i]	0.00 [i]	0.00 [i]	0.00 [i]
Fat (%)	0.31 [b]	0.39 [c]	0.57 [f]	-0.33 [g]	0.37 [g]	-0.12 [g]		0.63 [g]	0.77 [c]	-0.16 [g]	-0.16 [c]	0.49 [c]	-0.05 [c]	0.14 [c]	-0.05 [h]	0.14 [h]
Protein (%)	0.14 [b]	0.30 [c]	0.55 [f]	-0.31 [g]	0.08 [g]	0.10 [g]	0.54 [g]		0.98 [c]	-0.10 [g]	-0.24 [c]	0.58 [c]	-0.08 [c]	0.44 [c]	-0.08 [h]	0.44 [h]
Casein (%)	0.15 [c]	0.35 [c]	0.55 [h]							-0.03	-0.36 [c]	0.65 [c]	-0.22 [c]	0.53 [c]	-0.22 [h]	0.53 [h]
SCS	1.17 [b]	0.21 [b]	0.27 [f]	-0.08 [g]	-0.09 [g]	-0.05 [g]	-0.02 [g]	0.07 [g]	0.00 [c]		0.44 [c]	-0.08 [c]	0.25 [c]	-0.40 [c]	0.25 [h]	-0.40 [h]
pH	0.05 [c]	0.21 [c]	0.36 [d]									-0.68 [c]	0.81 [c]	-0.85 [c]	0.81 [h]	-0.85 [h]
TA (°SH/50 mL)	0.15 [c]	0.17 [c]	0.36 [h]										-0.50 [c]	0.66 [c]	-0.50 [h]	0.66 [h]
RCT (min)	2.22 [c]	0.25 [c]	0.45 [d]	-0.11 [c]	0.00 [i]	0.00 [i]	-0.11 [c]	-0.07 [c]	-0.19 [c]	0.17 [c]	0.52 [c]	-0.43 [c]		-0.89 [c]	0.93 [e]	-0.89 [h]
a_{30} (mm)	4.06 [c]	0.15 [c]	0.50 [d]	0.03 [c]	0.00 [i]	0.00 [i]	0.16 [c]	0.23 [c]	0.32 [c]	-0.14 [c]	-0.46 [c]	0.41 [c]	-0.76 [c]		-0.89 [h]	0.77 [e]
pRCT (min)	1.92 [e]	0.37 [e]	0.45 [h]	-0.11 [h]	0.00 [i]	0.00 [i]	-0.11 [h]	-0.07 [h]	-0.19 [h]	0.17 [h]	0.52 [h]	-0.43 [h]	0.67 [e]	-0.76 [h]		-0.89 [h]
pa_{30} (mm)	4.14 [e]	0.40 [e]	0.50 [h]	0.03 [h]	0.00 [i]	0.00 [i]	0.16 [h]	0.23 [h]	0.32 [h]	-0.14 [h]	-0.46 [h]	0.41 [h]	-0.76 [h]	0.51 [e]	-0.76 [h]	

[a] SCS = somatic cell score; TA = titratable acidity; RCT = rennet coagulation time; a_{30} = curd firmness after 30 min from coagulant addition; pRCT = RCT predicted by mid-infrared spectroscopy (MIRS); pa_{30} = a_{30} predicted by MIR.
[b] Interbull, 2011.
[c] Cassandro et al., 2008.
[d] Vallas et al., 2010.
[e] Cecchinato et al., 2009.
[f] Welper and Freeman, 1992.
[g] Castillo-Juarez et al., 2002.
[h] assumed values.
[i] unknown values.

Appendix B

Derivation of covariance matrixes in cow and bull pathways of selection.

