Eesti Maaülikool
Estonian University of Life Sciences

GENETIC AND MODELLING ASPECTS OF MILK COAGULATION PROPERTIES IN DAIRY CATTLE

PIIMA LAAPUMISOMADUSTE MODELLEERIMINE JA GENEETILINE DETERMINEERITUS

MIRJAM VALLAS

A Thesis
for applying for the degree of Doctor of Philosophy
in Animal Science

Väitekiri
filosoofiadoktori kraadi taotlemiseks
loomakasvatuse erialal

Tartu 2013

EESTI MAAÜLIKOOL
ESTONIAN UNIVERSITY OF LIFE SCIENCES

Eesti Maaülikool
Estonian University of Life Sciences

GENETIC AND MODELLING ASPECTS OF MILK COAGULATION PROPERTIES IN DAIRY CATTLE

PIIMA LAAPUMISOMADUSTE MODELLEERIMINE JA GENEETILINE DETERMINEERITUS

MIRJAM VALLAS

A Thesis
for applying for the degree of Doctor of Philosophy
in Animal Science

Väitekiri
filosoofiadoktori kraadi taotlemiseks
loomakasvatuse erialal

Tartu 2013

Institute of Veterinary Medicine and Animal Sciences
Estonian University of Life Sciences

According to verdict No 141 of May 23, 2013, the Doctoral Committee of the Agricultural and Natural Sciences of the Estonian University of Life Sciences has accepted the thesis for the defence of the degree of Doctor of Philosophy in Animal Science.

Opponent: Ass. Prof. Mauro Penasa, PhD
University of Padova, Italy

Supervisors: Elli Pärna, PhD
Estonian University of Life Sciences

Prof. Kalev Pärna, PhD
University of Tartu

Ass. Prof. Tanel Kaart, PhD
Estonian University of Life Sciences

Defence of the thesis:
Estonian University of Life Sciences, room A201, Kreutzwaldi 62, Tartu on June 27, 2013, at 10:00.

The English language was edited by David Arney and the Estonian by Nele Rand.

Publication of this dissertation is supported by the Estonian University of Life Sciences and by Bio-Competence Centre of Healthy Dairy Products LLC.

ISBN 978-9949-484-87-4 (trükis)
ISBN 978-9949-484-88-1 (pdf)

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on following publications referred to by Roman numerals in the text:

I **Vallas, M.**, Bovenhuis, H., Kaart, T., Pärna, K., Kiiman, H., Pärna, E. 2010. Genetic parameters for milk coagulation properties in Estonian Holstein cows. – Journal of Dairy Science, 93(8), 3789–3796.

II Pretto, D., Kaart, T., **Vallas, M.**, Jõudu, I., Henno, M., Ancilotto, L., Cassandro, M., Pärna, E. 2011. Relationship between milk coagulation property traits analyzed with different methodologies. – Journal of Dairy Science, 94(9), 4336–4346.

III **Vallas, M.**, Kaart, T., Värv, S., Pärna, K., Jõudu, I., Viinalass, H., Pärna, E. 2012. Composite β-κ-casein genotypes and their effect on composition and coagulation of milk from Estonian Holstein cows. – Journal of Dairy Science, 95(11), 6760–6769.

The contributions from the authors to the papers are as follows:

Paper	Original idea and structure	Data collection	Data analysis	Manuscript preparation
I	MV, HB, TK, EP	EP, MV	MV	All
II	DP, TK, MV, EP, MC, IJ	DP, LA, IJ, EP	TK, DP, MV, IJ	All
III	MV, TK	EP, MV, SV, IJ	MV	All

MV – Mirjam Vallas; HB – Henk Bovenhuis; TK – Tanel Kaart; EP – Elli Pärna; DP – Denis Pretto; MC – Martino Cassandro; IJ – Ivi Jõudu; LA – Lucia Ancilotto; SV – Sirje Värv; All – all authors of the paper.

ABBREVIATIONS

ASO-PCR	Allele-specific (oligo) PCR
a_{30}	Curd firmness, measured in volts
CRM	Computerized Renneting Meter
DNA	Deoxyribonucleic Acid
EARC	Estonian Animal Recording Centre
EHF	Estonian Holstein breed
E_{30}	Curd firmness, measured in millimetres
LAT	Lattodinamografo
MCP	Milk coagulation properties
NC	Non-coagulation
NIR	Near-infrared
OPT	Optigraph
PC	Poor coagulation
PCR	Polymerase Chain Reaction
RCT	Milk coagulation time
RFLP	Restriction Fragment Length Polymorphism
ROC	Receiver operating characteristic, statistical analysis
SCC	Somatic cell count
SCS	Somatic cell score, log-transformed SCC
α_{S1}-CN	Casein alpha S1, milk protein
β-CN	Casein beta, milk protein
β-LG	Lactoglobulin beta, milk protein
β-κ-CN	Composite beta- and kappa casein, milk protein
κ-CN	Casein kappa, milk protein

1. INTRODUCTION

Currently, genetic information on milk production, udder health, conformation, longevity, calving and female fertility traits is considered in dairy cattle. The dairy industry, however, is interested also in milk with good technological properties to guarantee better quality products and higher income.

Milk coagulation properties (MCP) have great importance for the dairy industry because of their influence on cheese outcome and quality (Aleandri *et al.*, 1989; Ng-Kwai-Hang *et al.*, 1989; Johnson *et al.*, 2001). At present, in Italy about 70% (referred by Pretto, 2012), in the Netherlands about 50% (referred by Heck, 2009), in Scandinavian countries 33% (referred by Wedholm *et al.*, 2006), and in Estonia 54% (according to the total milk and cheese production in 2011, reported by Statistikaamet, 2012) of milk is used for cheese production. Cheese production in Estonia has increased over the last decade. The production of cheese (with several cheese categories included) increased from 20,000 tons in the year 2000 to 40,630 tons in 2011 (Eurostat, 2013). To promote progress in the Estonian dairy sector, the Estonian Dairy Strategy 2012–2020 of the Ministry of Agriculture of Estonia includes the objective to increase annual milk production by one third and enhance development of milk products with added value. Among other activities, this needs scientific collaboration for the genetic and nutritional improvement of Estonian dairy herds to increase the efficiency of production and concurrently ensure sustainability in the dairy sector (Eesti piimanduse …, 2012).

Traditional processes of coagulation in the dairy industry can be divided into heat, acid and/or rennet coagulation. The last type of coagulation is of interest in the current study and will be referred as milk coagulation. The use of rennet to coagulate casein to form a curd that is then further processed into cheese is a traditional process that forms the initial step in the manufacture of a wide variety of cheese types (IDF, 2007).

Commonly, the main milk coagulation traits studied are milk rennet coagulation time, which is the time from the addition of coagulant to milk until the beginning of coagulation, and curd firmness, which is the strength of the curd 30 min after the addition of rennet. Milk with good

coagulation properties has a short coagulation time and forms a firm curd, yields more and higher quality cheese. Therefore, improvement of milk coagulation traits could be of economic advantage to the dairy industry. Besides the direct measurements, these milk coagulation traits are recorded using different systems based on optical, thermal, mechanical, and vibrational methods, which have been comprehensively reviewed by O'Callaghan *et al.* (2002) and Lucey (2002a). Different instruments are available commercially and are currently widely used in research institutes to record milk coagulation traits. No standard method for MCP determination, however, has been established. The most common method used for research in MCP has been the mechanical method. Optical methods, however, provide good potential for developing on-line methods for industrial applications for the continuous observation of MCP without interfering with the coagulation process. In the current study, milk coagulation traits were measured by using the optical method.

Interest in MCP in recent decades has resulted in numerous studies on methodology, genetic parameters of MCP and the associations of MCP with milk protein polymorphism, milk protein content and cheese-making properties.

As MCP are found to be influenced by genetic factors, genetic improvement of milk coagulation could be a consideration. Although it has been suggested that genetic factors affect milk coagulation, few studies have quantified the extent to which genetic factors play in this role. Furthermore, only a few studies in Finnish and Italian dairy cattle populations have reported genetic relationships between milk coagulation traits and milk production and composition traits. Associations of MCP with milk production and composition traits are important when considering selection possibilities for the improvement of MCP, to avoid deterioration in these other economically important traits. Reported heritability estimates for MCP vary from low to moderate, indicating potential for genetic improvement of these milk properties. Genetic correlation between MCP has been found to be strong, while there is some inconsistency in genetic correlations between MCP and milk production and composition traits.

Regular direct large-scale estimation of breeding values for these traits, however, is complicated due to the time consuming measuring method of MCP. Mid-infrared spectroscopy technology has been proposed as a

cheaper method to predict MCP routinely, and for large-scale recording (De Marchi *et al.*, 2009). However, this technology is based on the determination of a calibration equation predicted from spectral data and requires a MCP analytical methodology specific reference method, which could cause further complication.

An alternative indirect selection approach for the improvement of milk quality could be milk protein polymorphisms. Associations have been studied between milk protein polymorphisms and milk production traits and protein composition. One of the most striking effects of milk protein polymorphisms on traits of economic interest is their relationship with the cheese-making properties of milk (review by Caroli *et al.*, 2009), including milk coagulation properties.

In Estonia, previous research on MCP by Mihhejev (2002) and Jõudu (2008) has been focused on finding the factors influencing milk coagulation properties, comparing analytical procedures and providing an overview of milk coagulation, and variation in the milk protein content and milk protein polymorphism among dairy cattle breeds in Estonia (Estonian Native, Estonian Red and Estonian Holstein). According to the study by Jõudu (2008), MCP are better in the Estonian Native and Estonian Red cows. The Estonian Holstein breed, however, forms about 75% of the Estonian dairy cow population (Eesti jõudluskontrolli …, 2012) and has been under selection for higher milk yield. Other recent studies in Estonia have focused on the associations between MCP and milk and blood metabolomes (Harzia *et al.*, 2012) and feeding strategies (Harzia *et al.*, 2013).

The large-scale study described in this thesis provides more insight into the genetic information needed for the development of selection strategies in Estonian Holstein cattle, and is a part of the research project conducted by the Bio-Competence Centre of Healthy Dairy Products LLC.

2. REVIEW OF THE LITERATURE

2.1. Process and measurement of milk coagulation

Milk coagulation is a complicated process that is influenced by many factors. Rennet coagulation of milk may be divided into primary (enzymic hydrolysis) and secondary (aggregation) stages, although these stages normally overlap to some extent during cheese making. During the primary stage, κ-CN is cleaved by rennet to form para-κ-CN and rennet-altered micelles become susceptible to aggregation (Walstra, 1990). Overlap with the aggregation stage occurs (under conditions of normal pH and protein content) after at least about 87% of κ-CN is hydrolysed and aggregation of rennet-altered micelles starts. The aggregation process in the secondary stage leads to formulation of rennet coagulum. The nature of the attractive forces during the aggregation of casein micelles is still not completely understood (Lucey, 2002a,b).

Common milk coagulation traits are milk coagulation time (RCT), curd firming time and curd firmness (E_{30} or a_{30}). RCT characterizes the enzymic stage of the milk coagulation process and curd firmness describes the aggregation stage. Curd firming time, commonly defined as the time needed until the curd is firm enough to be cut (Ikonen, 2000), is quite often not reported because of the slow coagulation process, which results in a considerable proportion of samples not reaching curd firmness sufficient to be cut during the usual test period of 30 minutes.

A variety of instruments have been used for measuring milk coagulation in different studies: the Formagraph (Ikonen *et al.*, 1999; Malacarne *et al.*, 2006; Jõudu *et al.*, 2007, 2008), the Optigraph (Harzia *et al.*, 2012; Leitner *et al.*, 2006), the Computerized Renneting Meter (Ikonen *et al.*, 2004; Cassandro *et al.*, 2008; Cecchinato *et al.*, 2011; Dal Zotto *et al.*, 2008; Pretto *et al.*, 2013), the Bohlin VOR rheometer (Wedholm *et al.*, 2006; Hallén *et al.*, 2007) and the ReoRox4 rheometer (Frederiksen *et al.*, 2011a,b; Jensen *et al.*, 2012a,b).

The Formagraph (McMahon, Brown, 1982) was the first mechanical instrument that was widely used. The principle of this instrument is based on 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 Ikonen (2000), expressing curd firmness in millimetres.

Measurements made with the Optigraph are not based on a rheological method but on an optical signal in the near-infrared (NIR) wavelength. During a coagulation test, the light emitted through the milk gradually weakens, because of changes in the micellar structure of casein (CN). The Optigraph calculates the coagulation parameters (coagulation time, curd firmness, and speed of aggregation) by means of particular feature points extracted from the optical information acquired in real time (Optigraph User's Manual) and expresses values of curd firmness in volts.

After the manufacture of the Formagraph and its accessories ceased, a new instrument, the Optigraph, developed by the French research institute INRA was studied to compare the coagulation measurement techniques of these instruments and find possibilities to convert the electrical parameters obtained by the Optigraph into millimetres, which were used to estimate curd firmness by Formagraph and other mechanical instruments previously in use (Kübarsepp *et al.*, 2005a).

Klandar *et al.* (2007) compared five analytical techniques and concluded that NIR spectroscopy, an on-line sensor, could best assess rennet-induced coagulation of skimmed milk samples. The previous mechanical instrument, the Formagraph, has been replaced by more developed instrument, which has the same measurement principle. This instrument, the Lattodinamografo produced by Foss Italy, has also been referred to as a Formagraph in recent literature (Cecchinato *et al.*, 2013; Cipolat-Gotet *et al.*, 2012). Comparison of MCP measures of this mechanical device with NIR-based optical MCP measurements from the Optigraph showed considerable differences (Cipolat-Gotet *et al.*, 2012). Another optical method for predicting MCP measurements is based on mid-infrared spectroscopy technology (De Marchi *et al.*, 2009). This technology is based on the determination of a calibration equation predicted from spectral data and a reference method and, therefore, the lack of calibration equations for the different reference methods of MCP analysis could cause further complications.

2.2. Variation in milk coagulation properties

2.2.1. Non-coagulation and poor coagulation of milk in dairy cattle populations

Different study populations have been characterized by the proportion of milk samples with poor coagulation (PC) and non-coagulated (NC) samples. Definitions used for these classifications are discussed in the material and methods section 4.1.

Reports on the proportion of PC and NC milks vary between study populations. Tyrisevä (2008) observed that about 30% of Finnish Ayrshire cows and 12% of Holstein Friesian cows produced PC milk and about 10% of Finnish Ayrshire cows and 1% of Holstein Friesian cows produced NC milk. Proportions of PC and NC samples were 28% and 3%, respectively, in a study of Swedish Red-and-White, Swedish Holstein and Danish Holstein cows by Wedholm (2008). Jõudu (2008) reported 14 to 16% of milk samples with PC and 3 to 6% NC milk samples in the study of dairy cattle breeds in Estonia (Estonian Holstein, Red-and-White Holstein, Estonian Red and Estonian Native). The proportion of NC milk in the Italian cattle population of 4% for Italian Brown Swiss (Cecchinato *et al.*, 2009), 6.3% for Simmental (Bonfatti *et al.*, 2010a) and 9.7% for Italian Holstein Friesian cows (Cassandro *et al.*, 2008) were observed. According to Frederiksen *et al.* (2011a), the prevalence of NC milk was 3% and PC about 20% in Danish dairy breeds (Jersey, Danish Red and Danish Holstein Friesian).

These comparisons between studies are somewhat arbitrary, to some extent, due to differences in measurement procedures and instruments, and also to the somewhat different classifications used for PC milk. They give, however, a general overview of the phenotypic variation of MCP of cows in different study populations in discrete classes.

2.2.2. Environmental variation

The main compositional and processing factors affecting MCP, described by Lucey (2002b), include temperature, enzyme concentration, heat treatment of milk, calcium, NaCl, solid content and pH of milk. The main milk compositional characteristics, such as fat, protein, calcium

and phosphorus contents influence MCP as reported by McMahon *et al.* (1984), Okigbo *et al*. (1985a), Jõudu (2008), Hallén *et al*. (2010) and Jensen *et al*. (2012b).

Milk pH was the most significant factor that affected coagulation time and curd firmness in an experimental study by Okigbo *et al*. (1985b). An association between low pH and favourable MCP has been reported in several observational studies (Ikonen *et al*., 1997; Tyrisevä *et al*., 2003; Ikonen *et al*., 2004; Cassandro *et al*., 2008).

An effect of total protein content on MCP has been observed in several studies. Okigbo *et al.* (1985b) and Hallén *et al*. (2007) found that the concentration of total milk protein content was positively associated with milk coagulation as it increased curd firmness, while no association for protein content with MCP was reported by Ikonen *et al.* (2004) and moderate correlation with curd firmness (0.23) was observed by Cassandro *et al*. (2008). Thus, the use of total protein content as an indicator trait for MCP is limited. Protein composition, rather than total protein or total CN content, is important in milk coagulation (Hallén, 2008). According to Wedholm *et al*. (2006), MCP depend on the CN composition, and CN number (i.e. the proportion of CN to total protein) was significant for the transfer of protein from milk to cheese.

Somatic cell score (SCS) is used as a criterion for assessing milk hygienic quality. Several studies have reported an unfavourable association between SCS and MCP (Grandison, Ford, 1986; Politis, Ng-Kwai-Hang, 1998; O'Brien *et al*., 2001; Ikonen *et al*., 2004; Leitner *et al*., 2006, 2012; Cassandro *et al*., 2008). However, Jõudu (2008) found no clear effect of SCS on the MCP. Also Ikonen *et al*. (1997), Somers *et al*. (2002), Wedholm *et al*. (2006) and Leitner *et al*. (2008) found no significant effect of SCS on MCP. Despite the inconsistencies in association between SCS and MCP, an unfavourable effect of udder infection on MCP was observed by Leitner *et al*. (2006, 2012) and Merin *et al*. (2008). According to Merin *et al*. (2008), SCS actually reflects herd udder status, and thus, the deleterious effect is not the number of somatic cells present in the milk itself, but rather the intramammary infection status of the whole herd milked, and collected into the bulk tank.

The effect of lactation stage on MCP has been found to be significant in many studies, while the change in MCP during lactation has been found

to be somewhat different between studies. Most commonly reported is that poorest MCP is found in mid-lactation (Ostersen *et al.*, 1997; Ikonen *et al.*, 1999a, 2004; Tyrisevä *et al.*, 2003, 2004; Jõudu, 2008; Leitner *et al.*, 2012). According to Okigbo *et al.* (1985b), however, the mean RCT generally increased as lactation progressed and curd firmness was generally greatest in mid-lactation samples. De Marchi *et al.* (2007) also reported a better RCT of herd milk at the beginning of lactation. According to Coulon *et al.* (1998), maximum firmness of the coagulum of late-lactation milk was not different from that of either early or mid-lactation milks. A non-significant effect of lactation stage in some studies could be due to confounding effects of lactation stage and season (Ikonen *et al.*, 1999a).

Contradictory results have been reported for the effect of parity on MCP. A deterioration in MCP with increasing parity number was observed by Lindström *et al.* (1984) and Tyrisevä *et al.* (2003). An opposite effect of parity was reported by Schaar (1984), but in studies by Pagnacco, Caroli (1987), Ikonen *et al.* (1999a) and Tyrisevä *et al.* (2004) no significant effect of parity was found. No clear effect of parity was also found in Estonian dairy cattle by Jõudu (2008), and there was no effect of parity among Estonian Native cows (Jõudu *et al.*, 2007), whereas in a study involving other Estonian dairy breeds (Estonian Holstein, Red-and-White Holstein and Estonian Red) MCP were significantly influenced by parity (Kübarsepp *et al.*, 2005b).

In addition to the factors influencing MCP described above, some other factors are referred. Studies have also shown effects of daily milking frequency (Martin *et al.*, 2009) and renneting meter sensor of lactodynamograph (Cecchinato *et al.*, 2013). High milk yield as a risk factor was observed by Tyrisevä *et al.* (2003). A year-season effect, showing best MCP during the grazing season of June to August, was found by Tyrisevä *et al.* (2003). A season effect was also found in a herd milk study by De Marchi *et al.* (2007), which showed better MCP in September and October. Ikonen *et al.* (1999a) found little variation due to herd in RCT. The herd explained only a minor part of the variation in MCP also in studies by Tyrisevä *et al.* (2004) and Ikonen *et al.* (2004). Frequent feeding of the concentrate was associated with good MCP (Tyrisevä *et al.*, 2004). Macheboeuf *et al.* (1993) reported better MCP for group fed at a higher dietary energy level.

2.2.3. Variation among breeds

Significant differences in MCP between dairy cattle breeds have been observed in several studies (Ikonen *et al.*, 1999a; Auldist *et al.*, 2004; Tyrisevä *et al.*, 2004; Malacarne *et al.*, 2006; De Marchi *et al.*, 2008; Jõudu, 2008; Frederiksen *et al.*, 2011a). Several studies reported less favourable MCP for high-yielding dairy breed such as Holstein compared to indigenous dairy breeds (Macheboeuf *et al.*, 1993; Lien *et al.*, 1999; Auldist *et al.*, 2002; Kübarsepp *et al.*, 2005b; De Marchi *et al.*, 2007). This is explained by the lower frequency of the κ-CN B allele, associated with better MCP, observed in the Holstein population. Thus, effect of breed is, to some extent, associated with the effect of the other important genetic factor, milk protein polymorphism, due to different distributions of the genetic variants of milk proteins in different breeds (Lien *et al.*, 1999).

2.2.4. Variation due to protein polymorphism

General agreement exists of the favourable effect of the κ-CN B allele (Pagnacco, Caroli, 1987; Jakob, Puhan, 1992; Macheboeuf *et al.*, 1993; Mayer *et al.*, 1997; Walsh *et al.*, 1998; Ikonen *et al.*, 1997, 1999a; Comin *et al.*, 2008; Jensen *et al.*, 2012a) and the unfavourable effect of the κ-CN E allele on MCP (Ikonen *et al.*, 1997, 1999a; Caroli *et al.*, 2000; Ojala *et al.*, 2005; Wedholm *et al.*, 2006). In addition, several studies observed an association of genetic polymorphism of κ-CN with milk protein percentage (Aleandri *et al.*, 1990; Bovenhuis *et al.*, 1992; Ikonen *et al.*, 1999b; Heck *et al.*, 2009). Furthermore, Hallén *et al.* (2007) found the κ-CN B allele to be associated with a higher concentration of κ-CN, while the lowest κ-CN concentration was observed for the κ-CN E allele. A higher concentration of κ-CN associated with the κ-CN B allele was also reported by Heck *et al.* (2009), although a lower concentration of κ-CN was found for κ-CN A allele compared to the E allele in this study. An important role of κ-CN concentration can be presumed also in the association between β-CN and MCP, as indicated by Hallén (2008) who observed a favourable effect of the β-CN A1A2 genotype on MCP, and also a higher concentration of κ-CN for this genotype within the composite β-κ-CN genotype containing the β-CN A1A2 genotype. However, Heck *et al.* (2009) reported a higher relative concentration of β-CN to be related to the β-CN A1A2 genotype compared to the homozygous β-CN genotypes

of the A1 and the A2 alleles. Polymorphism of β-CN was also found to be related to milk fat percentage and milk fat and protein yields (Ng-Kwai-Hang *et al.*, 1984; Bovenhuis *et al.*, 1992; Ikonen *et al.*, 1999b; Comin *et al.*, 2008).

Jensen (2012) reported that a strong genetic contribution in relation to milk coagulation was clear, as the majority of poorly or NC samples for both Danish Jersey and Danish Holstein-Friesian breeds had the same composite BBA2A2AA genotype of α_{S1}-β-κ-CN. The same α_{S1}-β-κ-CN genotype was found to be most frequent (21.2%) among Estonian Native cows. However, this breed nevertheless showed the best MCP compared to other breeds in Estonia, due to the highest prevalence of the favourable κ-CN B allele (Jõudu *et al.*, 2007). Little research on the association between MCP and α_{S1}-CN genotypes has been carried out, because this locus is found to be rather monomorphic in cattle populations (Jõudu *et al.*, 2007).

β-lactoglobulin (β-LG) has shown an inconsistent effect on MCP in different studies. Marziali and Ng-Kwai-Hang (1986) reported the best MCP for the β-LG AA genotype, while Kübarsepp *et al.* (2005b) and Ikonen *et al.* (1999a) found a favourable effect of β-LG AA genotype, but this was only for RCT. However, Pagnacco, Caroli (1987), Ikonen *et al.* (1997) and Hallén *et al.* (2007) observed no effect of β-LG genotypes on MCP. Studies on milk protein composition have observed higher concentrations of CN with the β-LG BB genotype compared to the β-LG AA genotype (Ikonen *et al.*, 1997; Lundén *et al.*, 1997; Bobe *et al.*, 1999; Hallén *et al.*, 2008; Heck *et al.*, 2009). These inconsistencies between different studies might be due to the indirect influence of the epistatic effect between the κ-CN, β-CN and β-LG loci for milk total protein and casein content, observed by Mayer *et al.* (1997).

In review by Bittante *et al.* (2012) summarising different studies on milk protein genotype effects on MCP the authors reported a moderate effect of β-LG genotype on MCP. In particular, the average MCP values of the BB genotype were poorer than those of the AA genotype, and the MCP of heterozygous AB genotype were intermediate. However, the variability of estimates was found to be very large and the authors concluded that any effect of this gene needs to be studied further.

Because of the genetic linkage between the β-CN and κ-CN loci, composite β-κ-CN genotypes or haplotypes have been proposed for the estimation of casein genotype effects (Lundén *et al.*, 1997; Ojala *et al.*, 1997; Comin *et al.*, 2008) on milk performance traits. The data from these studies generally confirm previous results of estimates of casein genotype effects on MCP separately (review by Bittante *et al.*, 2012).

Recently, large-scale studies have been conducted (Comin *et al.*, 2008; Bonfatti *et al.*, 2010a; Penasa *et al.*, 2010), enabling more accurate adjustment for composite casein genotype effects in the estimation of the additive genetic variance component of milk composition and coagulation traits. However, few studies have reported the presence of the β-CN I allele (Bonfatti *et al.*, 2010a,b; Visker *et al.*, 2011), and no clear influence of this allele on milk coagulation traits has been reported. Furthermore, only the small-sample-size study by Ikonen *et al.* (1997) used β-κ-CN genotypes and repeated measurements, allowing the separation of variance components of additive genetic and permanent environmental effects in the model for analysis of variation in milk coagulation.

2.2.5. Polygenetic variation

Genetic parameters of MCP have been much less investigated compared to research findings available on associations between protein polymorphism and MCP (Table 1). Without considering the uncommonly high estimates derived by Ikonen *et al.* (1997) and heritability of combined MCP (Bittante *et al.*, 2002), the magnitude of heritability estimates for RCT has ranged from 0.13 to 0.38 and from 0.12 to 0.44 for curd firmness, indicating moderate genetic variation among cows in dairy cattle populations.

Only a few studies (Tervala *et al.*, 1985; Ikonen *et al.*, 1997; Cecchinato *et al.*, 2013) have provided information on heritability estimates of curd firming time. Estimates on scarce datasets were considerably different and varied from 0.02 (Tervala *et al.*, 1985) to 0.60 (Ikonen *et al.*, 1997). A recent study by Cecchinato *et al.* (2013) reported heritability estimates of 0.21 based on Formagraph measurements and 0.37 based on Optigraph measurements of curd firming time.

Some studies have observed an influence of protein polymorphism on the additive genetic variation of MCP (Ikonen *et al.*, 1997, 1999a; Bonfatti *et al.*, 2010a, 2011; Penasa *et al.*, 2010). After accounting for the effect of protein polymorphism moderate decreases in additive genetic variation estimates (20% and 24% for RCT and curd firmness, respectively) were observed by Ikonen *et al.* (1999a). These results are similar to those of Bonfatti *et al.* (2011) who reported a decrease in additive genetic variance of 27% and 21% for RCT and curd firmness, respectively. A moderate influence of protein polymorphism on the additive genetic variance of MCP was also reported by Ikonen *et al.* (1997) and Bonfatti *et al.* (2010a), while Penasa *et al.* (2010) found a considerably higher reduction in additive genetic variance (47% and 68% for RCT and curd firmness, respectively).

Additionally to Penasa *et al.* (2010), two other studies estimating heritabilities of MCP have accounted for protein polymorphism (Ikonen *et al.*, 1997; Bonfatti *et al.*, 2011). The lowest heritability estimates for curd firmness were reported in a large-scale studies by Penasa *et al.* (2010) and Bonfatti *et al.* (2011), while the largest heritabilities were estimated for both MCP in a small-sample-sized study by Ikonen *et al.* (1997).

Some studies on MCP used repeated measurements for the estimation of repeatabilities of MCP (Schaar, 1984; Caroli *et al.*, 1990; Ikonen *et al.*, 1997; Tyrisevä *et al.*, 2003). The repeatability estimates in these studies ranged from 0.43 to 0.66 for RCT and from 0.57 to 0.68 for curd firmness. Thus, MCP are repeatable and could be sampled at least three times per cow during lactation to derive reliable breeding value estimates of MCP, as suggested by Ikonen (2000) and Tyrisevä *et al.* (2003).

Due to the different sample sizes, dataset structures and availability of analytical techniques, different statistical models were used in the different studies. In earlier studies, simple sire models were applied, in later studies repeated measures, random regression or threshold animal models were applied. However, there is no clear association between heritability estimates of MCP and analytical or statistical methods used in different studies, as was also concluded by Bittante *et al.* (2012).

Studies were implemented also with different breeds and populations (Table 1) but no clear differences or similarities could be found.

The majority of studies on genetic parameters of MCP have also reported heritabilities for milk production and composition traits which were, in general, lower for milk production, acidity and SCS and similar for milk composition traits, when compared to the heritability estimates for MCP (Bittante *et al.*, 2012).

Table 1. Heritability estimates of milk coagulation time (RCT) and curd firmness (E_{30}) in different studies, with descriptive traits of experimental conditions and analytical technique used (modified from a review by Bittante *et al.*, 2012).

Reference	Breed[1]	Number of			Model	Instrument[2]	Type of rennet[3]	Rennet concentration, IMCU/mL[4]	Heritability	
		samples	cows	sires					RCT	E_{30}
Lindström *et al.* (1984)	Ay	731	731	20	Sire	Sommer M.	Hansen St.	–	0.27	–
Tervala *et al.* (1985)	Ay, HF, FC	319	319	18	Sire	Formagraph	Hansen St.	–	0.13	0.25
Oloffs *et al.* (1992)	HF	–	3,346	92	Sire	Formagraph	–	–	0.27	0.30
Oloffs *et al.* (1992)	An	–	1,507	45	Sire	Formagraph	–	–	0.38	0.39
Ikonen *et al.* (1997)	Ay	174	59	25	Animal	Formagraph	Renco l.	0.116	0.62	0.41
Ikonen *et al.* (1997)	HF	155	55	32	Animal	Formagraph	Renco l.	0.116	0.35	0.57
Ikonen *et al.* (1999a)	Ay, HF	895	895	287	Animal	Formagraph	RCRL	0.116	0.22	0.40
Bittante *et al.* (2002)	HF	6,909	517	–	Animal	Formagraph	–	–	0.40	–
Tyrisevä *et al.* (2004)	Ay, HF, CB	1,408	1,408	547	Animal	CRM	Hansen 190	0.114	0.21	0.22
Ikonen *et al.* (2004)	Ay	4,664	4,664	91	Animal	CRM	Hansen 190	0.114	0.28	0.22
Cassandro *et al.* (2008)	HF	1,042	1,042	54	Animal	CRM	Hansen 190	0.061	0.25	0.15
Cecchinato *et al.* (2009)	BS	1,200	1,200	50	Animal	CRM	Hansen 190	0.061	0.32	0.24
Kaart *et al.* (2010)	HF	18,825	5,007	–	Animal	Optigraph	Milase 750	0.150	0.34	0.44
Penasa *et al.* (2010)	HF	1,025	1,025	54	Animal	CRM	Hansen 190	0.061	0.25	0.12
Cecchinato *et al.* (2011)	BS	1,234	1,234	58	Animal	CRM	Hansen 190	0.061	0.24	0.15
Cecchinato *et al.* (2011)	HF	1,025	1,025	54	Animal	CRM	Hansen 190	0.061	0.21	0.17
Bonfatti *et al.* (2011)	Si	2,167	2,167	–	Animal	CRM	Hansen 190	0.061	0.29	0.12
Cecchinato *et al.* (2013)	BS	913	913	–	Animal	Formagraph	Hansen 160	0.051	0.23	0.17
Cecchinato *et al.* (2013)	BS	913	913	–	Animal	Optigraph	Hansen 160	0.051	0.24	0.21

[1] Ay – Ayrshire; HF – Holstein-Friesian; FC – Finncattle; An – Angler; CB – crossbreds (Ay x HF); BS – Brown Swiss; Si – Simmental.

[2] Formagraph (Foss Electric A/S, Hillerød, Denmark); CRM (Polo Trade, Monselice, Italy); Optigraph (Ysebaert SA, Frépillon, France).

[3] Renco rennet (Renco New Zealand, Eltham, New Zealand); Hansen 190 rennet (PacovisAmrein AG, Bern, Switzerland); Renco calf rennet liquid (RCRL, New Zealand Rennet Company Ltd., Eltham, New Zealand); Hansen 160 rennet (PacovisAmrein AG); Milase 750 rennet (CSK Food Enrichment BV, Ede, the Netherlands).

[4] IMCU – international milk clotting unit.

2.3. Selection for improvement of milk coagulation properties

2.3.1. Direct polygenetic selection

MCP are moderately heritable (Table 1), which suggests that direct selection on MCP could be considered for improvement in these economically important milk properties. Strong negative genetic correlation between RCT and curd firmness (–0.77 to –0.97) has been reported (Ikonen *et al.*, 1999a, 2004; Cassandro *et al.*, 2008; Penasa *et al.*, 2010; Cecchinato *et al.*, 2011; Bonfatti *et al.*, 2011; Cecchinato *et al.*, 2013) and indicates that selection for one of the MCP would also result in improvement in the other trait. Useful information for the consideration of direct polygenetic selection can be derived from genetic correlations between MCP and economically important traits, such as milk yield, milk composition and quality traits. Average correlations between MCP and milk production and composition traits reported in review by Bittante *et al.* (2012) showed no detrimental associations, supporting the potential for direct improvement of MCP. Moreover, MCP seem to have favourable associations with fertility traits (Kaart *et al.*, 2010). However, genetic associations found in previous studies are inconsistent and further research on the estimation of these associations is necessary. Furthermore, in the present situation, no well-established high capacity methodology exists for measuring MCP and performing mass selection routinely. As an alternative, Cassandro *et al.* (2008) suggested direct recording of MCP only in a random sample of available daughters per sire, and Ikonen (2000) proposed that breeding values for milk coagulation traits could be estimated for a selected important group of dairy animals, e.g., bull dams and young AI-bulls.

2.3.2. Indirect polygenetic selection

Investigation into the possibilities for indirect selection for improvement of MCP is based mainly on estimation of genetic correlations between MCP and milk composition traits, to identify milk traits which are sufficiently highly favourably genetically associated with MCP and which could be routinely recorded. Inclusion of such traits in the total merit index of breeding bulls would be the easiest way to indirectly improve MCP (Tyrisevä, 2008).

A review of studies on genetic and phenotypic relationships between milk quality and composition traits and MCP showed that RCT has negligible associations with milk traits (except for milk acidity), while a firmer curd is associated moderately with higher protein and casein contents (Bittante *et al.*, 2012). However, Ikonen *et al.* (2004) and Jõudu (2008) found that selection for higher protein content would improve MCP, but it would probably not reduce the prevalence of NC milk. To maintain both better MCP and reduce the occurrence of NC milk, Ikonen *et al.* (2004) suggested selection for low somatic cell count (SCC). A favourable genetic association between SCC and MCP was confirmed by Cecchinato *et al.* (2011).

Milk acidity is the most highly correlated characteristic with MCP in studies of genetic correlations between milk composition and quality and MCP (Bittante *et al.*, 2012). Thus, pH and titratable acidity of milk have been suggested for indirect selection for MCP (Ikonen *et al.*, 2004; Cassandro *et al.*, 2008; Cecchinato *et al.*, 2011; Bittante *et al.*, 2012).

2.3.3. Marker-assisted selection

Increasing the frequency of the κ-CN B allele in a dairy cattle population has been of interest to the dairy industry, especially to cheese manufacturers, for over two decades. Pinder *et al.* (1991) observed a low frequency of the κ-CN B allele in USA Holstein Friesian bulls and concluded that selection in favour of the B allele, which is superior for cheese production, could thus have a large effect. Ng-Kwai-Hang *et al.* (1991) suggested genotyping bulls for the κ-CN genotype, and using sires with the homozygous κ-CN B allele.

In a dairy cattle population with considerable frequency of the κ-CN E allele, selection against the E allele has been considered an option for improvement of MCP, as this allele is unfavourably associated with MCP and also with some milk composition traits (Ikonen, 2000), but it would not affect the occurrence of NC milk (Ikonen *et al.*, 1999a; Tyrisevä, 2008).

Some studies agree with the possibility of improving MCP by selecting for protein genetic variants β-CN B, κ-CN B (Comin *et al.*, 2008) and additionally β-LG B (Jensen, 2012). However, Heck (2009) found no influence of gene variants of β-LG on the composition of the casein

and thus suggested no influence of selection for the β-LG B on the milk coagulation process.

Recent studies have shown that heritability of MCP is still appreciable after adjustment for protein genotypes or haplotypes, suggesting that phenotype recording and polygenetic selection of these traits cannot be replaced solely by genotyping of animals only for milk protein variants (Penasa *et al.*, 2010; Bonfatti *et al.*, 2011). However, the constant monitoring of milk protein variation in different breeds of cattle is an essential practice in the aim of avoiding an increase in frequencies of mutations with unfavourable effects on cheese-making (Caroli *et al.*, 2009).

3. AIMS OF THE STUDY

Milk coagulation properties are found to have moderate genetic variation, which implies good potential for genetic improvement of these economically important milk properties. However, different analytical techniques are applied for recording milk coagulation properties, using instruments with different working principle, which may potentially influence study results.

Based on this knowledge, current study hypotheses were:

- Genetic variation within Estonian Holstein breed exists in milk coagulation properties and improvement of these milk properties by selective breeding is possible;
- Measurements of milk coagulation properties derived from different analytical techniques (mechanical and optical measurements) are transformable into common scale.

Considering this, the main aims of this study were:

- To estimate heritability and repeatability of milk coagulation properties in Estonian Holstein cattle (**I**);
- To estimate genetic correlation of milk coagulation properties with milk yield and composition (**I**);
- To estimate the effect of composite β-κ-casein genotypes on coagulation and composition of milk (**III**);
- To estimate the proportion of additive genetic variation of milk coagulation and composition traits described by β-κ-casein genotypes (**III**);
- To propose a method for the transformation of the values of milk coagulation traits, analysed using different analytical techniques (**II**).

4. MATERIALS AND METHODS

4.1. Data collection

Milk samples from Estonian Holstein (EHF) cows (**I**, **III**) were collected during routine milk recording by the Estonian Animal Recording Centre (EARC) as a part of a development project for the Bio-Competence Centre of Healthy Dairy products LLC during the period from April 2005 to May 2010 (Table 2). The individual milk samples were a mixture of all test-day milkings or only the morning, afternoon or evening milkings on the test-day. Repeated milk samples per cow for the first to third lactation were collected during 7 to 305 days in milk, generally with an interval of 2 to 3 months. Cows from different herds across the country were selected by the Animal Breeders' Association of Estonia. Pedigree information from three generations of ancestors was obtained from the EARC.

In the current study, poor coagulation is indicated by curd firmness that does not exceed 20 mm after the addition of rennet. Non-coagulation was defined if milk did not coagulate within 30 min, i.e. curd firmness is 0 mm. NC milk samples were excluded from analyses in studies **I** and **III**, i.e. 52 (0.3%) and 138 (0.6%) samples respectively. Additionally, milk samples with a pH level lower than 6.5, indicative of colostrum (Bhandari, Singh, 2002), and those samples outside expected ranges following suggestions from the International Committee for Animal Recording for the limits of milk yield, fat and protein percentage (ICAR, 2009), were eliminated as potentially inaccurate measurements and unreliable samples. Moreover, individual cow lactations with less than three measurements and herds with less than 10 and 5 cows, in studies **I** and **III** respectively, were excluded.

Milk samples from Italian Holstein cows (**II**) were collected in two freestall barns in Italy in October 2010 during the morning milking of the test-day. Days in milk of these cows varied from 5 to 703 in lactations 1 to 7.

Table 2. Basic description of studies.

Number of	Paper		
	I	II	III
samples	17,577	165	23,970
cows	4,191	165	2,859
sires	274	–	229
herds	73	2	78
lactations	1	7	3
Collection time	April 2005 to January 2009	October 2010	April 2005 to May 2010
Design	Repeated measures per cow	4 sub-samples per cow	Repeated measures per cow
Laboratories[1]	Lab_{EST1}, Lab_{EST2}	Lab_{EST1}, Lab_{IT1}, Lab_{IT2}	Lab_{EST1}, Lab_{EST2}
Studied traits[2]	MCP, MT, milk pH, urea	MCP, MT, milk pH	MCP, MT

[1] Lab_{EST1} – Laboratory of Milk Quality of the Estonian University of Life Sciences (Tartu, Estonia); Lab_{EST2} – the Milk Analysis Laboratory of EARC (Tartu, Estonia); Lab_{IT1} – the Milk Laboratory of the Veneto Region Breeders Association (Padova, Italy); Lab_{IT2} – the Milk Quality Laboratory of Veneto Agricoltura Institute (Thiene, Italy).

[2] MT – milk traits: yield, protein%, fat%, somatic cell count; MCP – milk coagulation properties: coagulation time and curd firmness [E_{30} in (**I**, **II**) and a_{30} in (**III**)].

The milk samples from all studies were preserved with Bronopol (Knoll Pharmaceuticals, Nottingham, UK) and stored at 4 °C during the transportation and analysis periods.

The milk samples were analysed for fat percentage, protein percentage, and urea using the MilkoScan 4000 and MilkoScan FT6000, and for SCC using the Fossomatic 400 and Fossomatic 5000 cell counter (all equipment from Foss, Hillerod, Denmark) at the Lab_{EST2} (Table 2; **I**, **III**) and at the Lab_{IT1} (Table 2; **II**), using methods suggested by the International Committee for Animal Recording (ICAR, 2009). SCC were log-transformed to SCS thus: $SCS = \log_2(SCC/100{,}000) + 3$.

Milk pH was determined using a Seven Multi pH meter equipped with InLab 413 electrode (Mettler Toledo GmbH, Greifensee, Switzerland) at a temperature of 20 °C (**I**, **III**) and a pH-Burette 24 (Crison Instruments, Barcelona, Spain) in study **II** before analysing the MCP.

Milk coagulation traits were determined at the Lab_{EST1} (Table 2; **I**, **II**, **III**). The Lab_{IT1} and the Lab_{IT2} (Table 2) were used for the parallel analysis of MCP (**II**).

In studies **I** and **III** milk coagulation traits were measured generally three days after sampling. The proportion of milk samples with an age of 7 to 12 days was very small (less than 1%). In study **II** milk coagulation analyses were performed on the second and third day after sampling. All the subsamples from each cow were analysed on the same day in every three laboratories.

The analytical technique used for milk coagulation analyses in the Lab_{EST1} (**I**, **III**) is described in publication **I**. In articles **I** and **III** the milk coagulation traits of milk coagulation time (RCT) and curd firmness (E_{30}, a_{30}) were studied. Curd firmness was measured originally with an optical signal, in volts (a_{30}), and transformed into millimetres (E_{30}) using a calibration equation (Kübarsepp *et al.*, 2005a). Transformed curd firmness E_{30} was analysed in articles **I** and **II** to be able to compare genetic parameters (**I**), descriptive statistics and phenotypic associations (**I**, **II**) with previous studies using mechanical measurements of milk coagulation. In article **III**, the original values of curd firmness a_{30} were used. Curd firmness in volts and in millimetres, are indicated as a_{30} and E_{30} respectively, throughout the results and discussion section of this thesis. In discussion about curd firmness in general, no abbreviations are used.

Different analytical techniques for milk coagulation analyses used in the study **II** are described in brief in Table 3.

Blood samples of 3,354 Estonian Holstein cows, those from which milk samples had been taken, were collected in tubes containing K_3EDTA during years 2007 to 2009. Whole blood was used to extract genomic DNA for genotyping. To evaluate the effect of the milk protein genetic variants on the MCP and composition of milk, single nucleotide polymorphisms in the casein gene cluster were studied using ASO-PCR (β-CN) and RFLP-PCR (κ-CN) of the relevant DNA regions (**III**). The samples were analysed for milk protein gene polymorphisms in the Laboratory of Animal Genetics of the Estonian University of Life Sciences.

Table 3. Description of analytical techniques used for milk coagulation analyses of sub-samples of 165 cows (methodologies A, B, C) and random sub-samples of 60 cows (methodology C*) in the study **II**.

Metho-dology	Laboratory	Instrument	Rennet	Rennet activity (IMCU/mL of milk)
A	Lab_{IT1}	Computerized renneting meter [Polo Trade (Monselice, Italy)]	Hansen standard 160[1]	0.051
B	Lab_{IT2}	Lattodinamografo [Foss-Italia (Padova, Italy)]	Hansen standard 160	0.051
C	Lab_{EST1}	Optigraph [Ysebaert (Frépillon, France)]	Milase MRS 600[2]	0.120
C*	Lab_{EST1}	Optigraph [Ysebaert (Frépillon, France)]	Hansen standard 160	0.051

[1] Pacovis Amrein AG (Bern, Switzerland); calf rennet containing 80% chymosin and 20% pepsin.
[2] CSK Food Enrichment B.V. (Leeuwarden, the Netherlands); microbial rennet containing 100% chymosin.

4.2. Statistical analyses

4.2.1. Animal models

Statistical analyses in studies **I** and **III** were carried out using repeatability animal model (**I**) and random regression animal model (**III**, for milk coagulation traits in study **I**) in ASReml (Gilmour *et al.*, 2002).

To account for the similarities between related animals, additive genetic relationship matrices including 17,185 and 20,791 animals were used in the animal models of studies **I** and **III**, respectively. All animal models considered random effects of herd, additive genetic component and permanent environment. Fixed effects in repeatability and random regression animal model in the study **I** included calving age, year-season of sampling and year-season of calving. The fixed effect of lactation stage was modelled as a quadratic polynomial of days in milk in the repeatability animal model and as a second-order Legendre polynomial in the random regression animal model. The latter also included second-order Legendre polynomials for additive genetic and environmental effect, except for

first-order Legendre polynomial for permanent environmental effect of RCT.

Two random regression animal models used in study **III** included fixed effects of calving age (nested within lactation), sample age (only for milk coagulation traits), year-season of sampling, year-season of calving and fixed lactation stage and composite β-κ-CN effects modelled as third-order Legendre polynomials (the reduced model excluded fixed effect of β-κ-CN). Random additive genetic and permanent environmental effects were modelled as zero to third order Legendre polynomials of days in milk, order depending on the trait.

4.2.2. Genetic parameters and composite genotype effects

Heritabilities (h^2) and repeatabilities (r) were calculated as described in paper **I**, using variance components from univariate repeatability animal models. Genetic correlations were estimated from bivariate repeatability animal models. All these calculations were performed in ASReml (Gilmour *et al.*, 2002).

Additional heritability and repeatability estimates with the random regression animal model in study **I** and dynamics of additive genetic variation in study **III** were calculated based on covariance matrixes of Legendre polynomials using SAS software version 9.2 (SAS Institute, 2008).

Composite genotype effects for milk coagulation and composition traits in study **III** were calculated in SAS software version 9.2 (SAS Institute, 2008) as sums of differences between daily values of average and genotype-specific lactation curves obtained by the random regression animal model.

4.2.3. Correlation and regression analysis

Correlation and regression analyses were used in study **II** for estimating associations between milk coagulation traits measured with different analytical techniques.

Two regression models were estimated for both milk coagulation traits. The first model was a single trait regression on the same coagulation

trait, measured with a different analytical technique. The second model included both coagulations traits, measured with a different analytical technique, as independent variables.

For estimation of NC probability of milk samples for different analytical techniques, logistic regression and ROC analyses were used. Additionally, sensitivity, specificity and area under the ROC curve were used to describe the accuracy of the estimation.

All these analyses were performed in SAS software version 9.2 (SAS Institute, 2008).

Figures were prepeared in MS Excel in publications **I** and **III** and in R software (version 2.10.1; http://www.r-project.org) in the publication **II**.

5. RESULTS AND DISCUSSION

5.1. Milk coagulation properties of Estonian Holstein cows

Joint dataset of studies **I** and **III** included milk samples from 4,228 EHF cows in their first lactation, 1,917 cows in second lactation and 1,071 cows in third lactation. On average, milk coagulation traits were stable during the three lactation periods, only RCT decreased very slightly and was somewhat favourable in the third lactation (Table 4). Average milk yield increased and average SCS and milk urea decreased in later lactations. The other milk composition traits were also stable throughout all lactations. Traits with stable lactation averages also had minor changes in variation in different lactations.

Table 4. Number of samples, mean and standard deviation (SD) of the milk production, composition and coagulation traits in the joint datasets from studies **I** and **III**.

Trait	I lactation			II lactation			III lactation		
	Count	Mean	SD	Count	Mean	SD	Count	Mean	SD
RCT, min	17,763	10.5	2.4	7,934	10.3	2.2	4,372	9.6	2.0
E_{30}, mm	17,763	26.9	7.4	7,934	26.7	8.0	4,372	26.7	8.1
a_{30}, V	17,763	13.7	4.0	7,934	13.7	4.3	4,372	13.7	4.3
Milk yield, kg	17,762	25.8	7.1	7,934	28.8	9.7	4,372	30.2	10.5
Fat, %	17,739	4.0	0.7	7,933	4.1	0.8	4,371	4.1	0.8
Protein, %	17,758	3.4	0.3	7,934	3.4	0.4	4,372	3.4	0.4
SCS	17,757	5.2	3.7	7,934	3.5	2.0	4,372	3.8	2.0
pH	17,763	6.6	0.1	7,934	6.7	0.1	4,372	6.6	0.1
Urea, mg/L	17,401	26.8	8.3	7,907	26.6	8.5	4,364	23.5	7.6

The largest portion (66.8%) of milk samples had good coagulation (Figure 1), forming curd with firmness of 20–35 mm. Although the proportions of milk samples in the different milk coagulation classes were very similar for the three lactation periods, there was a decrease of 6.2 percentage points in milk samples with good coagulation, while about a 3 percentage points increase of milk samples with poor or excellent coagulation occurred. The percentage of NC milk samples was 0.2%.

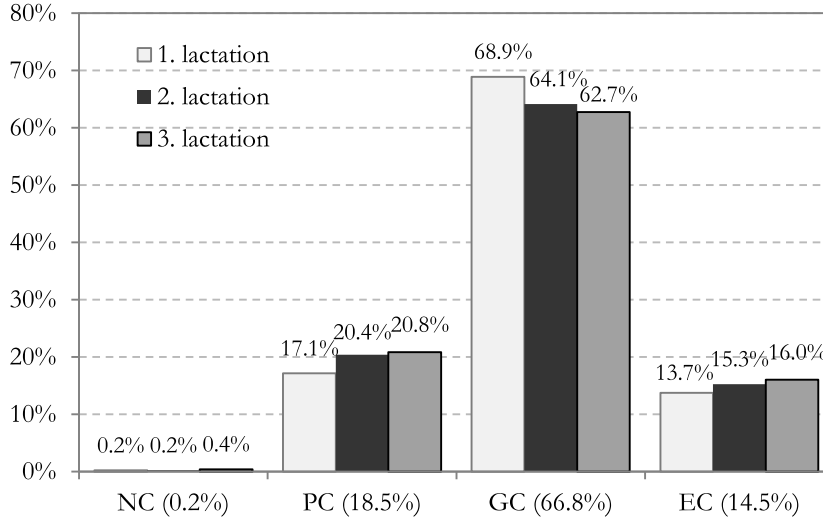

Figure 1. Distribution of samples in milk coagulation classes in three lactations and overall (behind the bars) in the joint dataset from studies **I** and **III**. Milk coagulation classes: NC – non-coagulation ($0 \le E_{30} < 3$ mm); PC – poor coagulation ($3 \le E_{30} < 19.9$ mm); GC – good coagulation ($20 \le E_{30} < 35$ mm); EC – excellent coagulation ($35 \le E_{30} < 53$ mm).

The proportions of daughter's milk sample coagulation classes corresponding to different sires are shown in Figure 2.

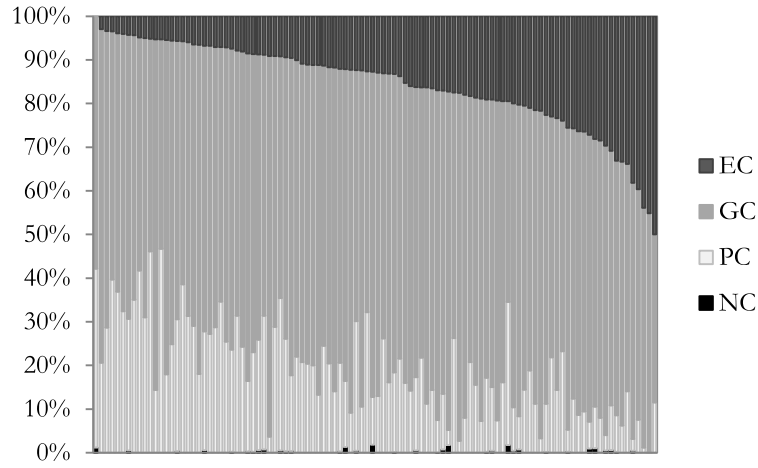

Figure 2. Distribution of milk sample coagulation classes of daughters by sire (at least 50 milk samples in total per sire in joint dataset of studies of **I** and **III**). Milk coagulation classes: NC – non-coagulation ($0 \le E_{30} < 3$ mm); PC – poor coagulation ($3 \le E_{30} < 19.9$ mm); GC – good coagulation ($20 \le E_{30} < 35$ mm); EC – excellent coagulation ($35 \le E_{30} < 53$ mm).

In general, the proportion of milk samples with good coagulation was quite similar for different sires, while the percentage of PC milk samples and excellently coagulating milk samples varied remarkably. This is an indication of genetic variation in the MCP of EHF cows. The percentage of NC milk samples from daughters per sire was marginal with maximum of 1.8%. Ikonen *et al.* (1999a) and Tyrisevä (2008) reported large differences between sires in proportions of daughters producing NC milk, also suggesting that cause for occurrence of NC is partly genetic.

In the first lactation (**I**), phenotypic correlation between milk coagulation traits RCT and E_{30} (–0.22) was considerably lower than the corresponding results in previous studies (Ikonen *et al.*, 1999a, 2004; Tyrisevä *et al.*, 2004; Cassandro *et al.*, 2008). An association of the same magnitude was confirmed in study **II** (–0.23). Phenotypic correlations of these milk coagulation traits with milk production and composition traits were low, except for moderately strong correlations for RCT with milk pH level and for E_{30} with milk protein percentage. Compared to previous studies (Ikonen *et al.*, 1999a, 2004; Cassandro *et al.*, 2008) a somewhat higher positive phenotypic correlation for protein percentage with both milk coagulation traits, and a lower phenotypic correlation between E_{30} and pH level, were found.

The association of E_{30} with protein percentage can be explained by the key role of casein in the milk coagulation process. Aggregation of casein micelles, the second phase of rennet coagulation process, starts only after about 87% of κ-casein is enzymatically degraded (Lucey, 2002b). Van Hooijdonk *et al.* (1986) reported that the optimum pH for hydrolysis of κ-casein is in the range from 5.1 to 5.3. This could explain the positive phenotypic correlation between RCT and pH levels, suggesting that a lower pH shortens RCT.

5.2. Influence of investigated environmental factors on milk coagulation traits

Rennet coagulation time increased toward the end of the first lactation and stabilized after day 180 to less favourable values. Curd firmness showed a different pattern with less favourable values during the first part of lactation, and more favourable (higher) values toward the end of the lactation (Figure 3, **I**). However, several studies have observed

poorest MCP in mid-lactation (Ikonen *et al.*, 2004; Tyrisevä *et al.*, 2004; Jõudu, 2008; Leitner *et al.*, 2012).