Notation:
$\sigma^2_{p\,ii}$ = phenotypic variance of trait i
$\sigma^2_{a\,ii}$ = genetic variance of trait i
$\sigma_{p\,ii}$ = phenotypic covariance between traits i and j
$\sigma_{a\,ij}$ = genetic covariance between traits i and j
n = number of phenotypic records per animal (own performance in cow pathways, performance of daughter in bull pathways)
r = repeatability of the trait
p =number of animal in progeny group
k = relationship among animal in progeny groups (half-sib= 0.25)
a = relationship among animal in progeny groups and animal to evaluate (bull to daughter=0.5)

Cow pathways

Elements in matrix P

$$\sigma_p{}^2{}_{ii} = \left[r + \frac{1-r}{n}\right] * \sigma^2_{p\,ii}$$

$$\sigma_{p\,ij} = \frac{\sigma_{p\,ij} + (n-1)*\sigma_{a\,ij}}{n}$$

Elements in matrix G
$\sigma^2_{a\,ii} = \sigma^2_{a\,ii}$
$\sigma_{a\,ij} = \sigma_{a\,ij}$

Bull pathways

Elements in matrix P

$$\sigma^2_p = \frac{\left[r+\frac{1-r}{n}\right]+(p-1)k*h^2}{p} * \sigma^2_p$$

$$\sigma_{p\,ij} = \frac{\frac{\sigma_{p\,ij}+(n-1)*\sigma_{a\,ij}}{n} + k*(p-1)*\sigma_{a\,ij}}{p}$$

Elements in matrix G

$\sigma^2_a = a*\sigma^2_a$
$\sigma_{a\,ij} = a*\sigma_{a\,ij}$

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GENERAL CONCLUSIONS

With the first work has been pointed out the problem that a standard definition for MCP traits analysis is needed to enable reliable comparisons between MCP traits recorded in different laboratories, and in different animal populations and breeds. The transformation of MCP traits analyzed with different methodologies is feasible but was more precise for RCT (R^2 0.77-0.82) than for a_{30} (R^2 0.28-0.63). The analyses of non-coagulated probabilities of milk samples showed that NC samples from one methodology were well distinguishable (with an accuracy of 0.972-0.996) based on the rennet coagulation time measured with the other methodology.

The effect of MCP in field condition for the one of the most important cheese product in Italy has been estimated. Milk characterized by high values of a_{30} resulted in higher cheese yield than milk with low values of a_{30}. Findings from our study indicate that MCP could be used as indicators of cheese making efficiency and proposed as traits to be included in multiple component milk pricing systems.

In agreement with previous study has been found the notable influence of composite *β*- and *κ*-casein genotypes on MCP. Moderate to negligible impact of composite *β*- and *κ*-casein genotypes on milk production traits were found. The inclusion of casein genotypes led to a decrease of 47, 68, 18, and 23% in the genetic variance for RCT, a_{30}, pH, and TA, respectively, and less than 6% for other traits. Heritability of RCT was still appreciable after adjustment for composite *β*- and *κ*-casein genotypes, suggesting that the recording of this trait cannot be replaced by genotyping of animals for milk protein variants. However, because MCP are affected by casein loci, and cheap and powerful techniques for simultaneous detection of the genetic variants are available, it can be argued that combining RCT data and casein information could provide a tool to better address future selection decisions aiming to improve MCP.

A method for calculating economic values of milk coagulation properties traits was proposed, based on the maximum impact that MCP properties could affect the cheese yield.
The weight for MCP in a possible sub-index for milk production and quality traits ranged from 2.1 to 8.1 % in Asiago cheese destination and from 2.2 to 8.3 % for Grana Padano cheese destination. These range of economic value could be used for simulate selection indexes for improve MCP traits and estimate the response of selection for all the traits.

Current selection index for Italian Holstein Friesian seems not affecting significantly MCP traits. Selection criteria with the implementation of MIRS prediction of MCP in the current recording systems was the option that allow to reach higher genetic response for MCP traits. The response was higher than measure once per cow MCP traits by direct instrument. An emphasis slightly more than 12.5% should reach an improvement of about 0.10 genetic standard deviation per year for both rennet coagulation time and curd firmness without lose improvement for milk yield, fat yield and protein yield. Moreover including MCP traits in the selection index can increase casein:protein ratio and could be an indirect way for decrease SCS.