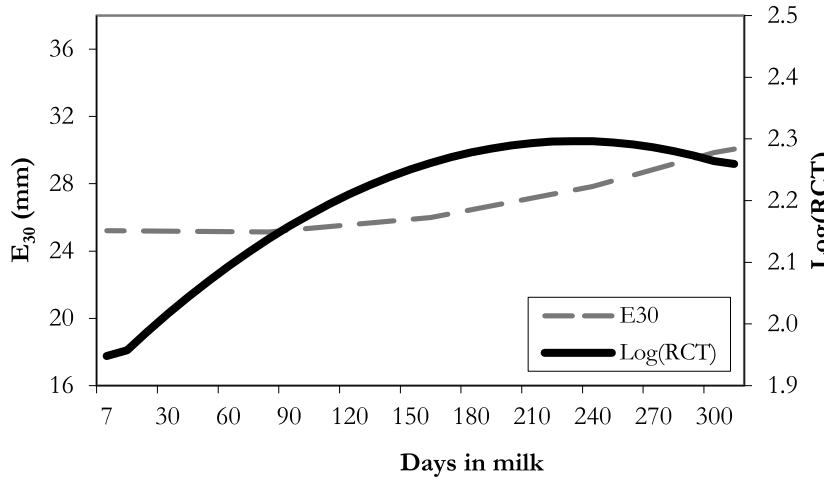

Figure 3. Lactation curve of log-transformed milk coagulation time [log(RCT)] and curd firmness (E_{30}) during the first lactation period from 7 to 305 days in milk (**I**).

RCT and E_{30} were not strongly influenced by differences between herds: the proportion of variation due to the herd was 0.04 for RCT and 0.03 for E_{30} (**I**). These proportions were smaller than those for milk production and composition traits. This is consistent with Tyrisevä *et al.* (2004). Milk coagulation properties therefore seem to be affected to a lesser extent by the herd environment than are milk composition traits. This suggests that it is difficult to improve MCP properties by herd management factors such as nutrition.

There was a significant effect ($p < 0.001$) of year-season of sampling and year-season of calving on both milk coagulation traits (**III**). RCT was also significantly affected by sample age and calving age within lactation ($p < 0.001$), while these effects on a_{30} were not significant ($p = 0.077$ and $p = 0.153$, respectively).

5.3. Polygenetic determination of milk coagulation traits

Heritability estimates for the first lactation milk coagulation traits were higher than those for milk yield and composition traits (**I**). Heritabilities of milk coagulation traits were 0.41 for curd firmness and 0.28 for RCT (Table 5). The heritability estimate for RCT was similar to that reported by Ikonen *et al*. (2004) and Bonfatti *et al*. (2011) but somewhat higher than other recent estimates (0.21 to 0.25) reported for Finnish and Italian dairy cattle populations (Table 1), with the exception of an estimate of 0.32 by Cecchinato *et al*. (2009). Also another study, by Kaart *et al*. (2010), on the Estonian Holstein cow population found a somewhat higher heritability for RCT (0.34). A heritability of E_{30} was consistent with the estimates 0.39 to 0.41 reported by Oloffs *et al*. (1992) and Ikonen *et al*. (1997, 1999a), and somewhat lower than the estimates of 0.44 and 0.57 by Kaart *et al*. (2010) and Ikonen *et al*. (1997), respectively. However, all recent studies in the last decade have reported considerably lower estimates (0.12 to 0.24) in Finnish and Italian dairy cattle (Table 1) than those found in Estonian Holstein cattle. These differences may be due to genetically different cattle populations that have been under different conditions of selective breeding. Breeding goal for Estonian dairy cattle includes only fat and protein yields (Piimaveiste põlvnemis- ja …, 2012). In addition to selection for fat and protein yields, Italian and Finnish selection indices have also included some weighting for protein percentage and SCS, which are moderately genetically correlated with curd firmness. Therefore, a slight indirect selection for curd firmness might have reduced additive genetic variation in curd firmness in these cow populations. The difference in analytical and statistical methods might also have some influence on the variation of milk coagulation traits.

Table 5. Genetic parameters with standard error (in brackets) of milk coagulation traits for first lactation Estonian Holstein cows (modified from **I**).

Parameter	E_{30}	RCT
Heritability	0.41 (0.04)	0.28 (0.04)
Repeatability	0.50 (0.01)	0.45 (0.01)
Genetic correlation with		
RCT, min	−0.16 (0.09)	–
Milk yield, kg	−0.29 (0.11)	−0.07 (0.12)
Fat, %	0.25 (0.09)	−0.10 (0.10)
Protein, %	0.48 (0.07)	0.19 (0.10)
SCS	−0.04 (0.15)	−0.06 (0.15)
Urea, mg/L	0.19 (0.12)	−0.00 (0.12)
pH	−0.06 (0.09)	0.69 (0.05)

Repeatabilities for RCT (0.45) and E_{30} (0.50) were high, and of the same order of magnitude as repeatabilities for milk yield (0.47) and protein percentage (0.46). Repeatabilities for RCT and E_{30}, reported in Finnish studies by Ikonen *et al.* (1997) and Tyrisevä *et al.* (2003), were somewhat higher (from 0.57 to 0.68) than those reported here, which may also reflect the different measurement aspects of coagulation in these studies. The high repeatability estimates for RCT and E_{30} in the current study, however, show that only a few measurements are needed for reliable genetic estimation of milk coagulation properties. Tyrisevä *et al.* (2003) proposed that, in order to estimate reliably the average milk coagulation ability of a cow in genetic evaluations, cows should be sampled at least three times during lactation.

Genetic correlation between the two milk coagulation traits was negligible (Table 5), suggesting that milk coagulation time and curd firmness, when measured by the Optigraph, are mainly influenced by different genes. Genetic correlations between milk coagulation and milk yield and composition traits were mainly low. Curd firmness had the highest genetic correlation with milk protein percentage (0.48), suggesting that a high protein percentage results in a favourable E_{30}. Cassandro *et al.* (2008) reported a correlation of 0.44 between E_{30} and protein percentage, which is in agreement with the current results. The genetic correlations of −0.24 and −0.07 reported by Ikonen *et al.* (1999a, 2004) for the same traits, however, are different. These inconsistencies indicate that many factors may influence the relationship between E_{30} and protein percentage, such as protein composition, sample size, breed, model and

instruments, and variation in time between milk sampling and analysis. Milk coagulation time had the strongest genetic correlation with pH (0.69). A high pH level is therefore associated with a less favourable RCT. This result is consistent with previous studies (Ikonen *et al.*, 1999a, 2004; Cassandro *et al.*, 2008), which also reported a moderate to high genetic correlation between RCT and pH (0.40 to 0.81). A moderate to high genetic correlation (–0.30 to –0.85) between E_{30} and pH observed in previous studies (Ikonen *et al.*, 1999a, 2004; Cassandro *et al.*, 2008), however, was not found in the current study, where a zero genetic correlation between E_{30} and pH was estimated. Curd firmness showed a weak positive genetic correlation with milk fat percentage (0.25) and a weak negative genetic correlation with milk yield (–0.29). Therefore, selection for improved curd firmness may be associated with a somewhat higher fat percentage and slightly reduced milk production. Genetic correlations for E_{30} with milk yield and fat percentage were negligible in previous studies (Ikonen *et al.*, 1999a, 2004; Cassandro *et al.*, 2008).

Some studies have reported moderate favourable genetic association between milk coagulation properties and SCS (Ikonen *et al.*, 2004; Cassandro *et al.*, 2008; Cecchinato *et al.*, 2011) indicating that lower SCS are associated with improved milk coagulation properties. This association was not confirmed in study **I**.

5.4. Frequencies and effects of composite β-κ-casein genotype of Estonian Holstein cows

There were two more frequent β-κ-casein composite genotypes: A2A2AA and A1A2AA, with prevalence of 27.4% and 23.1%, respectively (**III**). The percentages of the remaining 31 genotypes were less than 8%, including 20 genotypes with a percentage of less than 1%. Linkage between two CN loci was observed, as the κ-CN E allele predominantly occurred with the β-CN A1 allele and the β-CN B allele predominantly occurred with the κ-CN B allele as also found by Heck *et al.* (2009) in the Dutch Holstein Friesian population. Ikonen (2000) also reported a tight linkage disequilibrium between the κ-CN B allele and the β-CN A1 allele in Finnish Ayrshire and Finnish Friesian cattle, but this association was not confirmed by study **III**.

The more common β-CN A1 and A2 alleles comprised about 90% of the β-CN alleles in total, while the κ-CN A allele had a frequency of 73.8% (Table 6). Comparison with previous κ-CN allele frequencies in the EHF population showed slight increase of κ-CN B allele during the last decade, but the frequency of this allele was still lower than that reported by Toome (1972), who reported a frequency of 0.307 for this κ-CN variant in local Estonian Black-and-White breed prior the introduction of Holstein gene. The prevalence of the κ-CN B allele in 1972 was similar to that for Estonian Native cattle reported by Jõudu *et al.* (2007). In general, allele frequencies of β-CN and κ-CN reported in large scale studies (**III**; Visker *et al.*, 2011; Comin *et al.*, 2008) were similar for the EHF, Dutch and Italian Holstein Friesian populations. The prevalence of the κ-CN B allele, though, was higher in Dutch Holstein Friesian cows compared to the Italian Holstein Friesian and Estonian Holstein populations, which had similar proportions of this κ-CN variant. The rarely reported β-CN I allele appeared with a frequency of 0.062 in Estonian Holsteins. This was somewhat lower compared to the prevalence of 0.192 in Dutch Holstein Friesian cows (Visker *et al.*, 2011) and 0.12 in Italian Holstein Friesian cows Jann *et al.* (2002) while it is in line with the frequency of 0.058 presented by Bonfatti *et al.* (2011) in Simmental cattle.

Table 6. Allele frequencies of β-casein and κ-casein in different dairy cattle populations and in previous studies in EHF cows. The number of animals is presented in brackets.

Casein allele		Dutch HF[1] (1,854)	Italian HF[2] (973)	Simmental[3] (2,167)	Estonian Holstein[4] (2,859)	Estonian Holstein[5] (609)	Estonian Native[6] (118)
β-Cn	A1	0.283	0.367	0.188	0.318	–	0.318
	A2	0.504	0.575	0.596	0.586	–	0.644
	A3	0.001	0.003	–	0.001	–	–
	B	0.020	0.055	0.158	0.033	–	0.380
	I	0.192	–	0.058	0.062	–	–
κ-Cn	A	0.600	0.689	0.652	0.738	0.790	0.695
	B	0.310	0.217	0.348	0.195	0.138	0.305
	E	0.090	0.094	–	0.066	0.072	–

[1]Visker *et al.* (2011); [2]Comin *et al.* (2008); [3]Bonfatti *et al.* (2011); [4](**III**); [5]Kübarsepp *et al.* (2006); [6]Jõudu *et al.* (2007).

Study **III** showed that the most common β-κ-CN genotypes, A2A2AA and A1A2AA, were associated with poor milk coagulation properties (Figure 4). This was in agreement with a study on Danish Jersey and

Holstein Friesians by Jensen *et al*. (2012b), which reported a genetic contribution of the α_{S1}-β-κ-CN composite genotype BBA2A2AA to impaired milk coagulation.

Some studies on composite casein genotypes have reported a favourable effect of the B allele of both κ-CN and β-CN on milk coagulation traits (Comin *et al*., 2008; Bonfatti *et al*., 2010a). Jensen *et al*. (2012a) observed a high prevalence of B variants of both caseins in milk with good coagulation ability. As in the studies by Comin *et al*. (2008) and Bonfatti *et al*. (2010a), a favourable effect of the β-CN B allele on milk coagulation traits was found in study **III**. As indicated by groups based on κ-CN genotypes (Figure 4), however, κ-CN seemed to have a greater influence on these traits, as was also suggested by Comin *et al*. (2008). Furthermore, the order of the three κ-CN genotypes was clear for a_{30} in the β-κ-CN composite genotypes: BB > AB > AA (Figures 4 and 5). This order of κ-CN genotypes corresponded to the concentration and proportion of κ-CN described by McLean *et al*. (1984), Ikonen *et al*. (1997), Mayer *et al*. (1997), and Hallén *et al*. (2008). Wedholm *et al*. (2006), Hallén *et al*. (2010) and Jensen *et al*. (2012b) found a low κ-CN concentration to be a risk factor for NC of milk. Therefore, the association of the κ-CN B variant with milk coagulation traits may be due to a difference in protein composition, as was also suggested by Bonfatti *et al*. (2010a).

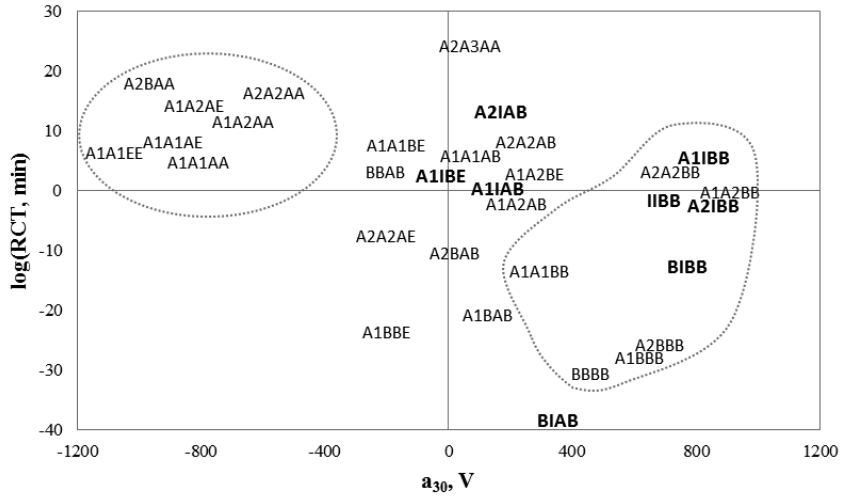

Figure 4. Estimated total β-κ-CN genotype effects for curd firmness (a_{30}) and log-transformed milk coagulation time [log(RCT)] as deviations from the overall mean. The genotypes in bold are those including the β-CN I allele; the two groups of genotypes surrounded by dotted lines comprise the κ-CN BB genotype (composite genotypes of favourable coagulation) or exclude the κ-CN B allele (except for two rare variants; composite genotypes of unfavourable coagulation), respectively (**III**).

There was also strong indication of the existence of the β-κ-CN IB haplotype, which had a favourable effect on curd firmness and protein percentage of milk (Figure 5). This is consistent with a moderate positive genetic correlation between E_{30} and protein percentage in study **I** suggesting the existence of common genes influencing these traits. Visker *et al*. (2011) found a positive effect of the β-CN I variant on protein percentage and suggested the association between β-κ-CN IB haplotype and protein percentage likely to result from the κ-CN B variant. In study **III**, the favourable association of β-κ-CN haplotype IB with protein percentage, however, seemed to derive from the positive effect of the β-CN I variant rather than from the effect of the κ-CN B variant (Figure 5). However, the frequency of this haplotype was too low to confidently draw any final conclusions. A recent study by Jensen *et al*. (2012a) reported a prevalence of the β-CN I variant in milk with both good and poor coagulation ability.

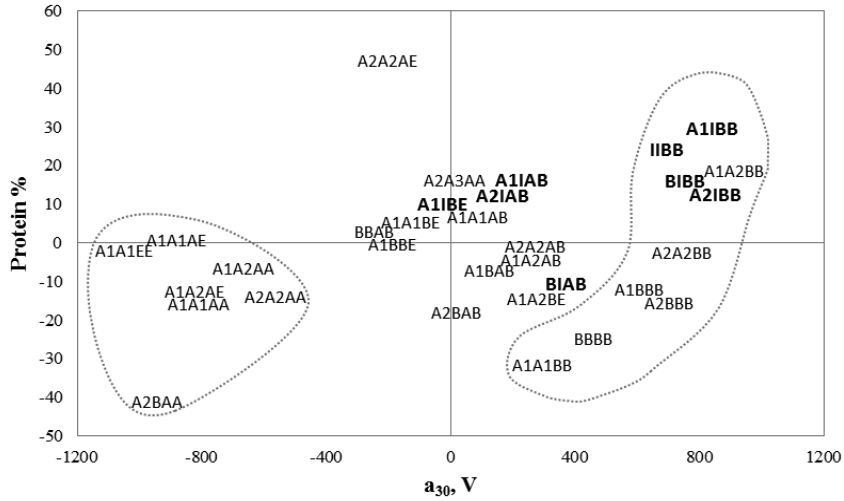

Figure 5. Estimated total β-κ-CN genotype effects for protein percentage and curd firmness (a_{30}) as deviations from the overall mean. Genotypes in bold are those including the β-CN I allele; the two groups of genotypes surrounded by dotted lines comprise the κ-CN BB genotype or exclude the κ-CN B allele (except for two rare variants), respectively (**III**).

Including the composite β-κ-CN genotype resulted in a decrease in the additive genetic variation of RCT and a_{30} by 12.9% and 51.1%, respectively (**III**). This change was a change of proportion, but the dynamics stayed the same throughout the lactation (Figure 6). A similar decrease of 68% for a_{30} was reported by Penasa *et al*. (2010). The decrease for RCT was comparable with the 20% decrease described by Ikonen *et al*. (1999a). However, the study by Ikonen *et al*. (1999a) included both Finnish Friesian and Finnish Ayrshire breeds and additionally considered a β-LG effect. The animal models used by Penasa *et al*. (2010) and Ikonen *et al*. (1999a) did not include a permanent environmental variance component, which may also cause some of the differences in their results regarding the effect of milk protein polymorphism on the additive genetic variance. Moreover, different methodologies used resulted in different variability of milk coagulation traits (**II**), which may also affect the changes in the genetic variability of milk coagulation traits. Similar to the reports by Penasa *et al*. (2010) and Ikonen *et al*. (1999a), the β-κ-CN genotype effects on milk yield, protein and fat percentages, and SCS additive genetic variance were marginal.

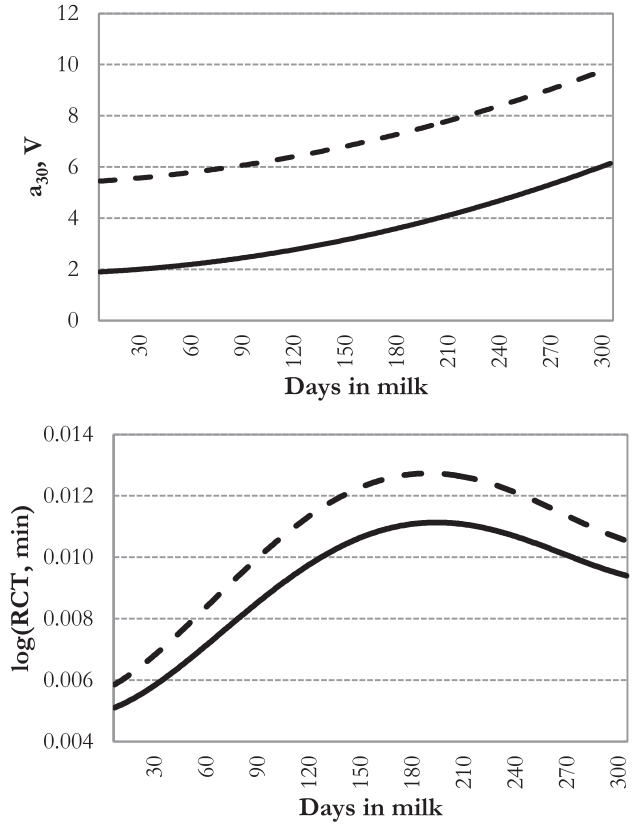

Figure 6. Dynamics of additive genetic variance of milk coagulation traits (log-transformed RCT and a_{30}) during lactation (7 to 305 days in milk) considering the β-κ-CN genotype effect (solid line) and excluding the β-κ-CN genotype effect (dashed line) as a third-order Legendre polynomial of days in milk (modified from **III**).

As stated by Caroli *et al.* (2009), milk protein polymorphism is important for possible technological use, and designing milk with different protein structures appropriate for its specific use is becoming more and more feasible for breeders and is an important task for animal geneticists. Ikonen *et al.* (1999b) suggested that it is important to know the structure of population with regard to the milk protein genotypes, before effects of casein are estimated and casein genotypes are considered for selection in a dairy cattle population. From the perspective of selective breeding one should consider the influence of casein polymorphism on possibly many economically important traits, while improving MCP. The current study suggested no negative influence on milk production and composition traits. The effect of the composite β-κ-CN genotype was significant for MCP and protein content ($p < 0.001$), while the significance probability

of the genotype effect for fat percentage was 0.096. No significant effects of these genotypes for milk yield and SCS were detected (**III**). Ojala *et al*. (2004) found a statistically significant effect of β-κ-CN genotype on body weight in Finnish Ayrshire cows, but the differences in body weight between the individual composite casein genotypes were negligible. The influence of β-κ-CN genotype on milk production and composition was statistically significant in studies by Ikonen *et al*. (2001) and Ojala *et al*. (2004), while Comin *et al*. (2008) presented results for Italian Holstein Friesians indicating no significant effect on these traits, except for milk and protein yield. Routtinen *et al*. (2004) concluded that selection based on β-κ-CN polymorphism should have no substantial impact on the fertility of Finnish Ayrshire heifers and cows. Similar results were reported for Dutch Holstein Friesian cattle by Demeter *et al*. (2010), who found no significant association between cow fertility and milk protein variants, suggesting no unfavourable effect of milk composition improvement on the reproductive performance of dairy cattle. Although contradictory effects of casein polymorphism on milk production and composition traits exist, the results of no detrimental genetic association of these traits with milk coagulation traits (**I**; Hallén *et al*., 2007; Cecchinato *et al*., 2011) suggest that inclusion of casein genotypes into breeding programmes is reasonable.

5.5. Transformation of milk coagulation traits and predicting non-coagulation

A variety of instruments are used in research on milk coagulation, as indicated in the review section 2.1. Also other aspects of analytical techniques accompanying the instruments, e.g. type and activity of coagulant used, vary. This may have an influence on the measurement values and variation, the proportion of NC milk samples and further estimates of genetic parameters of milk coagulation traits. The genetic association between milk coagulation traits measured with the Optigraph (OPT) was quite small (**I**), compared to the corresponding association between these traits derived by the Formagraph (Ikonen *et al*., 1999a, 2004) and by the Computerized Renneting Meter (Cassandro *et al*., 2008). This may indicate that coagulation aspects measured by the Optigraph device and other devices used may be different. Therefore, it would be important for comparisons between studies on MCP to examine the comparability of analytical techniques for measuring MCP.

Study **II** compared three different analytical techniques for measuring MCP used in Italian and Estonian laboratories (Table 3), identified as methodologies A, B, C. The results confirmed that the MCP measured with different methodologies, with different instruments and rennet activity, may give considerably different values (Table 7), and to use them in joint genetic evaluation, some transformation into one common scale is needed. Mean values of milk coagulation properties derived by methodologies used in Italy in this study were similar to those previously reported by Cassandro *et al.* (2008) for Italian Holstein Friesians, while using Estonian methodology resulted in mean values that were in accordance with values in study **I** for the Estonian Holstein population.

Table 7. Number of coagulated (CO) and non-coagulated (NC) milk samples, mean and standard deviation (SD) derived by using different methodologies for measuring RCT and E_{30} (modified from **II**).

Metho-dology	Number of			RCT[1], min		E_{30}[1], mm	
	CO	NC		Mean	SD	Mean	SD
A	158	7	(4.2%)[b]	15.6[b]	4.0[a]	36.2[a]	9.9[b]
B	146	19	(11.5%)[a]	18.2[a]	4.5[a]	29.6[b]	12.9[a]
C	164	1	(0.6%)[b]	8.0[c]	1.8[b]	29.7[b]	8.5[b]
C*	30	30	(50.0%)	21.0	4.9	5.9	3.1

[a-c] Proportions of noncoagulated samples, means and SD within column with different superscripts differ ($p < 0.05$).

[1] Only coagulated samples with A, B and C methodologies (n = 145) and C* methodology (n = 30).

Inconsistent associations between MCP presented in previous studies (Cassandro *et al.*, 2008; **I**) using different methodologies were confirmed (**II**). The E_{30} values measured with different methodologies were less related to each other than the RCT. This is in agreement with less favourable values for analytical repeatability and reproducibility for E_{30} compared with RCT, reported by Dal Zotto *et al.* (2008) for measures of milk coagulation traits obtained using the Computerized Renneting Meter (CRM). Further, changes in optical properties, monitored by the Optigraph, are not exclusively related to curd firming (O'Callaghan *et al.*, 1999). Therefore, curd firmness is more dependent on principles of measurement than RCT.

Higher consistency in RCT values than in E_{30} values between different studied methodologies (Figures 7 and 8) also resulted in better transformation ability

by regression analysis for RCT measurements (coefficient of determination, R^2, ranged from 0.77 to 0.82). The most reliable conversion of E_{30} values was between the two methodologies A and B using the mechanical technique (R^2 = 0.63). In case of transformation from E_{30} values derived by methodology C (optical technique) into either of methodologies using mechanical instrument, however, both milk coagulation traits derived by the mechanical technique should be included as arguments into the transformation equation (R^2 = 0.55 and R^2 = 0.73 for prediction of E_{30} values measured with methodologies A and B, respectively).

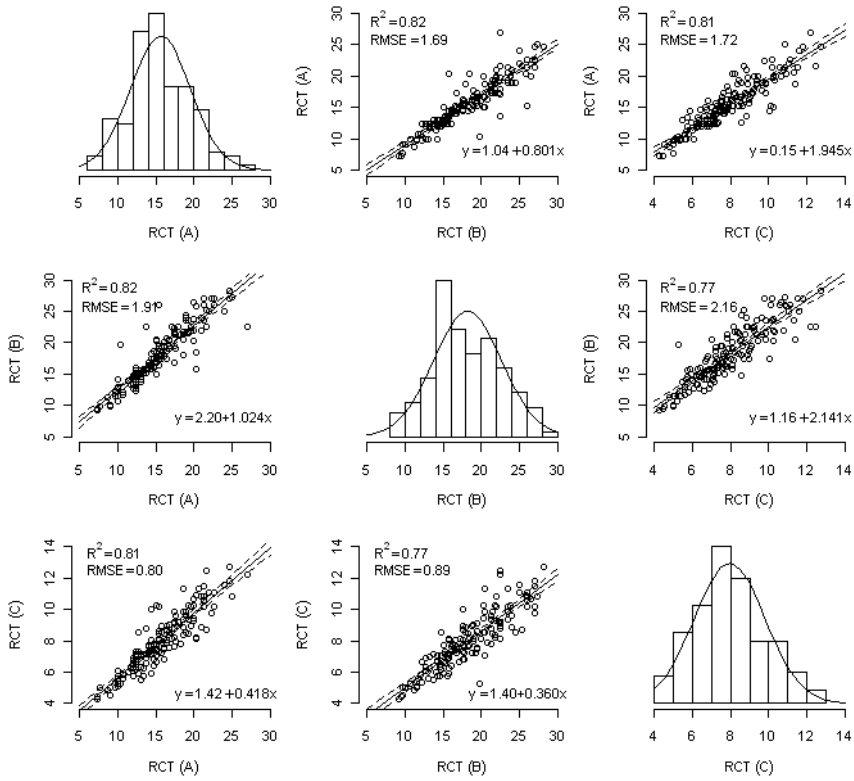

Figure 7. Distribution, regression parameters and regression line (solid line) shown with 95% confidence intervals (dashed lines) of milk rennet coagulation time (RCT, min) measured using three different methodologies indicated with A, B, and C in brackets, described in more detail in Table 3, for the coagulated samples with all methodologies (n = 145; $p < 0.05$ for all models). RMSE = root mean square error (**II**).

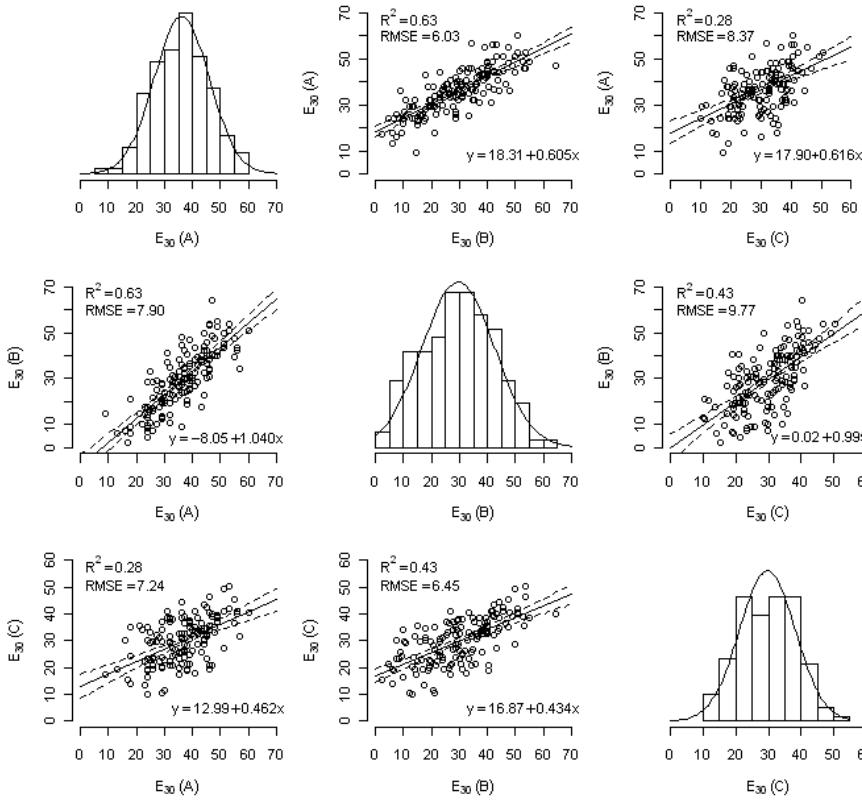

Figure 8. Distribution, regression parameters and regression line (solid line) shown with 95% confidence interval (dashed lines) of curd firmness (E_{30}, mm) measured with 3 different methodologies indicated with A, B, and C in brackets, described in more detail in Table 3, for the coagulated samples with all methodologies (n = 145; $p < 0.05$ for all models). RMSE = root mean square error (**II**).

Additional analysis comparing results of methodologies A, B and C* with uniformed coagulant activity showed similar mean values of RCT of coagulated samples for all methodologies. The mean for E_{30}, measured by OPT, however, decreased considerably compared to the mean value derived by methodology C with higher coagulant activity (Table 7). Moreover, the relationships between MCP measured with C* and other methodologies decreased compared with C, especially for E_{30}. From these results a conclusion was drawn that more rennet is needed for the OPT than the LAT and the CRM, because a higher activity of rennet in the milk is needed to cause differences in the optical signal. However, Cipolat-Gotet *et al.* (2012) reported similar mean values of RCT and E_{30} for both methodologies, while comparing the same mechanical and

optical techniques in uniform conditions with low rennet activity used in methodologies B and C* in the same laboratory, using milk samples of Brown Swiss cows. Furthermore, the percentages of NC milk samples were 11.5% and 50.0% for methodologies B and C* (**II**), respectively, while small differences of proportions of NC samples were observed by using these methodologies in the uniform experimental conditions of Cipolat-Gotet *et al.* (2012), reporting NC of 6.6% and 2.3% corresponding to methodologies B and C* in **II**, respectively.

Therefore, differences in mean values of E_{30} and proportions of NC milk samples by B and C* observed in study **II** compared to the study by Cipolat-Gotet *et al.* (2012), under more uniform analytical conditions may indicate the influence of experimental conditions (e.g. instrument settings, sample age and conservation, effect of storage conditions on rennet quality) on these indirect measures of E_{30} by OPT, rather than the effect of different type or concentration of rennet. Also, the instrument effect could have some influence on measurements, because there is no standard calibration available for measuring MCP in different laboratories. Moreover, an old version of the OPT device was used in study **II**. The higher proportions of NC milk samples observed in study **II** compared to the study by Cipolat-Gotet *et al.* (2012) may also reflect some breed effect. The milk from Italian Holstein Friesian cows, used in study **II**, had poorer MCP compared to the milk of the Brown Swiss cows (De Marchi *et al.*, 2007, 2008).

The proportion of NC samples differed between methodologies (Table 7), especially between methodologies C (0.6%) and C* (50.0%) using the OPT with different types and activities of rennet. In **II** it was proposed that coagulant activity or type of coagulant (calf rennet vs. microbial rennet) would explain this difference and calf rennet in lower activity is therefore unsuitable for the OPT instrument. However, Cipolat-Gotet *et al.* (2012) observed a small proportion of NC (2.3%) also in the conditions of calf rennet in lower activity. Considering this, it is possible to further speculate that experimental conditions other than activity and type of rennet (described above) are responsible for coagulation measurements using the OPT device. Curd firmness is measured indirectly by the OPT and, therefore, the OPT may need different calibration for high and low rennet activity to attain the same sensitivity of the instrument.

The probabilities of NC samples with methodology B, derived from logistic regression, were highly predictable based on the RCT measured with either C or A (**II**). Also, the probabilities of NC samples in A were highly predictable based on the RCT measured in C. NC samples were determined with a probability of 1.00, and coagulated samples with probabilities of 0.89 to 0.98. Predicting the NC probability based only on curd firmness resulted in less accurate predictions. Distinction of the coagulated and NC samples of methodology C* based on the MCP of the same samples measured with either A or B resulted in more than 95% of the correctly classified samples based on the RCT, and more than 82% based on the E_{30}. Therefore, the probability of NC samples seems to be well predictable irrespective of the instrument used, especially when based on the RCT.

Although direct comparisons of measurements of MCP using different analytical techniques could be somewhat complicated due to various confounding factors influencing the coagulation process and its recording, possibilities to transform measurements of milk coagulation traits derived by different instruments and analytical techniques exist (**II**) and should be studied further.

6. CONCLUSIONS

The following conclusions can be drawn from this study on some genetic and modelling aspects of MCP.

- Heritability estimates for first lactation MCP of Estonian Holstein cows were higher than those for milk production and composition traits. Of the variation of RCT, 28% was found to be additive genetic in its nature and 41% of variation was additive genetic for E_{30}. No detrimental genetic associations with milk production and composition traits were found. Considering the relatively high repeatability estimates (0.45 and 0.50 for RCT and E_{30}, respectively), only few measurements are needed for the reliable genetic estimation of MCP, and direct selection for improvement of MCP would be possible. However, a lack of high capacity equipment and analytical techniques for measuring MCP makes routine recording of these traits on whole dairy cow population unfeasible (**I**).

- Genetic associations between MCP, milk yield and composition traits were mainly negligible, except for a high favourable correlation of RCT with pH, a moderately high positive genetic association between E_{30} and protein content, and a moderate genetic association of E_{30} with fat content and milk yield. Therefore, selection for MCP is not expected to result in a strong deterioration of milk production and composition. No eligible milk composition trait for improvement of MCP by indirect selective breeding was established (**I**).

- Genetic correlation between milk coagulation traits was modest (–0.16) as well as the corresponding phenotypic correlation (–0.22 to –0.23). This differs from high genetic and phenotypic associations between MCP observed in previous studies using mechanical instruments for the measurement of MCP. However, phenotypic and genotypic associations between RCT and milk production and composition traits were in better accordance with estimates in previous studies than those of E_{30}. Moreover, the RCT showed a strong relationship between measurements from different laboratories, even when different analytical techniques

were used. On the contrary, the moderate correlations between E_{30} measured with Lattodinamografo or Computerized Renneting Meter and Optigraph are probably due to the different design principles of the instruments, accompanied with different types and concentrations of rennet used, resulting in the measurement of different aspects of the coagulation process. This demonstrates the possibility for good comparison of RCT between different analytical techniques for measuring MCP, while comparability of curd firmness would be modest (**I**, **II**).

- Considering that curd firmness a_{30} in volts derived by the new Optigraph device was measuring somewhat other aspects of coagulation than curd firmness E_{30} in millimetres derived by other devices (e.g. previous Formagraph device, Computerized Renneting Meter), the non-transformed values of curd firmness would be preferable for use in statistical analyses and further research is needed to study the effect of the Optigraph milk coagulation measurements on cheese yield and quality (**I**, **II**).

- The transformation method proposed provides the opportunity to convert MCP data obtained by different analytical techniques into comparable data sets across these MCP measurement techniques. The precision of transformation for both MCP is higher between analytical techniques using mechanical instruments for measuring MCP, and for RCT between all analytical techniques compared to the transformability of E_{30} (**II**). Direct comparisons of measurements of MCP using different analytical techniques could be somewhat complicated due to various confounding factors influencing the coagulation process and its recording. Considering that, establishing reliable transformation for milk coagulation measurements in studies using different analytical techniques, and their effect on genetic parameter estimates, would be useful for further comparison of MCP and their genetic parameters between studies. Thus, it is proposed that it is needed to investigate the possibilities for transformation of MCP measurements between different studies.

- Effect of the composite β-κ-CN genotype was statistically significant for MCP and protein content, while no significant effect of these genotypes for milk yield, fat percentage and SCS were detected (**III**).

- The most common β-κ-CN genotypes of Estonian Holstein cows A2A2AA and A1A2AA, both with a prevalence greater than 20%, were associated with poor MCP. These composite genotypes had a moderately positive effect on milk yield, albeit not statistically significant, which may indicate a result of selection in favour of higher milk yield. Considering the high frequency of unfavourable β-κ-CN genotypes for MCP, the low frequency of favourable κ-CN B allele on MCP and the positive association of the β-κ-CN IB haplotype with a_{30} and protein percentage, selection based on the β-κ-CN genotype for these traits could be a consideration. However, the association of rare markers, including β-κ-CN IB haplotype, with MCP and milk composition would need confirmation in further research (**III**).

- The composite β-κ-CN genotypes influenced the additive genetic variance of milk coagulation traits (reduction in additive genetic variance by 12.9% and 51.1% for RCT and a_{30}, respectively), whereas changes in the additive genetic variance of milk composition traits were marginal. Therefore, genetic improvement by means of selection for favourable β-κ-CN genotypes is expected to be higher for milk coagulation properties, especially for a_{30} (**III**).

To sum up the above for a final conclusion, the genetic improvement of milk coagulation properties of Estonian Holstein cows by selection would be possible on limited number of animals (e.g. bulls and bull dams). However, associations between milk coagulation properties derived by the Optigraph device and cheese outcome and quality need further investigation.

REFERENCES

Aleandri, R., Buttazzoni, L. G., Schneider, J. C., Caroli, A., Davoli, R. 1990. The effects of milk protein polymorphisms on milk components and cheese-producing ability. – Journal of Dairy Science, 73(2), 241–255.

Aleandri, R., Schneider, J. C., Buttazzoni, L. G. 1989. Evaluation of milk for cheese production based on milk characteristics and Formagraph measures. – Journal of Dairy Science, 72(8), 1967–1975.

Auldist, M. J., Johnston, K. A., White, N. J., Fitzsimons, W. P., Boland, M. J. 2004. A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Friesian and Jersey dairy cows. – Journal of Dairy Research, 71(1), 51–57.

Auldist, M., Mullins, C., O'Brien, B., O'Kennedy B. T., Guinee, T. 2002. Effect of cow breed on milk coagulation properties. – Milchwissenschaft, 57, 140–143.

Bhandari, V., Singh, H. 2002. Physical methods. In: Encyclopedia of Dairy Sciences. Roginski, H., Fuquay, J. W., Fox, P. F. (Eds.), Vol. 1. – Academic Press, San Diego, CA, 93–101.

Bittante, G., Marusi, M., Cesarini, F., Povinelli, M., Cassandro, M. 2002. Genetic analysis on milk rennet-coagulation ability in Italian Holstein cows. – CD-ROM communication No. 09-03 in Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, Montpellier, France.

Bittante, G., Penasa, M., Cecchinato, A. 2012. Invited review: Genetics and modeling of milk coagulation properties. – Journal of Dairy Science, 95(12), 6843–6870.

Bobe, G., Beitz, D. C., Freeman, A. E., Lindberg, G. L. 1999. Effect of milk protein genotypes on milk protein composition and its genetic parameter estimates. – Journal of Dairy Science, 82(12), 2797–2804.

Bonfatti, V., Cecchinato, A., Gallo, L., Blasco, A., Carnier, P. 2011. Genetic analysis of detailed milk protein composition and coagulation properties in Simmental cattle. – Journal of Dairy Science, 94(10), 5183–5193.

Bonfatti, V., Di Martino, G., Cecchinato, A., Degano, L., Carnier, P. 2010a. Effects of β-κ-casein (CSN2-CSN3) haplotypes and β-lactoglobulin (BLG) genotypes, and detailed protein composition on coagulation properties of individual milk of Simmental cows. – Journal of Dairy Science, 93(8), 3809–3817.

Bonfatti, V., Di Martino, G., Cecchinato, A., Vicario, D., Carnier, P. 2010b. Effects of β-κ-casein (CSN2-CSN3) haplotypes and β-lactoglobulin (BLG) genotypes on milk production traits and detailed protein composition of individual milk of Simmental cows. – Journal of Dairy Science, 93(8), 3797–3808.

Bovenhuis, H., van Arendonk, J. A. M., Korver, S. 1992. Associations between milk protein polymorphisms and milk production traits. – Journal of Dairy Science, 75(9), 2549–2559.

Caroli, A. M., Chessa, S., Erhardt, G. J. 2009. Invited review: milk protein polymorphisms in cattle: effect on animal breeding and human nutrition. – Journal of Dairy Science, 92(11), 5335–5352.

Caroli, A., Bolla, P., Budelli, E., Barbieri, G., Leone, P. 2000. Effect of κ-casein E allele on clotting aptitude of Italian Friesian milk. – Zootecnica e Nutrizione Animale, 26(3), 127–130.

Caroli, A., Bolla, P., Pagnacco, G., Rampilli, M., Degano, L. 1990. Repeatability of milk clotting aptitude evaluated by lactodynamographic analysis. – Journal of Dairy Research, 57(1), 141–142.

Cassandro, M., Comin, A., Ojala, M., Dal Zotto, R., De Marchi, M., Gallo, L., Carnier, P., Bittante, G. 2008. Genetic parameters of milk coagulation properties and their relationships with milk yield and quality traits in Italian Holstein cows. – Journal of Dairy Science, 91(1), 371–376.

Cecchinato, A., Cipolat-Gotet, C., Casellas, J., Penasa, M., Rossoni, A., Bittante, G. 2013. Genetic analysis of rennet coagulation time, curd-firming rate, and curd firmness assessed over an extended testing period using mechanical and near-infrared instruments. – Journal of Dairy Science, 96(1), 50–62.

Cecchinato, A., De Marchi, M., Gallo, L., Bittante, G., Carnier, P. 2009. Mid-infrared spectroscopy predictions as indicator traits in breeding programs for enhanced coagulation properties of milk. – Journal of Dairy Science, 92(10), 5304–5313.

Cecchinato, A., Penasa, M., De Marchi, M., Gallo, L., Bittante, G., Carnier, P. 2011. Genetic parameters of coagulation properties, milk yield, quality, and acidity estimated using coagulating and noncoagulating milk information in Brown Swiss and Holstein-Friesian cows. – Journal of Dairy Science, 94(8), 4205–4213.

Cipolat-Gotet, C., Cecchinato, A., De Marchi, M., Penasa, M., Bittante, G. 2012. Comparison between mechanical and near-infrared methods for assessing coagulation properties of bovine milk. – Journal of Dairy Science, 95(11), 6806–6819.

Comin, A., Cassandro, M., Chessa, S., Ojala, M., Dal Zotto, R., De Marchi, M., Carnier, P., Gallo, L., Pagnacco, G., Bittante, G. 2008. Effects of composite β- and κ-casein genotypes on milk coagulation, quality, and yield traits in Italian Holstein cows. – Journal of Dairy Science, 91(10), 4022–4027.

Coulon, J. B., Verdier, I., Pradel, P., Almena, M. 1998. Effect of lactation stage on the cheesemaking properties of milk and the quality of Saint-Nectaire-type cheese. – Journal of Dairy Research, 65(2), 295–305.

Dal Zotto, R., De Marchi, M., Cecchinato, A., Penasa, M., Cassandro, M., Carnier, P., Gallo, L., Bittante, G. 2008. Reproducibility and repeatability of measures of milk coagulation properties and predictive ability of mid-infrared reflectance spectroscopy. – Journal of Dairy Science, 91(10), 4103–4112.

De Marchi, M., Bittante, G., Dal Zotto, R., Dalvit, C., Cassandro, M. 2008. Effect of Holstein Friesian and Brown Swiss breeds on quality of milk and cheese. – Journal of Dairy Science, 91(10), 4092–4102.

De Marchi, M., Dal Zotto, R., Cassandro, M., Bittante, G. 2007. Milk coagulation ability of five dairy cattle breeds. – Journal of Dairy Science, 90(8), 3986–3992.

De Marchi, M., Fagan, C. C., O'Donnell, C. P., Cecchinato, A., Dal Zotto, R., Cassandro, M., Penasa, M., Bittante, G. 2009. Prediction of coagulation properties, titratable acidity, and pH of bovine milk using mid-infrared spectroscopy. – Journal of Dairy Science, 92(1), 423–432.

Demeter, R. M., Markiewicz, K., van Arendonk, J. A. M., Bovenhuis, H. 2010. Relationships between milk protein composition, milk protein variants, and cow fertility traits in Dutch Holstein-Friesian cattle. – Journal of Dairy Science, 93(11), 5495–5502.

Eesti jõudluskontrolli aastaraamat 2011/ Results of Animal Recording in Estonia 2011. 2012. Jõudluskontrolli Keskus. Kirjastus Elmatar, 52.

Eesti piimanduse strateegia 2012–2020. Põllumajandusministeerium. Tallinn, 2012, 37. http://www.agri.ee/public /juurkataloog/ ARENDUSTEGEVUS/strateegia_Eesti_piimandus_2012_2020.pdf (Accessed March 4, 2012, in Estonian)

Eurostat. 2013. Production of cheese. http://epp.eurostat.ec.europa. eu/ tgm/table.do?tab=table&init=1&language=en&pcode=tag00040&p lugin=1 (Accessed March 2, 2013)

Frederiksen, P. D., Andersen, K. K., Hammershoj, M., Poulsen, H. D., Sorensen, J., Bakman, M., Qvist, K. B., Larsen, L. B. 2011b. Composition and effect of blending of noncoagulating, poorly coagulating, and well-coagulating bovine milk from individual Danish Holstein cows. – Journal of Dairy Science, 94(10), 4787–4799.

Frederiksen, P., Hammershøj, M., Bakman, M., Andersen, P., Andersen, J., Qvist, K., Larsen, L. 2011a. Variations in coagulation properties of cheese milk from three Danish dairy breeds as determined by a new free oscillation rheometry-based method. – Dairy Science and Technology, 91(3), 309–321.

Gilmour, A. R., Gogel, B. J., Cullis, B. R. Welham, S. J., Thompson, R. 2002. ASReml User Guide Release 2.0. – VSN International, Hemel Hempstead, UK.

Grandison, A. S., Ford, G. D. 1986. Effects of variations in somatic cell count on the rennet coagulation properties of milk and on the yield, composition and quality of Cheddar cheese. – Journal of Dairy Research, 53(4), 645–655.

Hallén, E. 2008. Coagulation properties of milk – association with milk protein composition and genetic polymorphism. – PhD thesis, Swedish University of Agricultural Sciences, Faculty of Natural Resources and Agricultural Sciences, Department of Food Science, 64.

Hallén, E., Allmere, T., Näslund, J., Andrén, A., Lundén, A. 2007. Effect of genetic polymorphism of milk proteins on rheology of chymosin-induced milk gels. – International Dairy Journal, 17(7), 791–799.

Hallén, E., Lundén, A., Tyrisevä, A.-M., Westerlind, M., Andrén, A. 2010. Composition of poorly and non-coagulating bovine milk and effect of calcium addition. – Journal of Dairy Research, 77(4), 398–403.

Hallén, E., Wedholm, A., Andrén, A., Lundén, A. 2008. Effect of β-casein, κ-casein and β-lactoglobulin genotypes on concentration of milk protein variants. – Journal of Animal Breeding and Genetics, 125(2), 119–129.

Harzia, H., Kilk, K., Jõudu, I., Henno, M., Kärt, O., Soomets, U. 2012. Comparison of the metabolic profiles of noncoagulating and coagulating bovine milk. – Journal of Dairy Science, 95(2), 533–540.

Harzia, H., Kilk, K., Ariko, T., Kass, M., Soomets, U., Jõudu, I., Kaart, T., Arney, D., Kärt, O., Ots, M. 2013. Crude glycerol as a glycogenic precursor in feed: effects on milk coagulation properties and metabolic profiles of dairy cows. – Journal of Dairy Research, 80(2), 190–196.

Heck, J. M. L. 2009. Milk genomics, opportunities to improve the protein and fatty acid composition in raw milk. – PhD thesis, Wageningen University, 144.

Heck, J. M. L., Schennink, A., van Valenberg, H. J. F., Bovenhuis, H., Visker, M. H. P. W., van Arendonk, J. A. M., van Hooijdonk, A. C. M. 2009. Effects of milk protein variants on the protein composition of bovine milk. – Journal of Dairy Science, 92(3), 1192–1202.

Ikonen, T. 2000. Possibilities of genetic improvement of milk coagulation properties of dairy cows. – PhD thesis, University of Helsinki, Department of Animal Science, Publications No. 49, 34.

Ikonen, T., Ahlfors, K., Kempe, R., Ojala, M., Ruottinen, O. 1999a. Genetic parameters for the milk coagulation properties and prevalence of noncoagulating milk in Finnish dairy cows. – Journal of Dairy Science, 82(1), 205–214.

Ikonen, T., Bovenhuis, H., Ojala, M., Ruottinen, O., Georges, M. 2001. Associations between casein haplotypes and first lactation milk production traits in Finnish Ayrshire cows. – Journal of Dairy Science, 84(2), 507–514.

Ikonen, T., Ojala, M., Syväoja, E.-L. 1997. Effects of composite casein and β-lactoglobulin genotypes on renneting properties and composition of bovine milk by assuming an animal model. – Agricultural and Food Science, 6(4), 283–294.

Ikonen, T., Morri, S., Tyrisevä, A.-M., Ruottinen, O., Ojala, M. 2004. Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content and pH of milk. – Journal of Dairy Science, 87(2), 458–467.

Ikonen, T., Ojala, M., Ruottinen, O. 1999b. Associations between milk protein polymorphism and first lactation milk production traits in Finnish Ayrshire cows. – Journal of Dairy Science, 82(5), 1026–1033.

International Committee for Animal Recording. 2009. International agreement of recording practices. Guidelines approved by the General Assembly held June 18, 2008. http://www.icar.org/ Documents/ Rules%20and%20regulations/Guidelines/Guidelines_2009.pdf (Accessed May 13, 2010)

International Dairy Federation (IDF). 2007. Coagulation of milk: processes and characteristics. – Bulletin 420/2007. International Dairy Federation, Brussels, Belgium.

Jakob, E., Puhan, Z. 1992. Technological properties of milk as influenced by genetic polymorphism of milk proteins. A review. – International Dairy Journal, 2(3), 157–178.

Jann, O., Ceriotti, G., Caroli, A., Erhardt, G. 2002. A new variant in exon VII of bovine β-casein gene (CSN2) and its distribution among European cattle breeds. – Journal of Animal Breeding and Genetics, 119(1), 65–68.

Jensen, H. B. Milk proteomics. Linking genetic and isoform variation of milk proteins to technological properties of bovine milk. 2012. – PhD thesis, Aarhus University, Department of Food Science, 57.

Jensen, H. B., Holland, J. W., Poulsen, N. A., Larsen, L. B. 2012a. Milk protein genetic variants and isoforms identified in bovine milk representing extremes in coagulation properties. – Journal of Dairy Science, 95(6), 2891–2903.

Jensen, H. B., Poulsen, N. A., Andersen, K. K., Hammershoj, M., Poulsen, H. D., Larsen, L. B. 2012b. Distinct composition of bovine milk from Jersey and Holstein-Friesian cows with good, poor, or noncoagulation properties as reflected in protein genetic variants and isoforms. – Journal of Dairy Science, 95(12), 6905–6917.

Johnson, M. E., Chen, C. M., Jaeggi, J. J. 2001. Effect of rennet coagulation time on composition, yield, and quality of reduced-fat cheddar cheese. – Journal of Dairy Science, 84(5), 1027–1033.

Jõudu, I. 2008. Effect of milk protein composition and genetic polymorphism on milk rennet coagulation properties. – PhD thesis, Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, 114.

Jõudu, I., Henno, M., Kaart, T., Püssa, T., Kärt O. 2008. The effect of milk protein contents on the rennet coagulation properties of milk from individual dairy cows. – International Dairy Journal, 18(9), 964–967.

Jõudu, I., Henno, M., Värv, S., Kaart, T., Kärt, O., Kalamees, K. 2007. Milk protein genotypes and milk coagulation properties of Estonian native cattle. – Agricultural and Food Science, 16(3), 222–231.

Kaart, T., Vallas, M., Waldmann, A., Kiiman, H., Pärna, K., Pärna, E. 2010. Associations between milk quality traits and coagulation properties and fertility in Estonian Holstein heifers and first lactation cows. – Abstract 0567 in Proceedings of the 9th World Congress on Genetics Applied to Livestock Production, Leipzig, Germany, August 1-6, 2010.

Klandar, A. H., Lagaude, A., Chevalier-Lucia, D. 2007. Assessment of the rennet coagulation of skim milk: A comparison of methods. – International Dairy Journal, 17(10), 1151–1160.

Kübarsepp, I., Henno, M., Pärna, E., Viinalass, H., Sabre, D. 2006. Frequencies of κ-casein and β-lactoglobulin genetic variants among Estonian cattle breeds and their effect on the milk renneting properties. – Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, Brazil, August 13–18, 2006, Communication No 01–65.

Kübarsepp, I., Henno, M., Viinalass, H., Sabre, D. 2005b. Effect of κ-casein and β-lactoglobulin genotypes on the milk rennet coagulation properties. – Agronomy Research, 3(1), 55–64.

Kübarsepp, I., Henno, M., Kärt, O., Tupasela, T. 2005a. A comparison of the methods for determination of the rennet coagulation properties of milk. – Acta Agriculturae Scandinavica, Section A – Animal Science, 55(4), 145–148.

Leitner, G., Krifucks, O., Merin, U., Lavi, Y., Silanikove, N. 2006. Interactions between bacteria type, proteolysis of casein and physico-chemical properties of bovine milk. – International Dairy Journal, 16(6), 648–654.

Leitner, G., Merin, U., Lemberskiy-Kuzin, L., Bezman, D., Katz, G. 2012. Real-time visual/near-infrared analysis of milk-clotting parameters for industrial applications. – Animal, 6(7), 1170–1177.

Leitner, G., Silanikove, N., Jacobi, S., Weisblit, L., Bernstein, S., Merin, U. 2008. The influence of storage on the farm and in dairy silos on milk quality for cheese production. – International Dairy Journal, 18(2), 109–113.

Lien, S., Kantanen, J., Olsaker, I., Holm, L. E., Eythorsdottir, E., Sandberg, K., Dalsgard, B., Adalsteinsson, S. 1999. Comparison of milk protein allele frequencies in Nordic cattle breeds. – Animal Genetics, 30(2), 85–91.

Lindström, U. B., Antila, V., Syväjärvi, J. 1984. A note on some genetic and non-genetic factors affecting clotting time of Ayrshire milk. – Acta Agriculturae Scandinavica, 34(3), 349–355.

Lucey, J. A. 2002a. Formation and physical properties of milk protein gels. – Journal of Dairy Science, 85(2), 281–294.

Lucey, J. A. 2002b. Rennet coagulation of milk. In: Encyclopedia of Dairy Sciences. Roginski, H., Fuquay, J. W., Fox, P. F. (Eds.), Vol. 1. – Academic Press, San Diego, CA, 286–293.

Lundén, A., Nilsson, M., Janson, L. 1997. Marked effect of β-lactoglobulin polymorphism on the ratio of casein to total protein in milk. – Journal of Dairy Science, 80(11), 2996–3005.

Macheboeuf, D., Coulon, J.-B., D'Hour, P. 1993. Effect of breed, protein genetic variants and feeding on cows' milk coagulation properties. – Journal of Dairy Research, 60(1), 43–54.

Malacarne, M., Summer, A., Fossa, E., Formaggioni, P., Franceschi, P., Pecorari, M., Mariani, P. 2006. Composition, coagulation properties and Parmigiano-Reggiano cheese yield of Italian Brown and Italian Friesian herd milks. – Journal of Dairy Research, 73(2), 171–177.

Marziali, A. S., Ng-Kwai-Hang, K. F. 1986. Effects of milk composition and genetic polymorphism on coagulation properties of milk. – Journal of Dairy Science, 69(7), 1793–1798.

Martin, B., Pomiès, D., Pradel, P., Verdier-Metz, I., Rémond, B. 2009. Yield and sensory properties of cheese made with milk from Holstein or Montbéliarde cows milked twice or once daily. – Journal of Dairy Science, 92(10), 4730–4737.

Mayer, H. K., Ortner, M., Tschager, E., Ginzinger, W. 1997. Composite milk protein phenotypes in relation to composition and cheesemaking properties of milk. – International Dairy Journal, 7(5), 305–310.

McLean, D. M., Graham, E. R., Ponzoni, R. W., McKenzie, H. A. 1984. Effects of milk protein genetic variants on milk yield and composition. – Journal of Dairy Research, 51(4), 531–546.

McMahon, D. J., Brown, R. J. 1982. Evaluation of Formagraph for comparing rennet solutions. – Journal of Dairy Science, 65(8), 1639–1642.

McMahon, D. J., Brown, R. J., Richardson, G. H., Ernstrom, C. A. 1984. Effects of calcium, phosphate, and bulk culture media on milk coagulation properties. – Journal of Dairy Science, 67(5), 930–938.

Merin, U., Fleminger, G., Komanovsky, J., Silanikove, N., Bernstein, S., Leitner, G. 2008. Subclinical udder infection with Streptococcus dysgalactiae impairs milk coagulation properties: The emerging role of proteose peptones. – Dairy Science and Technology, 88(4-5), 407–419.

Mihhejev, K. 2002. Piima laapumist mõjutavad tegurid/ Factors influencing milk coagulation properties. – MSc thesis. Estonian Agricultural University, 72. (in Estonian)

Ng-Kwai-Hang, K. F., Hayes, J. F., Moxley, J. E., Monardes, H. G. 1984. Association of genetic variants of casein and milk serum proteins with milk, fat, and protein production by dairy cattle. – Journal of Dairy Science, 67(4), 835–840.

Ng-Kwai-Hang, K. F., Zadworny, D., Hayes, J. F., Kuhnlein, U. 1991. Identification of κ-casein genotype in Holstein sires: a comparison between analysis of milk samples from daughters and direct analysis of semen samples from sires by polymerase chain reaction. – Journal of Dairy Science, 74(8), 2410–2415.

Ng-Kwai-Hang, K. F., Politis, I., Cue, R. I. Marziali, A. S. 1989. Correlation between coagulation properties of milk and cheese yielding capacity and cheese composition. – Canadian Institute of Food Science and Technology Journal, 22, 291–294.

O'Brien, B., Meaney, W. J., McDonagh, D., Kelly, A. 2001. The effects of refrigerated storage on the quality of raw milk. – Australian Journal of Dairy Technology, 56, 213–218.

O'Callaghan, D. J., O'Donnell, C. P., Payne, F. A. 1999. A comparison of on-line techniques for determination of curd setting time using cheesemilks under different rates of coagulation. – Journal of Food Engineering, 41(1), 43–54.

O'Callaghan, D. J., O'Donnell, C. P., Payne, F. A. 2002. Review of systems for monitoring curd setting during cheesemaking. – International Journal of Dairy Technology, 55(2), 65–74.

Ojala, M., Tyrisevä, A.-M., Ikonen, T. 2005. Genetic improvement of milk quality traits for cheese production. In: Indicators of Milk and Beef Quality. Hocquette, J. F., Gigli, S. (Eds.). – European Association for Animal Production, Publication 112, Wageningen Academic Publishers, Wageningen, the Netherlands, 307–311.

Ojala, M., Seppänen, T., Tyrisevä, A.-M., Ikonen, T. 2004. Effects of milk protein genotypes on body weight and milk production traits in Finnish Ayrshire cows. – Proceedings of the 10th Baltic Animal Breeding Conference, Estonia, Tartu, 68–73.

Ojala, M., Famula, T. R., Medrano J. F. 1997. Effects of milk protein genotypes on the variation for milk production traits of Holstein and Jersey cows in California. – Journal of Dairy Science, 80(8), 1776–1785.

Okigbo, L. M., Richardson, G. H., Brown, R. J., Ernstrom, C. A. 1985a. Effects of pH, calcium chloride, and chymosin concentration on coagulation properties of abnormal and normal milk. – Journal of Dairy Science, 68(10), 2527–2533.

Okigbo, L. M., Richardson, G. H., Brown, R. J., Ernstrom, C. A. 1985b. Variation in coagulation properties of milk from individual cows. – Journal of Dairy Science, 68(4), 822–828.

Oloffs, K., Shulte-Coerne, H., Pabst, K., Gravert, H. O. 1992. Die Bedeutung der Proteinvarianten für genetische Unterschiede in der Käsereitauglichkeit der Milch. – Züchtungskunde, 64, 20–26.

Ostersen, S., Foldager, J., Hermansen, J. E. 1997. Effects of stage of lactation, milk protein genotype and body condition at calving on protein composition and renneting properties of bovine milk. – Journal of Dairy Research, 64(2), 207–219.

Pagnacco, G., Caroli, A. 1987. Effect of casein and β-lactoglobulin genotypes on renneting properties of milks. – Journal of Dairy Research, 54(4), 479–485.

Penasa, M., Cassandro, M., Pretto, D., De Marchi, M., Comin, A., Chessa, S., Dal Zotto, R., Bittante, G. 2010. Short communication: Influence of composite casein genotypes on additive genetic variation of milk production traits and coagulation properties in Holstein-Friesian cows. – Journal of Dairy Science, 93(7), 3346–3349.

Piimaveiste põlvnemis- ja jõudlusandmete kogumise, nende õigsuse kontrollimise, töötlemise ja säilitamise kord. 2012. Jõudluskontrolli Keskus. http://www.jkkeskus.ee/pages/pv/pv_kord_jk.pdf (Accessed October 12, 2012, in Estonian)

Pinder, S. J., Perry, B. N., Skidmore, C. J., Savva, D. 1991. Analysis of polymorphism in the bovine casein genes by use of the polymerase chain reaction. – Animal Genetics, 22(1), 11–20.

Politis, I., Ng-Kwai-Hang, K. F. 1988. Effects of somatic cell count and milk composition on cheese composition and cheese making efficiency. – Journal of Dairy Science, 71(7), 1711–1719.

Pretto, D. 2012. Improvement of milk coagulation properties in dairy chain. – PhD thesis, University of Padova, 106.

Pretto, D., De Marchi, M., Penasa, M., Cassandro, M. 2013. Effect of milk composition and coagulation traits on Grana Padano cheese yield under field conditions. – Journal of Dairy Research, 80(1), 1–5.

Ruottinen, O., Ikonen, T., Ojala, M. 2004. Associations between milk protein genotypes and fertility traits in Finnish Ayrshire heifers and first lactation cows. – Livestock Production Science, 85(1), 27–34.

SAS Institute. 2008. SAS/STAT 9.2 User's Guide. SAS Inst. Inc., Cary, NC.

Schaar, J. 1984. Effects of κ-casein genetic variants and lactation number on the renneting properties of individual milks. – Journal of Dairy Research, 51(3), 397–406.

Somers, J. M., Guinee, T. P., Kelly, A. L. 2002. The effect of plasmin activity and cold storage of cheese milk on the composition, ripening and functionality of mozzarella-type cheese. – Australian Journal of Dairy Technology, 55, 5–11.

Statistikaamet. 2012. Loomakasvatussaaduste tootmine. http://pub.stat.ee/px-web.2001/Database/Majandus/13Pellumajandus/06Pellumajandussaaduste_tootmine/02Loomakasvatussaaduste_tootmine/02Loomakasvatussaaduste_tootmine.asp (Accessed March 2, 2013)

Tervala, H.-L., Antila, V., Syväjärvi, J. 1985. Factors affecting the renneting properties of milk. – Meijeritieteellinen Aikakauskirja, XLIII, 1, 16–25.

Tyrisevä, A.-M., Ikonen, T., Ojala, M. 2003. Repeatability estimates for milk coagulation traits and non-coagulation of milk in Finnish Ayrshire cows. – Journal of Dairy Research, 70(1), 91–98.

Tyrisevä, A.-M., Vahlsten, T., Ruottinen, O., Ojala, M. 2004. Noncoagulation of milk in Finnish Ayrshire and Holstein-Friesian cows and effect of herds on milk coagulation ability. – Journal of Dairy Science, 87(11), 3958–3966.

Tyrisevä, A.-M. 2008. Options for selecting dairy cattle for milk coagulation ability. – PhD thesis, University of Helsinki, Department of Animal Science, Publications No. 93, 37.

Van Hooijdonk, A. C. M., Hagedoorn, H. G., Boerrigter, I. J. 1986. pH-induced physico-chemical changes of casein micelles in milk and their effect on renneting. 1. Effect of acidification on physicochemical properties. – Netherlands Milk and Dairy Journal, 40, 281–296.

Visker, M. H. P. W., Dibbits, B. W., Kinders, S. M., van Valenberg, H. J. F., van Arendonk, J. A. M., Bovenhuis, H. 2011. Association of bovine β-casein protein variant I with milk production and milk protein composition. – Animal Genetics, 42(2), 212–218.

Walsh, C. D., Guinee, T. P., Reville, W. D., Harrington, D., Murphy, J. J., O'Kennedy, B. T., FitzGerald, R. J. 1998. Influence of κ-casein genetic variant on rennet gel microstructure, Cheddar cheesemaking properties and casein micelle size. – International Dairy Journal, 8(8), 707–714.

Walstra, P. 1990. On the stability of casein micelles. – Journal of Dairy Science, 73(8), 1965–1979.

Wedholm, A. 2008. Variation in milk protein composition and its importance for the quality of cheese milk. – PhD thesis, Swedish University of Agricultural Sciences, Faculty of Natural Resources and Agricultural Sciences, Department of Food Science, 56.

Wedholm, A., Larsen, L. B., Lindmark-Mansson, H., Karlsson, A. H., Andrén, A. 2006. Effect of protein composition on the cheese-making properties of milk from individual dairy cows. – Journal of Dairy Science, 89(9), 3296–3305.

Тооме, А. А. 1972. Генетический полиморфизм β-лактоглобулинов и казеинов и возможности его использования в селекции пород крупного рогатого скота в Эстонской ССР. – Автореферат кандидата с.-х. наук, Тарту. 27 с. (in Russian)

SUMMARY IN ESTONIAN

PIIMA LAAPUMISOMADUSTE MODELLEERIMINE JA GENEETILINE DETERMINEERITUS

Piima laapumisomadustest sõltub juustu väljatulek ja kvaliteet, mistõttu on piima hea laapuvus vajalik eelkõige juustutööstusele. Nimelt on juustu (sh kohupiima) tootmismaht Eestis püsinud tõusvas trendis juba üle kümne aasta ning on selle aja jooksul enam kui kahekordistunud.

Piima laapumine on keeruline protsess ja standardset meetodit laapumisomaduste määramiseks pole veel välja töötatud. Erinevate määramismeetodite tõttu ei pruugi aga erinevate uuringute mõõtmistulemused olla üks-üheselt võrreldavad. Teadusuuringutes on siiani levinuimaks mehaaniline määramismeetod, kus piimaproovi sukeldatav andur registreerib piima viskoelastsete omaduste muutust ning võimaldab seeläbi piima laapumisomadusi otseselt mõõta. Viimasel ajal on kasutatud ka optilisi määramismeetodeid, mis registreerivad laapumisnäitajaid kaudselt ning laapumisprotsessi sekkumata, olles seega eriti sobilikud protsessi jälgimiseks juustutööstuses.

Varasemad piima laapumisalased uuringud Eestis on peamiselt keskendunud laapumist mõjutavate keskkonnafaktorite välja-selgitamisele. Geneetilisest aspektist lähtuvalt on uuritud piimavalkude genotüüpide mõju piima laapumisomadustele, kuid põhjalikumad uuringud piima laapumisomaduste parandamisvõimaluste kohta aretuse teel puuduvad. Itaalias ja Soomes läbi viidud uuringud on näidanud piima laapumisomaduste mõõdukat geneetilist varieeruvust, mis on soodne otseseks selektsiooniks laapumisnäitajate alusel. Samas on piima laapumisomaduste määramine töömahukas ja aeganõudev – erinevad määramismeetodid võimaldavad 30 minuti jooksul mõõta korraga vaid ühe kuni kümne piimaproovi laapumisomadusi. Seega on kasulik uurida võimalusi ka kaudseks selektsiooniks laapumisomadustega tugevas geneetilises seoses olevate rutiinselt määratavate piimanäitajate alusel.

Eelpool toodust lähtuvalt olid doktoritöö hüpoteesid:

- eesti holsteini tõugu lehmade piima laapumisomadustel esineb tõusisene geneetiline varieeruvus ning neid piima omadusi on võimalik parandada aretuse teel;
- erinevate määramismeetoditega mõõdetud laapumisnäitajad on teisendatavad ühtsele skaalale.

Sellest tulenevalt olid uuringu eesmärgid järgmised:

- hinnata piima laapumisomaduste päritavust ja korduvust (**I**);
- hinnata geneetilised korrelatsioonid laapumisnäitajate ning piima toodangu, pH ja koostisnäitajate (rasva- ja valgusisaldus, somaatiliste rakkude arv, karbamiidi sisaldus) vahel (**I**);
- hinnata β-κ-kaseiini genotüübi mõju piima toodangule, laapumis- ja koostisnäitajatele (**III**);
- hinnata β-κ-kaseiini genotüübi poolt kirjeldatava varieeruvuse osakaalu piima toodangu, laapumis- ja koostisnäitajate aditiivgeneetilises varieeruvuses (**III**);
- anda hinnangud valemitele, mille abil saab ühe määramismeetodi järgi määratud laapumisnäitajaid teisendada teise meetodi skaalale (**II**).

Geneetiliseks hindamiseks (**I**, **III**) määrati laapumisnäitajad optilise meetodiga (kasutades Optigraafi) ning kasutati korduvmõõtmistele tuginevat tavalist ja juhuslike regressioonikordajatega loomamudelit, arvestades vaatlusaluste lehmade kolme eellaste põlvkonna põlvnemisandmeid. Geneetilised parameetrid leiti 4191 eesti holsteini tõugu lehma esimese laktatsiooni piimaproovide alusel (kokku 17577 proovi) ning β-κ-kaseiini genotüübi mõju hindamine baseerus 2859 genotüpiseeritud eesti holsteini tõugu lehma esimese kolme laktatsiooni piimaproovidel (kokku 23970 proovi). Laapumisnäitajate teisendamisvõimaluste uurimiseks (**II**) teostati 165 itaalia holstein-friisi tõugu lehma piimaproovide laapumisnäitajate mõõtmised erinevate mehaaniliste meetoditega paralleelselt kahes Itaalia laboris ning optilise määramismeetodiga Eesti laboris.

Uuringu peamised tulemused olid järgmised.

- Päritavuse hinnangud olid eesti holsteini tõugu lehmade esimese laktatsiooni piima laapumisomadustel suuremad kui piima toodangu, pH ja koostisnäitajate vastava parameetri hinnangud, olles kalgendi tugevusel 41% ja laapumisajal 28%. Võttes arvesse laapumisomaduste suhteliselt kõrget korduvust (laapumisajal 0,45 ja kalgendi tugevusel 0,50), saab järeldada, et usaldusväärne geneetiline hinnang konkreetse lehma piima laapumisomadustele on antav juba väikse arvu mõõtmiste alusel. Seega on selektsioon piima laapumisomaduste alusel võimalik. Kuigi kogu populatsiooni lehmade piima laapumisomaduste rutiinne mõõtmine on tõhusate tehniliste vahendite ja standardse määramismeetodi puudumise tõttu hetkel veel keeruline, on sellele vaatamata võimalik geneetiliselt hinnata piiratud arvu aretusloomade (vaid valik pulle ja/või pulliemasid) piima laapumisomadusi (**I**).

- Geneetilised korrelatsioonid piima laapumisnäitajate ning rutiinselt määratavate piimanäitajate (piima toodang, pH, rasva- ja valgusisaldus, somaatiliste rakkude arvu skoor ja karbamiidi sisaldus) vahel olid üldjuhul nõrgad. Erandiks oli tugev positiivne seos laapumisaja ja piima pH vahel ning mõõduka tugevusega positiivne geneetiline seos kalgendi tugevuse ja piima valgusisalduse vahel. Kalgendi tugevusel esines ka nõrk positiivne geneetiline seos piima rasvasisaldusega ning nõrk negatiivne geneetiline seos piima toodanguga. Sellest tulenevalt võib järeldada, et selektsiooniga piima laapumisomaduste alusel ei kaasne teiste nimetatud piimanäitajate märgatav halvenemine. Samas välistab tugevate geneetiliste seoste puudumine piima laapumisnäitajate ja rutiinselt määratavate piimanäitajate vahel võimaluse piima laapumisomaduste parandamiseks kaudse selektsiooni teel (**I**).

- Võrreldes varasemate mehaanilisel meetodil läbi viidud uuringutega, oli antud uuringus nii geneetiline kui ka fenotüübiline korrelatsioon (vastavalt –0,16 ja –0,22 kuni –0,23) optilisel meetodil määratud piima laapumisnäitajate vahel nõrk. Laapumisaja geneetilised ja fenotüübilised seosed piima toodangu ja koostisnäitajatega olid varasemates uuringutes

leitud vastavate seostega paremas kooskõlas kui kalgendi tugevusel. Samuti esines laapumisaja puhul tugev seos erinevate määramismeetodite mõõtmistulemuste vahel, samas kui kalgendi tugevuse puhul oli mehaaniliste ja optiliste mõõtmistulemuste vaheline seos mõõdukas. Seega võib järeldada, et kalgendi tugevuse mõõtmistulemused sõltuvad määramismeetodist enam kui laapumisaja mõõtmistulemused, ning optiline ja mehaaniline määramismeetod kirjeldavad kalgendi tugevuse erinevaid aspekte. Sellest tulenevalt võib öelda, et Optigraafiga määratud kalgendi tugevuse mõõtmistulemuste teisendamisel mehaanilise meetodi määramisskaalale, tuleb eelistada selle näitaja algseid optilisi mõõtmistulemusi. Samuti vajab varasemate sarnaste uuringute puudumisel Optigraafil mõõdetud laapumisomaduste seos juustu väljatuleku ja kvaliteedinäitajatega täiendavaid uuringuid (**I**, **II**).

- Töö uurimistulemused näitavad, et erinevate määramismeetodite abil saadud laapumisomaduste mõõtmistulemused on regressioonivõrrandeid kasutades teisendatavad omavahel võrreldavateks näitajateks. Mõlema laapumisnäitaja (laapumisaeg ja kalgendi tugevus) osas on teisendustäpsus suurem mehaanilist meetodit kasutavate määramismeetodite vahel (R^2 vastavalt 0,82 ja 0,63). Erinevat tüüpi (mehaaniline *versus* optiline) määramismeetodite vaheline teisendustäpsus oli laapumisaja korral suurem (R^2 0,7 kuni 0,81) kui kalgendi tugevusel (R^2 0,28 kuni 0,43). Kuna laapumisnäitajate otsene võrdlemine erinevaid määramismeetodeid kasutades on mitmete segavate ja raskesti kontrollitavate eksperimentaalsete mõjutegurite tõttu küllaltki keeruline, lihtsustab sobivate teisendusmeetodite kasutamine laapumisnäitajate ning nende geneetiliste parameetrite võrdlemist. Sellest tulenevalt on põhjendatud edasised uuringud erinevate määramismeetodite vaheliste laapumisnäitajate teisendusmeetodite väljatöötamiseks (**II**).

- Statistiliselt oluline oli β-κ-kaseiini genotüübi mõju laapumisnäitajatele ning valgusisaldusele, samas kui statistiliselt olulist mõju sellel genotüübil piima toodangule, rasvasisaldusele ja somaatiliste rakkude arvu skoorile ei ilmnenud (**III**).

- Eesti holsteini tõugu lehmade kõige enam levinud β-κ-kaseiini genotüübid A2A2AA ja A1A2AA (mõlema genotüübi esinemissagedus oli üle 20%) seostusid piima halva laapuvusega. Nende genotüüpide suur esinemissagedus ja märgatav positiivne (kuigi statistiliselt mitteoluline) mõju piimatoodangule viitab eesti holsteini tõu piimatoodangu tõstmisele suunatud pikaajalise aretustöö tulemusele. Arvestades nimetatud suure sagedusega genotüüpide negatiivset mõju ning madala sagedusega κ-kaseiini B alleeli soodsat mõju laapumisnäitajatele ja β-κ-kaseiini IB haplotüübi positiivset seost kalgendi tugevuse ja piima valgusisaldusega, oleks võimalik piima laapumisomaduste parandamine eesti holsteini populatsioonis loomi β-κ-kaseiini genotüübi alusel selekteerides. IB haplotüübi ja teiste harva esinevate β-κ-kaseiini haplotüüpide ja genotüüpide mõju laapumisnäitajatele vajab täiendavaid uuringuid (**III**).

- Võttes statistilises mudelis arvesse β-κ-kaseiini genotüübi mõju, vähenes laapumisaja aditiivgeneetiline varieeruvus 13% ning kalgendi tugevuse aditiivgeneetiline varieeruvus 51%. Samas oli vastav mõju piima toodangu ja koostisnäitajate aditiivgeneetilisele varieeruvusele marginaalne. Seega avaldab selektsioon β-κ-kaseiini genotüübi alusel mõju eelkõige laapumisnäitajatele, eriti kalgendi tugevusele (**III**).

Kokkuvõttes saab öelda, et eesti holsteini tõugu lehmade piima laapumisomaduste geneetiline determineeritus võimaldab laapumisomaduste parandamist nii otsese selektsiooni teel kui ka geneetiliste markerite alusel (kaseiini variandid). Selektsiooniks soodne oli ka populatsiooni geneetiline struktuur, kuna laapumisomadustele negatiivset mõju avaldavad β-κ-kaseiini genotüübid olid suure esinemissagedusega. Praktilisest seisukohast on kogu populatsiooni laapumisomaduste mõõtmine ja rutiinne registreerimine tõhusa määramismeetodi puudumise tõttu hetkel küll raskendatud, kuid piiratud arvu aretusloomade (vaid valik pulle ja/või pulliemasid) geneetiline hindamine siiski võimalik. Täiendavalt vajavad uurimist Optigraafi abil mõõdetud laapumisomaduste seosed juustu väljatuleku ning kvaliteediga.

ACKNOWLEDGEMENTS

The research leading to these results was co-financed by the European Community's Regional Development Fund in the framework of the Competence Centre Programme of the Enterprise Estonia under project Nos EU22868; EU27789; EU28662; EU30002 of the Bio-Competence Centre of Healthy Dairy Products LLC (Tervisliku Piima Biotehnoloogiate Arenduskeskus OÜ), and by Targeted Finance Projects 1080045s07 and 1080022s07.

The people from Estonian Animal Recording Centre, Animal Breeders' Association of Estonia, Institute of Veterinary Medicine and Animal Sciences of the Estonian University of Life Sciences are warmly thanked for their contribution in the collection and laboratory analysis of the data for the databases COAGEN®, COAPED® and COACAS®.

I am grateful to Johan van Arendonk from Animal Breeding and Genomics Centre, Wageningen University, for the opportunity of the research period at the Animal Breeding and Genomics Centre of Wageningen University. The Archimedes Foundation DoRa scholarship that enabled this visit is gratefully acknowledged.

Great thanks to my colleagues from the Department of Animal Genetics and Breeding and from the Department of Nutrition and Animal Products Quality, who contributed in different ways into the work underlying this thesis. Many thanks go to Sirje Värv and Ivi Jõudu for their advice and helpful comments.

Thank you, David Arney and Nele Rand, for language editing in this thesis.

My special thanks go to Henk Bovenhuis from Animal Breeding and Genomics Centre, Wageningen University, for valuable discussions, inspiration and scientific contribution regarding my first paper.

I am very grateful to my supervisors Elli Pärna, Kalev Pärna and Tanel Kaart for their support and statistically significant contribution to this thesis.

I

ORIGINAL PUBLICATIONS

Vallas, M., Bovenhuis, H., Kaart, T., Pärna, K., Kiiman, H., Pärna, E. 2010.
GENETIC PARAMETERS FOR MILK COAGULATION PROPERTIES IN ESTONIAN HOLSTEIN COWS.
Journal of Dairy Science, 93(8), 3789–3796.

J. Dairy Sci. 93:3789–3796
doi:10.3168/jds.2009-2435

Genetic parameters for milk coagulation properties in Estonian Holstein cows

M. Vallas,*†[1] H. Bovenhuis,‡ T. Kaart,*† K. Pärna,†§ H. Kiiman,*† and E. Pärna*†
*Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, 51014 Tartu, Estonia
†Bio-Competence Centre of Healthy Dairy Products, Kreutzwaldi 1, 51014 Tartu, Estonia
‡Animal Breeding and Genomics Centre, Wageningen University, PO Box 338, 6700 AH Wageningen, the Netherlands
§Institute of Mathematical Statistics, University of Tartu, J.Liivi 2, 50409 Tartu, Estonia

ABSTRACT

The objective of this study was to estimate heritabilities and repeatabilities for milk coagulation traits [milk coagulation time (RCT) and curd firmness (E_{30})] and genetic and phenotypic correlations between milk yield and composition traits (milk fat percentage and protein percentage, urea, somatic cell count, pH) in first-lactation Estonian Holstein dairy cattle. A total of 17,577 test-day records from 4,191 Estonian Holstein cows in 73 herds across the country were collected during routine milk recordings. Measurements of RCT and E_{30} determined with the Optigraph (Ysebaert, Frepillon, France) are based on an optical signal in the near-infrared region. The cows had at least 3 measurements taken during the period from April 2005 to January 2009. Data were analyzed using a repeatability animal model. There was substantial variation in milk coagulation traits with a coefficient of variation of 27% for E_{30} and 9% for the log-transformed RCT. The percentage of variation explained by herd was 3% for E_{30} and 4% for RCT, suggesting that milk coagulation traits are not strongly affected by herd conditions (e.g., feeding). Heritability was 0.28 for RCT and 0.41 for E_{30}, and repeatability estimates were 0.45 and 0.50, respectively. Genetic correlation between both milk coagulation traits was negligible, suggesting that RCT and E_{30} have genetically different foundations. Milk coagulation time had a moderately high positive genetic (0.69) and phenotypic (0.61) correlation with milk pH indicating that a high pH is related to a less favorable RCT. Curd firmness had a moderate positive genetic (0.48) and phenotypic (0.45) correlation with the protein percentage. Therefore, a high protein percentage is associated with favorable curd firmness. All reported genetic parameters were statistically significantly different from zero. Additional univariate random regression analysis for milk coagulation traits yielded slightly higher average heritabilities of 0.38 and 0.47 for RCT and E_{30} compared with the heritabilities of the repeatability model.

Key words: milk coagulation, heritability, genetic correlation, Estonian Holstein dairy cattle

INTRODUCTION

Recent trends indicate that an increasing percentage of milk produced in European countries is used for manufacturing cheese (Eurostat, 2009). At present, in Italy more than 75% (De Marchi et al., 2008), in Scandinavian countries 33% (Wedholm et al., 2006), and in Estonia 60% (Statistics Estonia, 2009) of milk is being used for cheese production. Milk coagulation properties are important to the quality and yield of cheese (Leitner et al., 2006; Wedholm et al., 2006; De Marchi et al., 2009); therefore, these properties become more important if an increasing percentage of milk is used for cheese manufacturing. Milk coagulation properties are commonly defined by milk coagulation time and curd firmness. A short milk coagulation time and a firmer curd are favorable for cheese production. Shorter milk coagulation time may be beneficial to cheesemaking because it leaves more time for curd firming during the coagulation process. A firmer curd during clotting is positively correlated with cheese yield (Aleandri et al., 1989; Martin et al., 1997). Ikonen et al. (1999a) observed greater cheesemaking efficiency from milk with good coagulation properties.

Milk coagulation is a complex process and is not understood in detail, but studies have indicated that it is affected by numerous factors including casein composition (Okigbo et al., 1985a), SCC (Politis and Ng-Kwai-Hang, 1988), concentrations of total casein and calcium (Storry et al., 1983; Summer et al., 2002), pH (Najera et al., 2003), genetic polymorphism of milk proteins (Van den Berg et al., 1992; Mayer et al., 1997; Ikonen et al., 1999b), stage of lactation (Okigbo et al., 1985b; Ostersen et al., 1997), season (O'Brien et al., 1999), and breed (Auldist et al., 2002, 2004; De Marchi et al., 2008). Although it has been suggested that genetic factors affect milk coagulation, few studies have quantified the extent to which genetic factors play a

Received May 30, 2009.
Accepted April 6, 2010.
[1]Corresponding author: mirjam.vallas@emu.ee

role. Reported heritability estimates for milk coagulation traits range from 0.15 to 0.40 (Ikonen et al., 1999c, 2004; Tyrisevä et al., 2004; Cassandro et al., 2008). Furthermore, only a few studies have reported genetic relationships between milk coagulation traits and milk production and composition traits (Ikonen et al., 1999c, 2004; Cassandro et al., 2008). Knowledge of the genetic parameters of milk coagulation traits and their associations with milk production is important when considering ways to improve milk coagulation by means of genetic selection. Before including milk coagulation traits in the breeding goal, the economic value of these traits needs to be determined. There is a large amount of information about the application of biotechnology to cheese manufacturing but very little about the feasibility of enhancing cheese production efficiency by the genetic improvement of milk coagulation.

The aim of this study was to estimate the heritability and repeatability for milk coagulation traits and their genetic correlation with milk yield and composition, SCS, urea, and pH levels.

MATERIALS AND METHODS

Data Collection

First-lactation milk samples were collected during routine milk recording as part of a development project for the Bio-Competence Centre of Healthy Dairy Products in Estonia during the period from April 2005 to January 2009. The herds were milked 2 or 3 times a day. The individual milk samples collected from the cows were a mixture of all test-day milkings or only the morning, afternoon, or evening milkings on the test-day. Milk samples were immediately preserved with Bronopol (Knoll Pharmaceuticals, Nottingham, UK) and stored at 4°C during the transportation and analyzing periods. Milk samples with a pH <6.5, indicative of colostrum (Bhandari and Singh, 2002), and noncoagulated milk samples (n = 52) were excluded from the analysis. Additionally, farms with fewer than 10 cows and cows with fewer than 3 test-day records were removed. The final data set used for analyses consisted of 17,577 test-day records from 4,191 Estonian Holstein cows located in 73 herds across the country and that were daughters of 274 sires. The number of daughters per sire ranged from 1 to 267. Each cow has 3 to 6 measurements collected during the different stages (7 to 305 DIM) of the first lactation.

Information about the cows, herds, and pedigrees was obtained from the Estonian Animal Recording Centre (EARC) and the Animal Breeders' Association of Estonia, and entered into the database COAGEN of the Bio-Competence Centre of Healthy Dairy Products.

Laboratory Analysis

The test-day milk yield was recorded and individual milk samples were analyzed for fat percentage, protein percentage, and urea using the MilkoScan 4000 and MilkoScan FT6000, and for SCC using the Fossomatic 400 and Fossomatic 5000 cell counter (all equipment from Foss, Hillerød, Denmark) at the Milk Analysis Laboratory of EARC, using methods suggested by the International Committee for Animal Recording (2009). Values of SCC were log-transformed to SCS: SCS = $\log_2(\text{SCC}/100{,}000) + 3$.

The pH and milk coagulation properties were determined at the Laboratory of Milk Quality of the Estonian University of Life Sciences (Tartu, Estonia) generally 3 d after sampling. The proportion of milk samples with a maximum age of 7 d was very small (<1%). The pH level of the milk was determined using a pH meter (Seven Multi, Mettler Toledo GmbH, Greifensee, Switzerland) at a temperature of 20°C before analyzing the milk coagulation properties. The latter were milk coagulation time (**RCT**, min) and curd firmness ($\mathbf{E_{30}}$, mm). Before assessment of the milk coagulation properties, milk samples were heated to the renneting temperature (35°C). The rennet (Milase MRS 750 international milk clotting units/mL; CSK Food Enrichment B.V., Ede, the Netherlands) used in the analyses was diluted 1:100 (vol/vol) with distilled water, and 0.2 mL of the solution was added to 10 mL of milk. The milk coagulation properties were determined using the Optigraph (Ysebaert, Frepillon, France), which was developed by Ysebaert Dairy Division in partnership with Institut National de la Recherche Agronomique, Laboratoire de Génie et Microbiologie des Procédés Alimentaires (Thiverval-Grignon, France), to define coagulation characteristics in the laboratory, especially to answer the needs of cheese makers (Ysebaert Dairy Division, 2009).

Measurements made with the Optigraph are not based on a rheological measures but on an optical signal in the near-infrared region. During a coagulation test, the light emitted through the milk gradually weakens because of changes in the micellar structure of casein. The Optigraph then calculates the coagulation parameters (coagulation time, curd firmness, and speed of aggregation) by means of particular feature points extracted from the optical information acquired in real time. Optigraph system parameters were set as follows: R slope = 1.784 and R offset = −2.303. Milk coagulation time was recorded directly based on the maximum first derivative of the signal. To determine the firmness of the curd, the Optigraph signal 30 min after the addition of the rennet was converted into millimeters using a calibration equation (Kübarsepp et al., 2005).

Table 1. Number of observations (n), means, and coefficients of variation (CV) for test-day milk coagulation, production, and composition traits

Trait	n	Mean	CV (%)
Curd firmness (E_{30}) (mm)	17,577	27.0	27
Milk coagulation time (RCT)[1] (min)	17,577	2.3	9
Milk yield (kg)	17,575	25.9	28
Fat (%)	17,536	4.05	17
Protein (%)	17,567	3.38	9
SCS[2]	17,567	2.9	65
Urea (mg/L)	17,223	26.8	31
pH	17,577	6.6	1

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

Statistical Analysis

Statistical analysis was carried out in ASReml (Gilmour et al., 2002) using the following repeatability animal model:

$$Y_{ijklmn} = \mu + \beta_1 DIM_i + \beta_2 DIM_i^2 + \beta_3 age + ys_j + cys_k + herd_l + animal_m + pe_n + e_{ijklmn},$$

where Y_{ijklmn} = dependent variable (log-transformed RCT, E_{30}, milk yield, milk protein and fat percentages, SCS, urea, pH); μ = overall mean; DIM_i = ith day in milk, modeled with quadratic polynomial; age = linear regression on age at calving; β_1, β_2, and β_3 = regression coefficients; ys_j = fixed effect of sampling year-season (j = 1,..., 16 with 3-mo classes from April 2005 to January 2009); cys_k = fixed effect of calving year-season (k = 1,..., 15 with 3-mo classes from December 2004 to August 2008); $herd_l$ = random effect of herd (l = 1,..., 73), $herd_l \sim N(0, \mathbf{I}\sigma_h^2)$; $\mathbf{I}$ = identity matrix; $animal_m$ = random effect of animal (m = 1,..., 17,185), $animal_m \sim N(0, \mathbf{A}\sigma_a^2)$; $\mathbf{A}$ = additive genetic relationship matrix; pe_n = random permanent environmental term (n = 1, ..., 4,191), $pe_n \sim N(0, \mathbf{I}\sigma_{pe}^2)$; and e_{ijklmn} = residual random error term, $e_{ijklmn} \sim N(0, \mathbf{I}\sigma_e^2)$. For normality assumption, log-transformation of RCT was used.

Three generations of ancestors were included in the analysis and 17,185 animals were included in the relationship matrix. Heritabilities were estimated with univariate models, and genetic correlations were derived from bivariate models.

Heritability (h^2) was calculated as

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2},$$

repeatability (r) was calculated as

$$r = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2},$$

and the proportion of variation due to herds (hrd) was calculated as

$$hrd = \frac{\sigma_h^2}{\sigma_h^2 + \sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2},$$

where σ_a^2 = additive genetic variation; σ_{pe}^2 = permanent environmental variation; σ_e^2 = residual variation; and σ_h^2 = herd variation.

RESULTS AND DISCUSSION

Descriptive Statistics

Table 1 shows descriptive statistics of the milk coagulation traits, and milk production and composition traits. There was substantial variation in milk coagulation traits as indicated by the coefficient of variation (**CV**) of 27% for E_{30} and 9% for the log-transformed RCT. Milk yield had a CV similar to that of E_{30} (28%), whereas the CV was higher for urea (31%). There was little variation in pH (CV = 1%).

Systematic Environmental Effects

Table 2 shows the fraction of total phenotypic variance due to herd, reflecting the relative importance of differences between herds, probably mainly because of differences in feeding. Milk coagulation properties were not strongly influenced by differences between herds: the proportion of variation due to the herd was 0.04 for RCT and 0.03 for E_{30}. The proportion of the variance explained by herd was also low for milk composition traits. For milk yield, a substantial percentage of the variation was due to the herd (25%). Tyrisevä et al. (2004) reported slightly higher proportions of variation explained by herd for RCT (6%) and E_{30} (9%). Ikonen et al. (2004) reported similar results as in the present study with a small fraction of the variation explained by herd (5%) for RCT and E_{30}. Herd effects for milk yield, fat percentage, and protein percentage in studies by Tyrisevä et al. (2004) and Ikonen et al. (2004) were higher (ranging from 15 to 48%) than our estimates. Milk coagulation properties therefore seem to be affected to a smaller extent by the herd environment than are milk composition traits. This suggests that it is difficult to improve milk coagulation properties by herd management factors such as nutrition.

The effect of DIM on milk coagulation properties is shown in Figure 1. Rennet coagulation time increased toward the end of lactation and stabilized after d 180 to less favorable values. Curd firmness showed a different pattern with less favorable values during the first part of lactation and more favorable (higher) values toward the end of the lactation.

Variation in the time between milk sampling and analysis may also have an effect on milk coagulation properties. Ikonen et al. (2004) reported a significant effect of the time between milk sampling and analysis on curd firmness. We were not able to adjust for this effect, because this information was not available for all samples. Based on the design of the current study, we may state that the time between milk sampling and analysis could have varied from 1 to 7 d. This variation was, however, much smaller than in the study by Ikonen et al. (2004) (0 – 27 d) and therefore is expected to have less effect on milk coagulation properties.

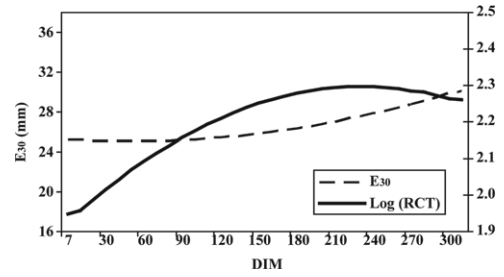

Figure 1. Lactation curve of log-transformed milk coagulation time [log (RCT)] and curd firmness (E_{30}) during the lactation period 7 to 305 DIM.

Heritabilities and Repeatabilities

Heritabilities and repeatabilities of all traits studied were statistically significantly different from zero ($P < 0.05$). Heritabilities of milk coagulation properties were 0.41 for curd firmness and 0.28 for milk coagulation time (Table 2). The heritability estimate for RCT was similar to that reported by Ikonen et al. (2004) but somewhat higher than other estimates (0.21 to 0.25) reported for Finnish and Italian dairy cattle populations (Ikonen et al., 1999c; Tyrisevä et al., 2004; Cassandro et al., 2008). Heritability of E_{30} was consistent with the estimate (0.4) reported by Ikonen et al. (1999c) but higher than other estimates (0.15 to 0.22) available in literature (Ikonen et al., 2004; Tyrisevä et al., 2004; Cassandro et al., 2008). These differences may be due to genetically different cattle populations. The difference in methods might also have influenced variation in milk coagulation traits. Coefficients of variation for milk coagulation traits in the studies (Ikonen et al., 1999c) using the Formagraph (Foss) or in the studies (Ikonen et al., 2004; Tyrisevä et al., 2004; Cassandro et al., 2008) using Computerized Renneting Meter (Polo Trade, Monselice, Italy) are larger than in our study (ranging from 27 to 50%). In our study, heritabilities for milk coagulation traits were higher than those for milk yield and composition traits. The highest heritability for a milk composition trait was for protein percentage (0.28); that for milk yield was 0.15. Heritability was low for SCS (0.05) and urea (0.07).

Repeatabilities for RCT (0.45) and E_{30} (0.50) were high and of the same order of magnitude as repeatabilities for milk yield (0.47) and protein percentage (0.46). Urea had a low repeatability of 0.19. The repeatability for SCS was moderately high (0.37).

Repeatabilities for RCT and E_{30} reported in Finnish studies by Ikonen et al. (1997) and Tyrisevä et al. (2003) are somewhat higher (from 0.57 to 0.68) than ours,

Table 2. The estimates of heritability (h^2), repeatability (r), proportion of variance due to herd (hrd), and total phenotypic variance (σ_P^2)[1] for the traits studied

Trait	h^2 (SE)	r (SE)	hrd (SE)	σ_P^2
Curd firmness (E_{30}) (mm)	0.41 (0.04)	0.50 (0.01)	0.03 (0.01)	49.27
Milk coagulation time (RCT)[2] (min)	0.28 (0.04)	0.45 (0.01)	0.04 (0.01)	3.15
Milk yield (kg)	0.15 (0.03)	0.47 (0.01)	0.25 (0.03)	38.00
Fat (%)	0.19 (0.03)	0.32 (0.01)	0.06 (0.01)	0.47
Protein (%)	0.28 (0.04)	0.46 (0.01)	0.06 (0.01)	0.06
SCS[3]	0.05 (0.02)	0.37 (0.01)	0.03 (0.01)	3.50
Urea (mg/L)	0.07 (0.02)	0.19 (0.01)	0.11 (0.02)	64.73
pH	0.24 (0.03)	0.36 (0.01)	0.06 (0.01)	0.31

[1]$\sigma_P^2 = \sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2$. σ_a = additive genetic variance, σ_{pe} = permanent environmental variance, and σ_e = residual variance.
[2]Log-transformed.
[3]SCS = [3 + $\log_2$(SCC/100,000)].

Table 3. Phenotypic (above the diagonal) and genetic correlations (below the diagonal; SE in parentheses) between milk coagulation properties, production, and composition traits[1]

Item	E_{30} (mm)	RCT	Milk yield (kg)	Fat (%)	Protein (%)	SCS	Urea (mg/L)	pH
Curd firmness (E_{30}) (mm)		−0.22	−0.06	0.11	0.45	0.01	0.02	−0.15
Milk coagulation time (RCT)[2] (min)	−0.16 (0.09)		−0.01	−0.04	0.15	0.10	0.03	0.61
Milk yield (kg)	−0.29 (0.11)	−0.07 (0.12)		−0.33	−0.18	−0.12	0.09	−0.08
Fat (%)	0.25 (0.09)	−0.10 (0.10)	−0.65 (0.09)		0.25	0.12	−0.03	−0.05
Protein (%)	0.48 (0.07)	0.19 (0.10)	−0.51 (0.09)	0.49 (0.08)		0.16	−0.11	−0.02
SCS[3]	−0.04 (0.15)	−0.06 (0.15)	0.12 (0.18)	−0.05 (0.16)	−0.02 (0.15)		−0.17	0.12
Urea (mg/L)	0.19 (0.12)	−0.00 (0.12)	0.13 (0.15)	−0.21 (0.13)	−0.12 (0.13)	0.12 (0.19)		0.00
pH	−0.06 (0.09)	0.69 (0.05)	0.07 (0.12)	−0.26 (0.10)	−0.12 (0.10)	−0.22 (0.14)	0.07 (0.12)	

[1]SE of phenotypic correlations were 0.01 to 0.02.

[2]Log-transformed.

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

which may also reflect the different measuring aspects of coagulation in these studies. The high repeatability estimates for RCT and E_{30} in our study, however, show that only a few measurements are needed for reliable genetic estimation of milk coagulation properties.

Genetic and Phenotypic Correlations

Genetic correlation between the 2 milk coagulation properties (Table 3) was negligible, suggesting that milk coagulation time and curd firmness measured by the Optigraph are mainly influenced by different genes. Other correlations reported here were significantly different from zero ($P < 0.05$). Phenotypic correlation between the 2 milk coagulation properties was slightly higher (−0.22). Both estimates were lower than those reported in previous studies (Ikonen et al., 1999c, 2004; Tyrisevä et al., 2004; Cassandro et al., 2008), ranging from −0.89 to −0.96. This result may indicate that the Optigraph device used in our study measures somewhat different aspects of milk coagulation compared with the devices used in previous studies (Formagraph and Computerized Renneting Meter), whose measurements of coagulation traits are based on direct measurement of viscosity. In our research, the Optigraph uses an optical signal, which is transformed to values for E_{30} (mm) by using a calibration equation (Kübarsepp et al., 2005).

Genetic correlations between milk coagulation and milk yield and composition traits were mainly low. Curd firmness had the highest genetic correlation with milk protein percentage (0.48), suggesting that a high protein percentage results in a favorable E_{30}. Cassandro et al. (2008) reported a correlation of 0.44 between E_{30} and protein percentage, which is in agreement with our results. The genetic correlations of −0.24 and −0.07 reported by Ikonen et al. (1999c, 2004) on the same traits, however, are different. These inconsistencies indicate that many factors may influence the relationship between E_{30} and protein percentage, such as sample size, breed, model and devices, and variation in time between milk sampling and analysis.

Milk coagulation time had the strongest genetic correlation with pH (0.69). A high pH level is therefore associated with a less favorable RCT. This result is consistent with previous studies (Ikonen et al., 1999c, 2004; Cassandro et al., 2008), which also reported a moderate to high genetic correlation between RCT and pH (0.40 to 0.81). A moderate to high genetic correlation (−0.30 to −0.85) between E_{30} and pH observed in previous studies (Ikonen et al., 1999c, 2004; Cassandro et al., 2008), however, was not found in our study; we estimated a zero genetic correlation between E_{30} and pH.

Curd firmness showed a weak positive genetic correlation with milk fat percentage (0.25) and a weak negative genetic correlation with milk yield (−0.29). Therefore, selection for improved curd firmness may be associated with a somewhat higher fat percentage and slightly reduced milk production. Genetic correlations for E_{30} with milk yield and fat percentage were negligible in previous studies (Ikonen et al., 1999c, 2004; Cassandro et al., 2008).

Phenotypic correlations between milk coagulation properties and milk composition traits were similar or somewhat lower than the corresponding genetic correlations, except for slightly stronger phenotypic correlations between coagulation properties (−0.22) and for E_{30} with pH (−0.15). Previous studies (Ikonen et al., 1999c, 2004; Cassandro et al., 2008) reported weak phenotypic correlations between milk coagulation traits and milk yield and composition traits, except for a moderately strong correlation between milk coagulation traits and pH and a moderate correlation for E_{30} with protein percentage. We found a somewhat higher

positive phenotypic correlation for protein percentage with both milk coagulation properties and a lower phenotypic correlation between E_{30} and pH level.

The association of E_{30} with protein percentage can be explained by the key role of caseins in the milk coagulation process. Aggregation of casein micelles, the second phase of rennet coagulation process, starts only after about 87% of κ-casein is enzymatically degraded (Lucey, 2002). Van Hooydonk et al. (1986) reported that the optimum pH for hydrolysis of κ-casein is in the range from 5.1 to 5.3. This could explain the positive phenotypic correlation between RCT and pH levels, suggesting that a lower pH shortens RCT.

To our knowledge, no genetic and phenotypic correlations between milk coagulation traits and urea have been published. All genetic and phenotypic correlations of urea with other milk composition traits and milk yield were low and nonsignificant in our study. Our results were of the same magnitude as the values reported by Stoop et al. (2007), except for the high genetic correlation between urea and SCS (0.85) reported by Stoop et al. (2007).

The average SCS in our study was slightly lower than the values derived from the monthly reported average SCC (Estonian Animal Recording Centre, 2008) for cows in milk recording during 2008 (92% of all cows in Estonia). For this reason, probably, we did not find any nonzero genetic correlations between SCS and milk coagulation traits. Ikonen et al. (2004) and Cassandro et al. (2008), however, reported that lower SCS are associated with improved milk coagulation properties. Some authors report that SCC has an unfavorable effect on milk coagulation properties at the phenotypic level (Politis and Ng-Kwai-Hang, 1988; O'Brien et al., 2001); Somers et al. (2002), however, did not find a significant effect of SCC on milk coagulation properties. To investigate a possible nonlinear relationship for SCS with RCT and E_{30}, we performed additional analyses in which SCS was included as a linear and as a quadratic covariate in the model. The results showed a small unfavorable effect of higher SCS ($P < 0.05$) on RCT and E_{30}. Adjusting for SCS differences in the model did not affect the estimated heritabilities.

In general, genetic and phenotypic correlations among milk yield and composition traits were in accordance with previous studies (Ikonen et al., 1999c, 2004; Cassandro et al., 2008).

Genetic Parameters with a Random Regression Analysis

In this article we used a repeatability model, which showed a good fit to the data (standard deviation of residuals was 1.17 for log-transformed RCT and 4.40 for E_{30}) and residual independence of DIM (residual and squared residual correlations across DIM were <0.05 for both milk coagulation traits). Because residuals were independent of lactation stage, variation due to DIM was sufficiently described by a repeatability model. For milk coagulation traits, however, additional univariate random regression models with second-order Legendre polynomials for the additive genetic and permanent environmental effects (except for first-order Legendre polynomial for permanent environmental effect of RCT) were applied to compare heritability and repeatability estimates and residuals with results from repeatability model analysis. Residual standard deviations of the random regression model were somewhat smaller than residuals from the repeatability model (standard deviation of residuals from repeatability and random regression model were 1.17 and 1.06, respectively, for RCT and 4.40 and 3.40, respectively, for E_{30}). Based on monthly averages of residuals, differences between both models were small; however, the random regression model showed a somewhat better fit in the last 2 (mo 9 and 10) lactation months for curd firmness. Average heritability across lactation was 0.38 for RCT and 0.47 for E_{30}. Heritability of RCT increased toward the end of lactation and had a maximum of 0.44 in lactation mo 6 and 7. The heritability for E_{30} showed an increase during lactation and a maximum of 0.51 in mo 8 to 10 of lactation. Average repeatability estimates were 0.53 for RCT and 0.58 for E_{30}.

CONCLUSIONS

In our study of 17,577 test-day records from 4,191 first-lactation Estonian Holstein cows, we found RCT and E_{30} to be highly heritable. Genetic correlations of the milk coagulation traits with milk production and composition traits were weak; therefore, selection for milk coagulation properties is not expected to result in a strong deterioration of milk production and composition. Genetic and phenotypic correlations between milk coagulation time and curd firmness were different from those reported in previous studies, indicating that coagulation aspects measured by the new Optigraph device and other devices used may be different. Therefore, further research is needed to study the effect of the Optigraph milk coagulation measurements on cheese yield and quality.

ACKNOWLEDGMENTS

The research leading to these results was co-financed by the European Community's Regional Development Fund in the framework of the Competence Centre Programme of the Enterprise Estonia under project no.

EU22868, carried out by the Bio-Competence Centre of Healthy Dairy Products, and by the Targeted Finance Project 1080045s07. The people from EARC (Tartu, Estonia), Animal Breeders' Association of Estonia (Keava), Institute of Veterinary Medicine and Animal Sciences of the Estonian University of Life Sciences (Tartu), especially Ivi Jõudu, are warmly thanked for their contribution in the collection and laboratory analysis of the data and the database COAGEN. Johan van Arendonk (Department of Animal Sciences, Wageningen University and Research Centre, the Netherlands) is warmly thanked for the opportunity of the research period at the Animal Breeding and Genomics Centre of Wageningen University. The Archimedes Foundation DoRa scholarship that enabled this visit is gratefully acknowledged.

REFERENCES

Aleandri, R., J. S. Schneider, and L. G. Buttazzoni. 1989. Evaluation of milk for cheese production based on milk characteristics and Formagraph measures. J. Dairy Sci. 72:1967–1989.

Auldist, M. J., K. A. Johnston, N. J. White, W. P. Fitzsimons, and M. J. Boland. 2004. A comparison of the composition, coagulation characteristics and cheese making capacity of milk from Friesian and Jersey dairy cows. J. Dairy Res. 71:51–57.

Auldist, M., C. Mullins, B. O'Brien, B. T. O'Kennedy, and T. Guinee. 2002. Effect of cow breed on milk coagulation properties. Milchwissenschaft 57:140–143.

Bhandari, V., and H. Singh. 2002. Physical Methods. Pages 93–101 in Encyclopedia of Dairy Sciences. Vol. 1. H. Roginski, J. W. Fuquay, and P. F. Fox, ed. Academic Press, San Diego, CA.

Cassandro, M., A. Comin, M. Ojala, R. Dal Zotto, M. De Marchi, L. Gallo, P. Carnier, and G. Bittante. 2008. Genetic parameters of milk coagulation properties and their relationships with milk yield and quality traits in Italian Holstein cows. J. Dairy Sci. 91:371–376.

De Marchi, M., G. Bittante, R. Dal Zotto, C. Dalvit, and M. Cassandro. 2008. Effect of Holstein-Friesian and Brown Swiss breeds on quality of milk and cheese. J. Dairy Sci. 91:4092–4102.

De Marchi, M., C. C. Fagan, C. P. O'Donnell, A. Cecchinato, R. Dal Zotto, M. Cassandro, M. Penasa, and G. Bittante. 2009. Prediction of coagulation properties, titratable acidity, and pH of bovine milk using mid-infrared spectroscopy. J. Dairy Sci. 92:423–432.

Estonian Animal Recording Centre. 2008. Results of animal recording in Estonia 2008. http://www.jkkeskus.ee/english/sisu/aastaraamat_2008.pdf Accessed June 28, 2009.

Eurostat. 2009. Production of cheese. http://epp.eurostat.ec.europa.eu/tgm/table.do?tab=table&init=1&plugin=1&language=en&pcode=tag00040 Accessed May 25, 2009.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, S. J. Welham, and R. Thompson. 2002. ASReml User Guide Release 2.0. VSN International, Hemel Hempstead, UK.

Ikonen, T., K. Ahlfors, R. Kempe, M. Ojala, and O. Ruottinen. 1999c. Genetic parameters for the milk coagulation properties and prevalence of non-coagulating milk in Finnish dairy cows. J. Dairy Sci. 82:205–214.

Ikonen, T., A. Morri, A.-M. Tyrisevä, O. Ruottinen, and M. Ojala. 2004. Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content and pH of milk. J. Dairy Sci. 87:458–467.

Ikonen, T., M. Ojala, and O. Ruottinen. 1999b. Associations between milk protein polymorphism and first lactation milk production traits in Finnish Ayrshire cows. J. Dairy Sci. 82:1026–1033.

Ikonen, T., M. Ojala, and E.-L. Syväoja. 1997. Effects of composite casein and beta-lactoglobulin genotypes on renneting properties and composition of bovine milk by assuming an animal model. Agric. Food Sci. Finl. 6:283–294.

Ikonen, T., O. Ruottinen, E.-L. Syväoja, K. Saarinen, E. Pahkala, and M. Ojala. 1999a. Effect of milk coagulation properties of herd bulk milks on yield and composition of Emmental cheese. Agric. Food Sci. Finl. 8:411–422.

International Committee for Animal Recording. 2009. International agreement of recording practices. Guidelines approved by the General Assembly held June 18, 2008. http://www.icar.org/Documents/Rules%20and%20regulations/Guidelines/Guidelines_2009.pdf Accessed May 13, 2010.

Kübarsepp, I., M. Henno, O. Kärt, and T. Tupasela. 2005. A comparison of the methods for determination of the rennet coagulation properties of milk. Acta Agric. Scand. Anim. Sci. 55:145–148.

Leitner, G., O. Krifucks, U. Merin, Y. Lavi, and N. Silanikove. 2006. Interactions between bacteria type, proteolysis of casein and physico-chemical properties of bovine milk. Int. Dairy J. 16:648–654.

Lucey, J. A. 2002. Rennet coagulation of milk. Pages 286–293 in Encyclopedia of Dairy Sciences. Vol. 1. H. Roginski, J. W. Fuquay, and P. F. Fox, ed. Academic Press, San Diego, CA.

Martin, B., J. F. Chamba, J. B. Coulon, and E. Perreard. 1997. Effect of milk chemical composition and clotting characteristics on chemical and sensory properties of Reblochon de Savoie cheese. J. Dairy Res. 64:157–162.

Mayer, H. K., M. Ortner, E. Tschager, and W. Ginzinger. 1997. Composite milk protein phenotypes in relation to composition and cheese making properties of milk. Int. Dairy J. 7:305–310.

Najera, A. I., M. de Renobales, and L. J. R. Barron. 2003. Effects of pH, temperature, $CaCl_2$ and enzyme concentrations on the rennet-clotting properties of milk: A multifactorial study. Food Chem. 80:345–352.

O'Brien, B., W. J. Meaney, D. McDonagh, and A. Kelly. 2001. The effects of refrigerated storage on the quality of raw milk. Aust. J. Dairy Technol. 56:213–218.

O'Brien, B., R. Mehra, J. F. Connolly, and D. Harrington. 1999. Seasonal variation in the composition of Irish manufacturing and retail milks 1. Chemical composition and renneting properties. Ir. J. Agric. Food Res. 38:53–64.

Okigbo, L. M., G. H. Richardson, R. J. Brown, and C. A. Ernstrom. 1985a. Casein composition of cow's milk of different chymosin coagulation properties. J. Dairy Sci. 68:1887–1892.

Okigbo, L. M., G. H. Richardson, R. J. Brown, and C. A. Ernstrom. 1985b. Variation in coagulation properties of milk from individual cows. J. Dairy Sci. 68:822–828.

Ostersen, S., J. Foldager, and J. E. Hermansen. 1997. Effects of stage of lactation, milk protein genotype and body condition at calving on protein composition and renneting properties of bovine milk. J. Dairy Res. 64:207–219.

Politis, I., and K. F. Ng-Kwai-Hang. 1988. Effects of somatic cell counts and milk composition on the coagulating properties of milk. J. Dairy Sci. 71:1740–1745.

Somers, J. M., T. P. Guinee, and A. L. Kelly. 2002. The effect of plasmin activity and cold storage of cheese milk on the composition, ripening and functionality of mozzarella-type cheese. Aust. J. Dairy Technol. 55:5–11.

Statistics Estonia. 2009. Agriculture statistical database. http://pub.stat.ee/px-web.2001/I_Databas/Economy/01AGRICULTURE/01AGRICULTURE.asp Accessed May 25, 2009.

Stoop, W. M., H. Bovenhuis, and J. A. M. van Arendonk. 2007. Genetic parameters for milk urea nitrogen in relation to milk production traits. J. Dairy Sci. 90:1981–1986.

Storry, J. E., A. S. Grandison, D. Millard, A. J. Owen, and G. D. Ford. 1983. Chemical composition and coagulating properties of renneted milks from different breeds and species of ruminant. J. Dairy Res. 50:215–229.

Summer, A., M. Malacarne, F. Martuzzi, and P. Mariani. 2002. Structural and functional characteristics of Modenese cow milk

in Parmigiano-Reggiano cheese production. Ann. Fac. Med. Vet. Univ. Parma 22:163–174.
Tyrisevä, A.-M., T. Ikonen, and M. Ojala. 2003. Repeatability estimates for milk coagulation traits and non-coagulation of milk in Finnish Ayrshire cows. J. Dairy Res. 70:91–98.
Tyrisevä, A.-M., T. Vahlsten, O. Ruottinen, and M. Ojala. 2004. Non-coagulation of milk in Finnish Ayrshire and Holstein-Friesian cows and effect of herds on milk coagulation ability. J. Dairy Sci. 87:3958–3966.
Van den Berg, G., J. T. M. Escher, P. J. de Koning, and H. Bovenhuis. 1992. Genetic polymorphism of κ-casein and β-lactogobulin in relation to milk composition and processing properties. Neth. Milk Dairy J. 46:145–168.
Van Hooydonk, A. C. M., H. G. Hagedoorn, and I. J. Boerrigter. 1986. pH-induced physico-chemical changes of casein micelles in milk and their effect on renneting. 1. Effect of acidification on physico-chemical properties. Neth. Milk Dairy J. 40:281–296.
Wedholm, A., L. B. Larsen, H. Lindmark-Månsson, A. H. Karlsson, and A. Andrén. 2006. Effect of protein composition on the cheese-making properties of milk from individual dairy cows. J. Dairy Sci. 89:3296–3305.
Ysebaert Dairy Division. 2009. Optigraph. http://www.ysebaert.com/ysebaert/optigraphuk.htm Accessed Dec. 10, 2009.

II

Pretto, D., Kaart, T., **Vallas, M.**, Jõudu, I., Henno, M., Ancilotto, L., Cassandro, M., Pärna, E. 2011.
RELATIONSHIP BETWEEN MILK COAGULATION PROPERTY TRAITS ANALYZED WITH DIFFERENT METHODOLOGIES.
Journal of Dairy Science, 94(9), 4336–4346.

J. Dairy Sci. 94:4336–4346
doi:10.3168/jds.2011-4267

Relationships between milk coagulation property traits analyzed with different methodologies

D. Pretto,*[1] T. Kaart,†‡ M. Vallas,†‡ I. Jõudu,†‡ M. Henno,† L. Ancilotto,* M. Cassandro,* and E. Pärna†‡
*Department of Animal Science, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy
†Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, 51014 Tartu, Estonia
‡Bio-Competence Centre of Healthy Dairy Products, Kreutzwaldi 1, 51014 Tartu, Estonia

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 noncoagulation (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 2 freestall barns in Italy, and sent to 3 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 international milk clotting units (IMCU)/mL of coagulant activity; B, with a Lattodinamografo (Foss-Italia, Padova, Italy) using 0.051 IMCU/mL of coagulant activity; and C, with an Optigraph (Ysebaert, Frépillon, France) 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, 2 regression models were applied: model 1 was a single regression model, where the dependent and independent variables were the same MCP trait determined by 2 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). Milk coagulation properties have been widely studied in recent years and have been proposed as technological traits 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. Proposals to include these traits in payment systems of milk used for cheese production also exist (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 min 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). Different instruments are available commercially and are currently widely used in research

Received February 14, 2011.
Accepted April 29, 2011.
[1]Corresponding author: denis.pretto@unipd.it

institutes to record MCP traits for genetic studies. The computerized renneting meter (**CRM**; Polo Trade, Monselice, Italy) was used, for example, in the work of Ikonen et al. (2004) and Cassandro et al. (2008); the Optigraph (**OPT**; Ysebaert, Frépillon, France) was used in the work of Vallas et al. (2010) and the Lattodinamografo (**LAT**; Foss-Italia, Padova, Italy), 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 CN. The OPT calculates the coagulation parameters (coagulation time, curd firmness, and 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 of 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 milliliter of milk. Because 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 that 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 to 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 to 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 to 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,b; 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 to 0.045 IMCU/mL is usually used, whereas in the manufacturing processes of some of the main North European cheeses, such as Edam and Gouda, it is on 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, as no standard methods for MCP analyses exist, 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 method 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 MCP measured with these methodologies (Laporte et al., 1998; Kübarsepp et al., 2005a; Klandar et al., 2007). Nevertheless, a critical feature of MCP data are the presence of noncoagulated (**NC**) milk records; that is, when milk does

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

Trait	Mean	CV (%)	Minimum	Maximum
Parity	2.5	57.7	1	7
DIM	181	85.8	5	703
Milk yield (kg/d)	32.2	27.8	7.9	56.4
Fat in milk (%)	3.93	20.0	2.04	6.91
Protein in milk (%)	3.50	14.4	2.46	5.34
SCC (10^3 cells/mL)	200.9	179.6	8	2,945
SCS[1] (U)	2.80	63.5	−0.64	7.88
pH	6.68	1.0	6.45	6.88

[1]SCS = [3 + $\log_2$ (SCC/10^5)].

not coagulate at all within a standard 30-min testing time (Tyrisevä et al., 2004). 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 noncoagulation 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 2 freestall 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; laboratory 1). For MCP analysis, 1 random subsample pack was sent to the milk quality laboratory of Veneto Agricoltura Institute (Thiene, Italy; laboratory 2); 1 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; laboratory 3); and the remaining 2 random subsample packs were kept in laboratory 1 for MCP analysis and the determination of milk fat and protein content (MilkoScan FT 6000; Foss Electric, Hillerød, Denmark), SCC (Fossomatic 5000; Foss Electric), and pH (pH-Burette 24; Crison Instruments, Barcelona, Spain). The temperature of the samples was maintained at 4°C throughout transport and storage. The subsample for laboratory 3 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 laboratory 3 were time consuming, MCP analysis was performed in 2 parts: 2 d (50% of samples) and 3 d (50% of samples) after collection. The analyses of the 4 subsamples of a same cow were performed in the same day in all 3 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; AIA, 2009).

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 identified as A, B, and C. In method A, a CRM and standard rennet were used (Hansen standard 160 IMCU/mL, with 80% chymosin and 20% pepsin; Pacovis Amrein AG, Bern, Switzerland), which were 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., Leeuwarden, the 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 refrigerator 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, for which the coagulant solution was prepared 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 of 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 NC.

Only samples that coagulated (**CO**) with all methodologies were used 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 the McNemar 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 2 different methodologies, whereas 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 noncoagulation 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) 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; http://www.r-project.org).

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 using A did not coagulate at the same time as B, whereas the only NC sample using C coagulated both with A and B. This could be related to the different principles of analysis for OPT than for 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 those of Vallas et al. (2010), where, using the same equipment (OPT), 0.3% of samples did not coagulate within 30 min after the addition of coagulant.

The means of RCT measured on the CO samples differed significantly between the 3 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 with 15.6 and 18.2 min). This large difference is probably due to the higher concentration of coagulant in C compared with both A and B. The variation coefficients for RCT were 23, 24, and 25% for A, B, and C, respectively, and the standard deviation was significantly lower for the analyses of C compared with both A and B. The means of a_{30} were similar for both B and C (29.6 and 29.7 mm, respectively), whereas 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 using B compared with 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.

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

	Method			
Item	A	B	C	C*[1]
Methodology				
Instrument	Computerized renneting meter[2]	Lattodinamografo[3]	Optigraph[4]	Optigraph
Coagulant	Hansen standard 160[5]	Hansen standard 160	Milase MRS 600[6]	Hansen standard 160
Coagulant activity (IMCU/mL of milk)	0.051	0.051	0.120	0.051
No.[7]				
CO	158	146	164	30
NC	7 (4.2%)[b]	19 (11.5%)[a]	1 (0.6%)[b]	30 (50.0%)
RCT[8] (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}[8] (mm)				
Mean	36.2[a]	29.6[b]	29.7[b]	5.1
SD	9.9[b]	12.9[a]	8.5[b]	

[a–c]Proportions of noncoagulated samples, means, and SD within a row for A, B, and C methodologies with different superscripts differ ($P < 0.05$).

[1]60 random samples were analyzed with this method.

[2]By Polo Trade (Monselice, Italy).

[3]By Foss-Italia (Padova, Italy).

[4]By Ysebaert (Frépillon, France).

[5]Produced by Pacovis Amrein AG (Bern, Switzerland).

[6]Produced by CSK Food Enrichment B.V. (Leeuwarden, the Netherlands).

[7]CO = coagulated samples; NC = noncoagulated samples.

[8]Only coagulated samples with A, B, and C methodologies (n = 145).

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).

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} compared with 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), whereas 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 coefficient of determination value by more than 0.01 to 0.03. The transformation of a_{30} values between pairs of methodologies was most reliable between A and B

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)[1]

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

[1]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).

($R^2 = 0.63$; 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 shown in figure: $a_{30\ A} = 44.74 + 0.473\ a_{30\ C} - 2.839\ RCT_C$; $a_{30\ B} = 37.09 + 0.797\ a_{30\ C} - 3.921\ RCT_C$).

Prediction of Noncoagulation Probability

The logistic regression and ROC analysis results for RCT in 3 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 with 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 to 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.

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 data set. 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 in NC samples could be that coagulant activity or type of coagulant (calf rennet vs. microbial coagulant) used in A and B are unsuitable for the Optigraph instrument.

The random sample contained 6 NC samples from B and 5 NC samples from the A analyses, and of all of 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 noncoagulated samples seems to be quite well predictable irrespective of the instrument used, especially based on the RCT.

Possible Causes of Difference for MCP Traits

Three 3 main reasons certainly exist that could cause a difference in the values of the MCP traits: the first is the different equipment used in each laboratory; the second is the difference in coagulant activity in the milk to induce coagulation, and the third is the type of coagulant used.

In this study, it was difficult to distinguish the effect of instrument and coagulant effects on the MCP for all of the methodologies. The instrument effect is clear in the A (CRM) and B (LAT) comparison because, in these analyses, the same coagulant activity and type of coagulant was used. The CRM had different sensitivity compared with the LAT, because, 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 2 other types of equipment investigated, could be the cause for the weak correlation of C with the other 2 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 with that of a microbial coagulant to ensure a specified curd-cutting time. Even in the case of a coagulant effect, a strong relationship should exist 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, as 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

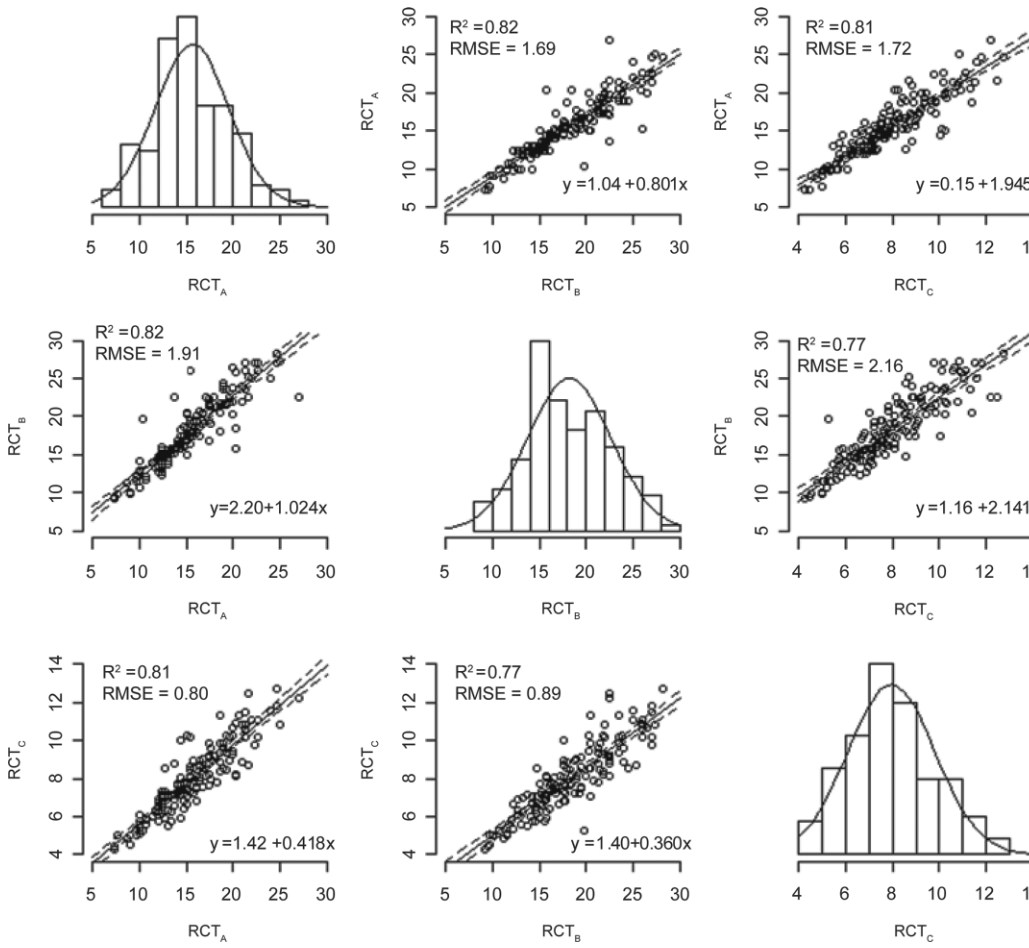

Figure 1. Distribution, regression parameters and regression line (solid line) shown with 95% confidence interval (dashed lines) of milk rennet coagulation time (RCT, min) measured with 3 different methodologies (A, B, and C subscripts) for the coagulated samples with all methodologies (n = 145; $P < 0.05$ 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.

that whereas the characteristic of optical change accompanies 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) in whose study 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 with mechanical systems, may be different.

It is supposed from the results of samples analyzed with uniform coagulant activity (methodology C*)

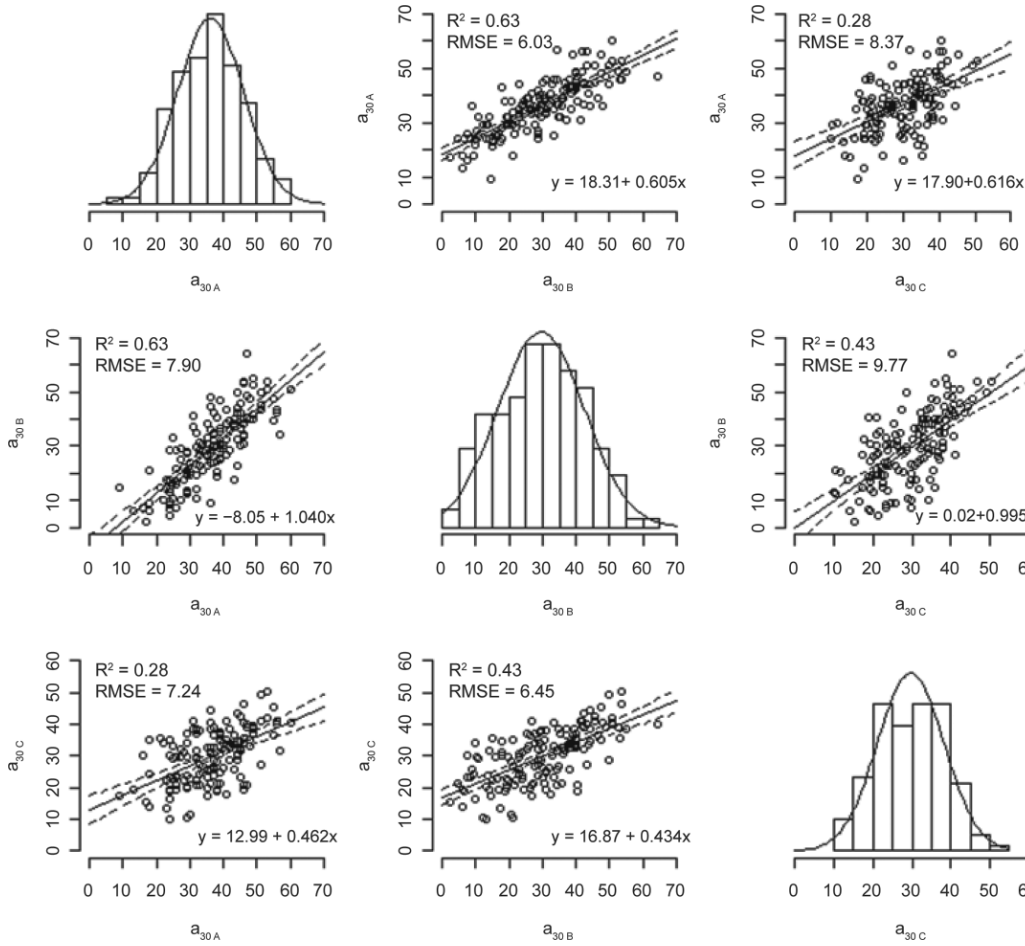

Figure 2. Distribution, regression parameters and regression line (solid line) shown with 95% confidence interval (dashed lines) of curd firmness (a_{30}, min) measured with 3 different methodologies (A, B, and C subscripts) for the coagulated samples with all methodologies (n = 145; $P < 0.05$ for all 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.

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, because 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

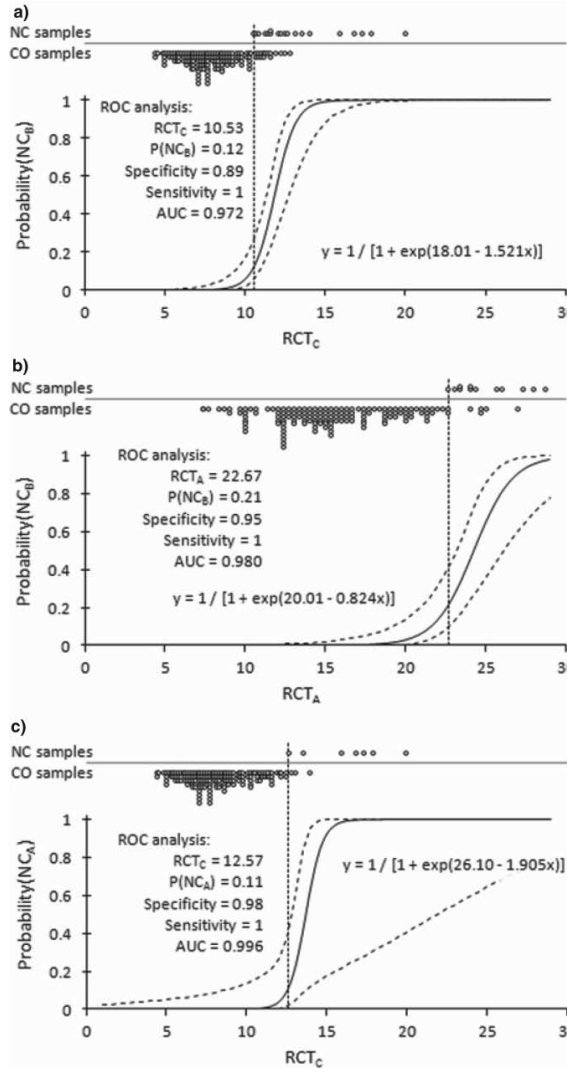

Figure 3. Results of logistic regression analyses predicting (a) the probability of noncoagulated (NC) samples in method B when the analyses for milk rennet coagulation time (RCT, min) are performed by method C, (b) the probability of NC samples in method B when the analyses for RCT are performed by 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).

initial OPT analyses in which higher coagulant activity was used.

CONCLUSIONS

The method proposed provides the opportunity to convert MCP data obtained by different methodologies into comparable data sets across different methodologies. The results of this study have shown that MCP traits analyzed with different methodologies have significantly different values due to the diversity of the instruments and the coagulant activity. The type of coagulant could have a further effect, as 2 different coagulants were used: calf rennet that contains 2 milk-clotting enzymes and one of microbial origin, which contained a single milk-clotting enzyme; but more investigations are needed 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, whereas the C measurements could be transformed into the other scales with moderate accuracy only when both curd firmness and rennet coagulation time were included in the model. The method proposed for harmonization of noncoagulation probabilities 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.

ACKNOWLEDGMENTS

The authors acknowledge financial support by Distretto Lattiero Caseario del Veneto (Venice, Italy): TRACCIABIANCA project (DGR 2549, 04/08/2009, Mis. 2A - Regione Veneto) and PRIN project 2007-prot. 2007898KYN_004, of the Italian Ministry of University and Research (MIUR). The research leading to these results was co-financed also by the European Community's Regional Development Fund in the framework of the Competence Centre Programme of Enterprise Estonia under project no. EU30002 carried out by the Bio-Competence Centre of Healthy Dairy Products (Tartu, Estonia), and by the Targeted Finance Projects 1080045s07 and 0170165s08.

REFERENCES

AIA (Associazione Italiana Allevatori). 2009. Milk recording activity: Official Statistics. Associazione Italiana Allevatori (AIA), Rome, Italy.

Aleandri, R., J. C. Schneider, and L. G. Buttazzoni. 1989. Evaluation of milk for cheese production based on milk characteristics and Formagraph measures. J. Dairy Sci. 72:1967–1975.

Brown, R. J., and S. K. Collinge. 1986. Actual milk coagulation time and inverse of chymosin activity. J. Dairy Sci. 69:956–958.

Cassandro, M., A. Comin, M. Ojala, R. Dal Zotto, M. De Marchi, L. Gallo, P. Carnier, and G. Bittante. 2008. Genetic parameters of milk coagulation properties and their relationships with milk yield and quality traits in Italian Holstein cows. J. Dairy Sci. 91:371–376.

Cecchinato, A., M. De Marchi, L. Gallo, G. Bittante, and P. Carnier. 2009. Mid-infrared spectroscopy predictions as indicator traits in breeding programs for enhanced coagulation properties of milk. J. Dairy Sci. 92:5304–5313.

Dal Zotto, R., M. De Marchi, A. Cecchinato, M. Penasa, M. Cassandro, P. Carnier, L. Gallo, and G. Bittante. 2008. Reproducibility and repeatability of measures of milk coagulation properties and predictive ability of mid-infrared reflectance spectroscopy. J. Dairy Sci. 91:4103–4112.

De Marchi, M., G. Bittante, R. Dal Zotto, C. Dalvit, and M. Cassandro. 2008. Effect of Holstein Friesian and Brown Swiss breeds on quality of milk and cheese. J. Dairy Sci. 91:4092–4102.

De Marchi, M., C. C. Fagan, C. P. O'Donnell, A. Cecchinato, R. Dal Zotto, M. Cassandro, M. Penasa, and G. Bittante. 2009. Prediction of coagulation properties, titratable acidity, and pH of bovine milk using mid-infrared spectroscopy. J. Dairy Sci. 92:423–432.

Hallén, E., T. Allmere, J. Näslund, A. Andrén, and A. Lundén. 2007. Effect of genetic polymorphism of milk proteins on rheology of chymosin-induced milk gels. Int. Dairy J. 17:791–799.

Hallén, E., A. Lundén, A.-M. Tyrisevä, M. Westerlind, and A. Andrén. 2010. Composition of poorly and non-coagulating bovine milk and effect of calcium addition. J. Dairy Res. 77:398–403.

ICAR (International Committee for Animal Recording). 2009. International agreement of recording practices. Guidelines approved by the General Assembly held in Niagara Falls, USA 18 June 2008.

Ikonen, T., K. Ahlfors, R. Kempe, M. Ojala, and O. Ruottinen. 1999. Genetic parameters for the milk coagulation properties and prevalence of noncoagulating milk in Finnish dairy cows. J. Dairy Sci. 82:205–214.

Ikonen, T., S. Morri, A.-M. Tyrisevä, O. Ruottinen, and M. Ojala. 2004. Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content, and pH of milk. J. Dairy Sci. 87:458–467.

ISO/IDF (International Standards Organization/International Dairy Federation). 2007. Milk—Determination of total milk-clotting activity by bovine rennets. ISO 11815/IDF 157. International Dairy Federation, Brussels, Belgium.

Jacob, M., D. Jaros, and H. Rohm. 2010. The effect of coagulant type on yield and sensory properties of semihard cheese from laboratory, pilot and commercial scale productions. Int. J. Dairy Technol. 63:370–380.

Johnson, M. E., C. M. Chen, and J. J. Jaeggi. 2001. Effect of rennet coagulation time on composition, yield, and quality of reduced-fat Cheddar cheese. J. Dairy Sci. 84:1027–1033.

Jõudu, I. 2008a. Effect of milk protein composition and genetic polymorphism on milk rennet coagulation properties. PhD Dissertation. Estonian University of Life Sciences, Tartu, Estonia. Accessed Jan. 15, 2011. http://dspace.emu.ee/jspui/bitstream/10492/113/1/Thesis_Joudu_2008.pdf.

Jõudu, I., M. Henno, T. Kaart, T. Püssa, and O. Kärt. 2008b. The effect of milk proteins contents on the rennet coagulation properties of milk from individual dairy cows. Int. Dairy J. 18:964–967.

Karlsson, A. O., R. Ipsen, and Y. Ardö. 2007. Rheological properties and microstructure during rennet induced coagulation of UF concentrated skim milk. Int. Dairy J. 17:674–682.

Klandar, A. H., A. Lagaude, and D. Chevalier-Lucia. 2007. Assessment of the rennet coagulation of skim milk: A comparison of methods. Int. Dairy J. 17:1151–1160.

Kübarsepp, I., M. Henno, O. Kärt, and T. Tupasela. 2005a. A comparison of the methods for determination of the rennet coagulation properties of milk. Acta Agric. Scand. A Anim. Sci. 55:145–148.

Kübarsepp, I., M. Henno, H. Viinalass, and D. Sabre. 2005b. Effect of κ-casein and β-lactoglobulin genotypes on the milk rennet coagulation properties. Agron. Res. 3:55–64.

Laporte, M. F., R. Martel, and P. Paquin. 1998. The near-infrared optic probe for monitoring rennet coagulation in cow's milk. Int. Dairy J. 8:659–666.

Lucey, J. A. 2002. Formation and physical properties of milk protein gels. J. Dairy Sci. 85:281–294.

Martin, B., J.-F. Chamba, J.-B. Coulon, and E. Perreard. 1997. Effect of milk chemical composition and clotting characteristics on chemical and sensory properties of Reblochon de Savoie cheese. J. Dairy Res. 64:157–162.

Ng-Kwai-Hang, K. F., I. Politis, R. I. Cue, and A. S. Marziali. 1989. Correlations between coagulation properties of milk and cheese yielding capacity and cheese composition. Can. Inst. Food Sci. Technol. J. 22:291–294.

O'Callaghan, D. J., C. P. O'Donnell, and F. A. Payne. 2002. Review of systems for monitoring curd setting during cheesemaking. Int. J. Dairy Technol. 55:65–74.

Pretto, D., and M. Cassandro. 2010. Estimation of economic values for milk production, SCS and milk coagulation properties in Italian Holstein Friesian dairy cattle. Page 325 in Proc. 9th World Congr. Genet. Appl. Livest. Prod., Leipzig, Germany. German Society for Animal Science, Giessen, Germany.

Tyrisevä, A.-M., T. Vahlsten, O. Ruottinen, and M. Ojala. 2004. Non-coagulation of milk in Finnish Ayrshire and Holstein-Friesian cows and effect of herds on milk coagulation ability. J. Dairy Sci. 87:3958–3966.

Vallas, M., H. Bovenhuis, T. Kaart, K. Pärna, H. Kiiman, and E. Pärna. 2010. Genetic parameters for milk coagulation properties in Estonian Holstein cows. J. Dairy Sci. 93:3789–3796.

Wedholm, A., L. B. Larsen, H. Lindmark-Månsson, A. H. Karlsson, and A. Andrén. 2006. Effect of protein composition on the cheesemaking properties of milk from individual dairy cows. J. Dairy Sci. 89:3296–3305.

Zannoni, M., and S. Annibaldi. 1981. Standardization of the renneting ability of milk by Formagraph. Sci. Tecn. Latt. Cas. 32:79–94.

III

Vallas, M., Kaart, T., Värv, S., Pärna, K., Jõudu, I., Viinalass, H., Pärna, E. 2012.
COMPOSITE β-κ-CASEIN GENOTYPES AND THEIR EFFECT ON COMPOSITION AND COAGULATION OF MILK FROM ESTONIAN HOLSTEIN COWS.
Journal of Dairy Science, 95(11), 6760–6769.

J. Dairy Sci. 95:6760–6769
http://dx.doi.org/10.3168/jds.2012-5495

Composite β-κ-casein genotypes and their effect on composition and coagulation of milk from Estonian Holstein cows

M. Vallas,*†[1] T. Kaart,*† S. Värv,*† K. Pärna,‡ I. Jõudu,*† H. Viinalass,*† and E. Pärna*†
*Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, 51014 Tartu, Estonia
†Bio-Competence Centre of Healthy Dairy Products, Kreutzwaldi 1, 51014 Tartu, Estonia
‡Institute of Mathematical Statistics, University of Tartu, J. Liivi 2, 50409 Tartu, Estonia

ABSTRACT

The objective of this study was to estimate the effect of composite β-κ-CN genotypes on milk coagulation and composition traits, and on the additive genetic variation of these traits in Estonian Holstein dairy cattle. A total of 23,970 milk samples, repeated measurements from the first to third lactation from 2,859 Estonian Holstein cows from 78 herds across the country, were analyzed for milk yield, milk fat and protein percentages, somatic cell count, and milk coagulation properties (milk coagulation time and curd firmness). Each cow had at least 3 measurements per lactation. Two single-trait random regression animal models were fitted for the traits studied. The first model considered fixed effects of year-season of sampling and year-season of calving, calving age (nested within lactation), sample age (only for milk coagulation traits) and days in milk, and random herd, additive genetic, and permanent environmental effects. The animal and permanent environmental effects were modeled over the lactation period by using Legendre polynomials. The second model had the additional fixed β-κ-casein effect in the form of a third-order Legendre polynomial. The 2 most frequent β-κ-casein composite genotypes were A2A2AA and A1A2AA, both with prevalence greater than 20%. Percentages of the remaining 31 genotypes were less than 8%, including 20 genotypes with percentages less than 1%. The β-κ-casein genotype-specific lactation curves were significantly different for milk coagulation traits and milk protein percentage. The B variant of κ-casein showed a favorable effect on both milk coagulation traits, whereas the IB haplotype had an increasing effect on curd firmness and protein percentage. Inclusion of the β-κ-casein genotype effects in the model resulted in decreases in the mean additive genetic variations for milk coagulation time and curd firmness of 12.9 and 51.1%, respectively.

Received March 1, 2012.
Accepted July 22, 2012.
[1]Corresponding author: mirjam.vallas@emu.ee

Key words: casein polymorphism, milk coagulation, Estonian Holstein dairy cattle

INTRODUCTION

Milk protein polymorphisms are often investigated in connection with milk performance traits. Some of these traits are hard to measure; therefore, milk protein polymorphisms promote a better understanding of the genetic background of milk production and offer an alternative indirect selection approach for improving milk quality. Many researchers have investigated associations between milk protein polymorphisms and milk production traits (Ng-Kwai-Hang et al., 1984; Bovenhuis et al., 1992; Boettcher et al., 2004) and protein composition (McLean et al., 1984; Heck et al., 2009; Bonfatti et al., 2010b). One of the most striking effects of milk protein polymorphisms on traits of economic interest is their relationship with the cheese-making properties of milk (see review by Caroli et al., 2009), including milk coagulation properties.

Some studies have found an association of genetic polymorphism of κ-CN with milk coagulation traits (Macheboeuf et al., 1993; Ikonen et al., 1997; Mayer et al., 1997) and protein percentage (Aleandri et al., 1990; Bovenhuis et al., 1992; Ikonen et al., 1999b), whereas the β-CN polymorphism was found to be related to fat percentage and fat and protein yields (Bovenhuis et al., 1992; Ikonen et al., 1999b). Many of the studies, however, had small sample sizes, with small coverage of the study population and limited variation in CN genotypes. Additionally, possibly because of the different breeds and different mathematical models used in the different studies, some results are inconsistent. Furthermore, because of the genetic linkage between the β-CN and κ-CN loci, composite β-κ-CN genotypes or haplotypes have been proposed for the estimation of CN genotype effects (Lundén et al., 1997; Ojala et al., 1997; Comin et al., 2008) on milk performance traits.

Recently, large-scale studies have been conducted (Comin et al., 2008; Bonfatti et al., 2010a; Penasa et al., 2010), enabling a more accurate adjustment for

CN genotype effects in the estimation of the additive genetic variance component of milk composition and coagulation traits. However, few studies have reported the presence of the β-CN I allele (Bonfatti et al., 2010a,b; Visker et al., 2010), and no clear influence of this allele on milk coagulation traits has been reported. Furthermore, the authors are aware of only the small-sample-size study by Ikonen et al. (1997) using β-κ-CN genotypes and repeated measurements, allowing the separation of variance components of additive genetic and permanent environmental effects in the model for analysis of variation in milk coagulation.

The aim of the present large-scale study, with repeated measurements, was to estimate the effect of composite β-κ-CN genotypes on the coagulation properties and composition of milk and on the additive genetic variation of these traits in the Estonian Holstein dairy population.

MATERIALS AND METHODS

Data

Milk samples from first- to third-lactation Estonian Holstein cows were collected during routine milk recording, as part of a development project for the Bio-Competence Centre of Healthy Dairy Products in Estonia, from April 2005 to May 2010. The herds were milked 2 or 3 times a day. The individual milk samples from each cow were collected either as a mixture of all test-day milkings from the cow or as only the morning, afternoon, or evening milking of the cow on the test day. Milk samples were immediately preserved with Bronopol (Knoll Pharmaceuticals, Nottingham, UK) and stored at 4°C during the transportation and analysis periods. The individual milk samples of the cow were collected and analyzed for milk composition and coagulation traits, with intervals of 2 to 3 mo during lactation (7 to 305 DIM).

Milk samples were excluded if the pH was lower than 6.5, indicative of colostrum (Bhandari and Singh, 2002), the sample age was more than 12 d and, according to the recommendations of the International Committee for Animal Recording (ICAR, 2009), milk yield was less than 3 kg, fat content was outside the range of 1.5 to 9%, and protein content was outside the range of 1 to 7%. Noncoagulated milk samples (n = 138) were also removed. Further, individual cow lactations with fewer than 3 samples and herds with fewer than 5 cows were excluded.

The number of test-day records in the final data set was 23,970. Milk samples were collected from 2,859 Estonian Holstein cows (daughters of 229 sires) from 78 herds across the country. Each cow had 3 to 7 measurements per lactation. The number of daughters per sire varied from 1 to 177. Additionally, a pedigree of 4 generations of animals was used, which included 20,791 animals.

Information about the cows, herds, and pedigree was obtained from the Estonian Animal Recording Centre (Tartu) and the Animal Breeders' Association of Estonia (Keava), and the database COAGEN, of the Bio-Competence Centre of Healthy Dairy Products (Tartu, Estonia), was formed from these data.

Milk Sample Analysis

The test-day milk yield was recorded, and individual milk samples were analyzed for fat and protein percentages by using MilkoScan 4000 and MilkoScan FT6000 instruments (FOSS, Hillerød, Denmark), and for SCC by using Fossomatic 4000 and Fossomatic 5000 cell counters (FOSS), at the Milk Analysis Laboratory of the Estonian Animal Recording Centre, using methods suggested by the International Committee for Animal Recording (ICAR, 2009). Milk sample analysis included all individual milk samples, including milk samples collected as a mixture of all test-day milkings of the cow. Somatic cell count values were log-transformed to SCS: $SCS = \log_2(SCC/100{,}000) + 3$.

The pH and milk coagulation properties were determined at the Milk Quality Laboratory of the Estonian University of Life Sciences, as described by Vallas et al. (2010). Briefly, a pH meter (Seven Multi, Mettler Toledo GmbH, Greifensee, Switzerland) was used for determination of the pH level of the milk before measuring milk coagulation time (**RCT**, min) and curd firmness ($\mathbf{a_{30}}$, V), which were determined using an Optigraph (Ysebaert, Frepillon, France).

Genotyping of Milk Protein Variants

Genotyping was carried out in the Animal Genetics Laboratory at the Estonian University of Life Sciences and the database COACAS, of the Bio-Competence Centre of Healthy Dairy Products, was formed. Blood samples were collected in tubes containing K_3EDTA. Deoxyribonucleic acid was extracted from whole blood using a commercial Genomic DNA Purification Kit (MBI Fermentas, Vilnius, Lithuania) and a Puregene DNA Purification System for Laboratory Use Blood Kit (Gentra Systems, Minneapolis, MN). The CN gene polymorphisms (SNP) were analyzed on the basis of nucleotide exchanges in codons 67, 93, 106, and 122 of exon 7 in the β-CN gene (determining the variants A1, A2, A3, B, and I) and in codons 148 and 155 of

exon 4 in the κ-CN gene (A, B, and E variants). The primer design was based on GenBank sequence X14711 for primer-specific PCR (allele-specific oligonucleotide-PCR) to detect polymorphisms in the β-CN gene. Using the present set of primers enabled discrimination of the I allele, previously genotyped as A2. Polymorphisms in the κ-CN gene were analyzed according to the method described by Velmala et al. (1993), applying restriction analysis (restriction fragment length polymorphism-PCR). The restriction fragment length polymorphism-PCR and allele-specific oligonucleotide-PCR products were visualized by electrophoresis on 2% agarose gel and documented using Bio-Capt v. 12.5 software (Vilbert Lourmant, France). Samples with various milk protein genotypes were verified using DNA sequencing of the relevant chromosomal regions. Sequencing was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA) and analyzed using an ABI Prism 3130 Genetic Analyzer (Applied Biosystems).

Statistical Analysis

Two single-trait random regression animal models were applied to the studied traits. The full model was

$$y_{ijklmn}(t) = \mu + \sum_{p=1}^{3} b_p \lg_p(t) + \sum_{p=0}^{3} c_{ip} \lg_p(t) + F_j + h_k + \sum_{p=0}^{q} a_{lp} \lg_p(t) + \sum_{p=0}^{r} pe_{mp} \lg_p(t) + \varepsilon_{ijklmn}(t),$$

where $y_{ijklmn}(t)$ is the observation of milk coagulation traits (RCT and a_{30}), milk yield, protein percentage, fat percentage, or SCS on the tth day in milk; μ is the overall mean; $\lg_p(t)$ is the pth-order Legendre polynomial of the tth DIM; b_p is a fixed regression coefficient of the pth-order Legendre polynomial; c_{ip} is a fixed genotype-specific regression coefficient; F_j indicates a subclass of other fixed effects, including calving age (nested within lactation), sample age (only for milk coagulation traits), year-season of sampling, and year-season of calving; h_k is random herd effect; a_{lp} is a random animal-specific regression coefficient of the qth-order Legendre polynomial; pe_{mp} is a random permanent environment-specific regression coefficient of the rth-order Legendre polynomial; and $\varepsilon_{ijklmn}(t)$ is the random error term. Orders q and r varied from 0 to 3, depending on the trait studied. The reduced model was the full model without the β-κ-CN genotype effect $\sum_{p=0}^{3} c_{ip} \lg_p(t)$.

The analyses were performed using ASReml (Gilmour et al., 2002). Further, composite genotype-specific lactation curves and dynamics of additive genetic variation during lactation were evaluated in SAS software version 9.2 (SAS Institute, 2008) based on the regression coefficients and covariance matrices of Legendre polynomials, respectively. Lactation total genotype effects for milk composition and coagulation traits were calculated as sums of differences between daily values of average and genotype-specific lactation curves obtained by the full model.

RESULTS AND DISCUSSION

Descriptive Statistics

Descriptive statistics for milk yield, composition, and coagulation traits are presented in Table 1. The number of test-day records was much higher in the first lactation; in the second and the third lactations, the number of cows decreased along with the number of test-day records.

Genotype frequencies of β-κ-CN genotypes are shown in Table 2. Two genotypes, A2A2AA and A1A2AA, were more frequent than the rest, with percentages of 27.4 and 23.1%, respectively. The percentages of the remaining 31 genotypes were less than 8%, including 20 genotypes with percentages less than 1%. These genotype frequencies were similar to those reported for Italian Holstein cows (Comin et al., 2008). Genotype A1IAE appeared only once and was therefore excluded

Table 1. Descriptive statistics for milk coagulation and composition traits per lactation (mean ± SD)

Trait	First lactation (n = 11,699)	Second lactation (n = 7,917)	Third lactation (n = 4,354)	Total (n = 23,970)
Milk coagulation time, min	10.65 ± 2.50	10.30 ± 2.04	9.51 ± 1.76	10.32 ± 2.27
Curd firmness, V	13.79 ± 3.93	13.69 ± 4.28	13.72 ± 4.29	13.74 ± 4.11
Milk yield, kg/d	25.76 ± 7.12	28.86 ± 9.72	30.28 ± 10.47	27.61 ± 8.91
Fat, %	4.05 ± 0.69	4.07 ± 0.80	4.07 ± 0.81	4.06 ± 0.75
Protein, %	3.36 ± 0.31	3.39 ± 0.35	3.38 ± 0.35	3.38 ± 0.33
SCS[1]	2.85 ± 1.88	3.52 ± 2.01	3.81 ± 2.01	3.25 ± 1.99

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

Table 2. Distribution of casein genotypes [no. (%)]: marginal genotype frequencies of β-casein (last column), κ-CN (upper row) and frequencies of composite β-κ-casein genotypes

	κ-CN genotype						
	AA	AB	AE	BB	BE	EE	Total no. (%)
β-CN genotype	1,561 (54.6)	812 (28.4)	287 (10.0)	114 (4.0)	77 (2.7)	8 (0.3)	2,859 (100)
A1A1	112 (3.9)	44 (1.5)	70 (2.4)	3 (0.1)	17 (0.6)	8 (0.3)	254 (8.9)
A1A2	659 (23.1)	210 (7.3)	213 (7.5)	18 (0.6)	29 (1.0)		1,129 (39.5)
A1B		43 (1.5)		12 (0.4)	9 (0.3)		64 (2.2)
A1I		79 (2.8)	1 (0.0)	16 (0.6)	22 (0.7)		118 (4.1)
A2A2	783 (27.4)	176 (6.2)	3 (0.1)	4 (0.1)			966 (33.8)
A2A3	5 (0.2)						5 (0.2)
A2B	2 (0.1)	76 (2.7)		10 (0.3)			88 (3.1)
A2I		179 (6.3)		19 (0.7)			198 (7.0)
BB		3 (0.1)		6 (0.2)			9 (0.3)
BI		2 (0.1)		14 (0.5)			16 (0.6)
II				12 (0.4)			12 (0.4)

from further statistical analyses. A Dutch Holstein-Friesian investigation inferring β-κ-CN haplotypes showed that β-CN protein variant I occurred only with κ-CN variant B (Visker et al. 2010). The very rare β-κ-CN genotype A1IAE showed the presence of different combinations of the β-CN I allele with the κ-CN variant other than the B allele in our study. However, of 344 animals having at least 1 β-CN I allele, 343 also had at least 1 κ-CN B allele. The rare κ-CN genotype EE was associated only with the β-CN A1A1 genotype. Genotypes BE and AE of κ-CN were also mainly associated with β-CN genotypes that included the A1 allele. The β-κ-CN A1E haplotype was also observed in the popular Finnish Ayrshire bull Mäkimattilan Inssi in the study by Velmala et al. (1995), in which the E allele was exclusively found in this haplotype.

β-κ-CN Genotype Effects on Milk Composition and Coagulation Traits

Associations between β-κ-CN genotypes and milk composition and coagulation traits are presented in

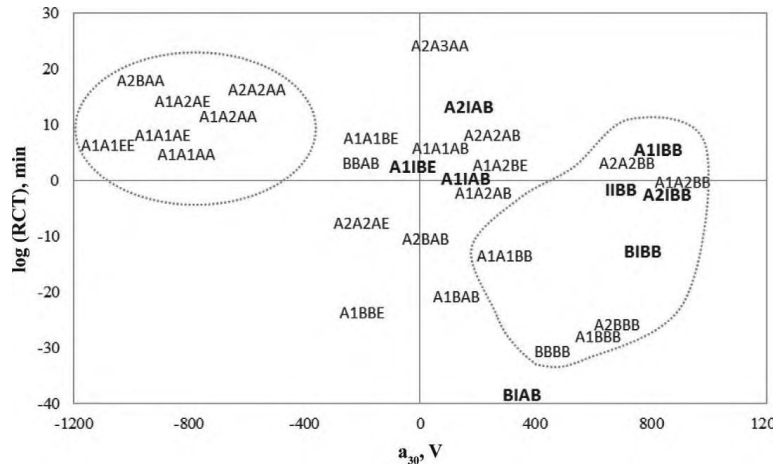

Figure 1. Estimated total β-κ-CN genotype effects for curd firmness (a_{30}) and log-transformed milk coagulation time [log(RCT)] as deviations from the overall mean. The genotypes in bold are those including the β-CN I allele; the 2 groups of genotypes surrounded by dotted lines comprise the κ-CN BB genotype (composite genotypes of favorable coagulation) or exclude the κ-CN B allele (except for 2 rare variants; composite genotypes of unfavorable coagulation), respectively.

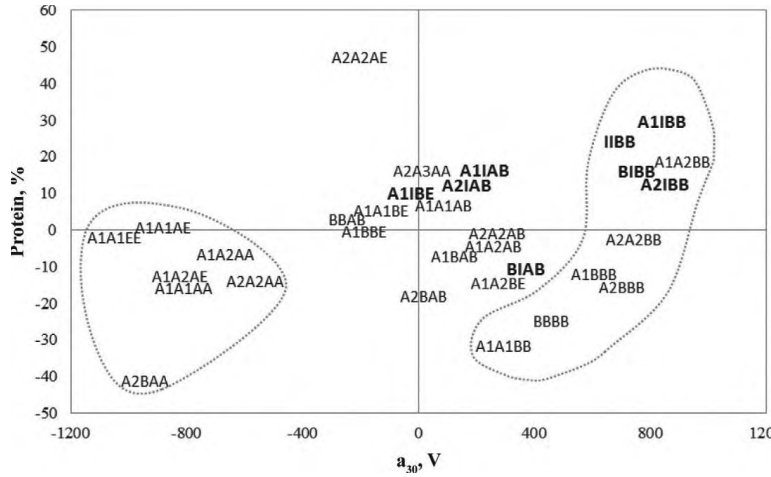

Figure 2. Estimated total β-κ-CN genotype effects for protein percentage and curd firmness (a_{30}) as deviations from the overall mean. Genotypes in bold are those including the β-CN I allele; the 2 groups of genotypes surrounded by dotted lines comprise the κ-CN BB genotype or exclude the κ-CN B allele (except for 2 rare variants), respectively.

Figures 1, 2, and 3. For the presented traits, the significance values of a β-κ-CN genotype effect were $P < 0.001$ for the RCT, a_{30}, and protein percentage and $P = 0.096$ for the fat percentage. Additionally, dynamics of β-κ-CN genotype effects on milk coagulation traits and protein percentage during lactation are shown in Figures 4, 5, and 6.

The association of β-κ-CN genotypes with milk coagulation traits indicates that the genotypes related to the higher values of a_{30} also corresponded to a shorter RCT (Figure 1). The favorable β-κ-CN genotypes increasing the a_{30} and decreasing the RCT comprised genotype BB of κ-CN, whereas the unfavorable genotypes decreasing the a_{30} and increasing the RCT excluded the B allele of κ-CN (including 2 more frequent genotypes, A2A2AA and A1A2AA). These groups, based on the κ-CN genotype in the β-κ-CN composite genotype, are designated with dotted lines in Figure 1. A similar effect of the κ-CN B allele was reported by Comin et al. (2008). As in the studies by Comin et al. (2008) and Bonfatti et al. (2010a), a favorable effect of the β-CN B allele on milk coagulation traits was found in the present study. As indicated by groups based on κ-CN genotypes (Figure 1), however, κ-CN seemed to have a greater influence on these traits, as was also suggested by Comin et al. (2008). Furthermore, the order of 3 κ-CN genotypes was clear for a_{30} in the β-κ-CN composite genotypes: BB > AB > AA (Figures 1 and 2). This order of κ-CN genotypes corresponded to the concentration and proportion of κ-CN described by McLean et al. (1984), Ikonen et al. (1997), and Hallén et al. (2008). Therefore, the association of the κ-CN B variant with milk coagulation traits may be due to an alteration in protein composition, as was also suggested by Bonfatti et al. (2010a). No clear superiority of genotypes was observed, including the β-CN I allele for either of the milk coagulation properties. However, genotypes including the I allele were all (with one exception) above the a_{30} mean and were close to the mean for RCT. Only the A1IBE genotype showed an unfavorable effect for both milk coagulation traits, which might have been caused by the negative effect of the κ-CN E allele on milk coagulation, as previously reported by Ojala et al. (2005) and Hallén et al. (2007).

Genotype BB of κ-CN showed no association with protein percentage, whereas the group of genotypes lacking the B allele of κ-CN (excluding the rare genotypes A2A2AE and A2A3AA, where the effect may be uncertain) corresponded to a lower protein percentage (Figure 2). The latter result is in accordance with previous studies of Bonfatti et al. (2010b) and Visker et al. (2010) but disagrees with the result of Comin et al. (2008), who found no association between protein percentage and κ-CN genotypes. The I allele of β-CN had a positive effect on protein percentage. This result confirms the report by Visker et al. (2010), who also

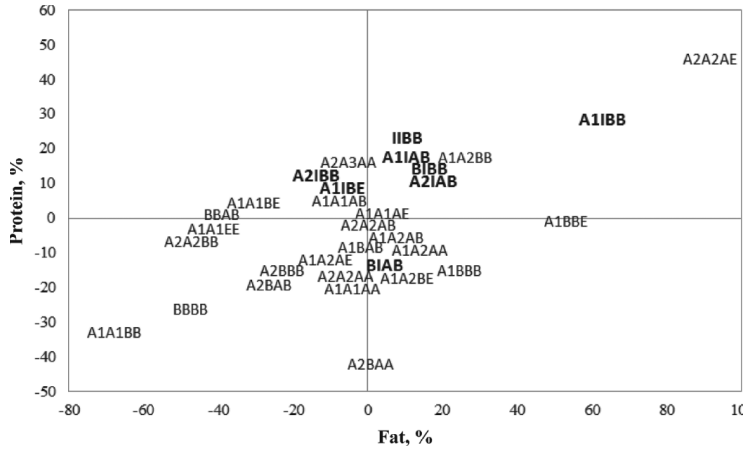

Figure 3. Estimated total β-κ-CN genotype effects for fat percentage and protein percentage as deviations from the overall mean. Genotypes in bold are those including the β-CN I allele.

found a positive effect of the β-CN I variant on protein percentage. The favorable association of β-κ-CN haplotype IB with protein percentage, however, seemed to occur from the positive effect of the β-CN I variant rather than from the effect of the κ-CN B variant (Figure 2). At the same time, all genotypes (except one) potentially incorporating the β-κ-CN haplotype IB had a positive effect on a_{30} (Figures 1 and 2), confirming the favorable effect of this haplotype on a_{30}. The positive effect of β-κ-CN haplotype IB on a_{30} has not been reported previously. A slight increase in the protein percentage associated with haplotype IB has also been reported by Bonfatti et al. (2010b).

Genotypes related to a higher protein percentage were also related to a higher fat percentage (Figure 3), but no specific groups of genotypes could be determined. The effect of the β-κ-CN genotype on milk yield and SCS was not significant. These results agree with those in previous studies by Comin et al. (2008), Bonfatti et al. (2010b), and Visker et al. (2010). However, the 2 more frequent β-κ-CN genotypes, A2A2AA and A1A2AA, had a moderately positive effect on milk yield albeit not statistically significant.

β-κ-CN Genotype Effects on Additive Genetic Variance of Milk Traits

The β-κ-CN genotype-specific lactation curves were significantly different for milk coagulation traits and milk protein percentage ($P < 0.05$). The analysis of covariance functions showed that the β-κ-CN genotype

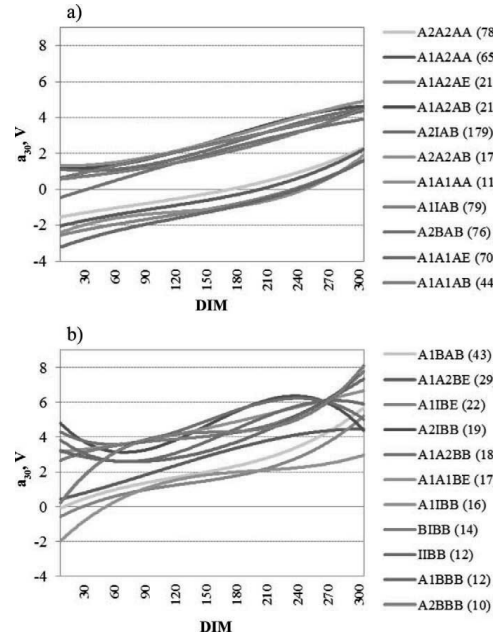

Figure 4. Genotype-specific curves for β-κ-CN genotypes with a frequency above 10 for curd firmness (a_{30}). The genotypes with higher frequencies are presented in panel a, and less frequent genotypes are presented in panel b. Genotype frequencies are presented in parentheses. Color version available in the online PDF.

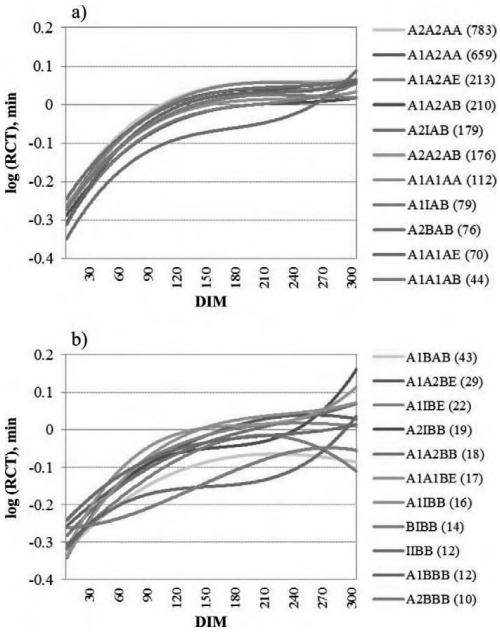

Figure 5. Genotype-specific curves for β-κ-CN genotypes with a frequency above 10 for log-transformed milk coagulation time [log(RCT)]. The genotypes with higher frequencies are presented in panel a, and less frequent genotypes are presented in panel b. Genotype frequencies are presented in parentheses. Color version available in the online PDF.

effect mainly caused changes in the proportion of additive genetic variation, whereas the dynamics (the shape of the curve) remained largely the same (Figure 7).

Inclusion of a β-κ-CN genotype effect into the model resulted in a decrease in the mean additive genetic variance by 12.9% for RCT, and by 51.1% for a_{30} (Table 3). The result for a_{30} was similar to the decrease of 68% reported by Penasa et al. (2010), whereas the decrease for RCT was comparable with the 20% decrease described by Ikonen et al. (1999a). However, the study by Ikonen et al. (1999a) included both Finnish Friesian and Finnish Ayrshire breeds and additionally considered a β-LG effect. Similar to the reports by Penasa et al. (2010) and Ikonen et al. (1999a), the β-κ-CN genotype effects on milk yield, protein and fat percentages, and SCS additive genetic variance were marginal. The cows in the studies by Penasa et al. (2010) and Ikonen et al. (1999a) were sampled only once; consequently, the animal models in these studies did not include a permanent environmental variance component, which may have caused some of the difference in their results compared with the present study regarding the effect of milk protein polymorphism on the additive genetic variance. Analyses of the permanent environmental variance showed that the inclusion of a β-κ-CN genotype effect caused an increase in this parameter (Table 3). Inclusion of the β-κ-CN genotype effect allowed more precise distinction of the additive genetic and the animal-specific permanent effects, which could explain the increase in permanent environmental variance.

CONCLUSIONS

This study included 2,859 Estonian Holstein cows. The most frequent β-κ-CN composite genotypes were A2A2AA and A1A2AA, both with prevalence greater than 20%. These genotypes had unfavorable effects on RCT and a_{30}. Of the 344 animals that had at least 1

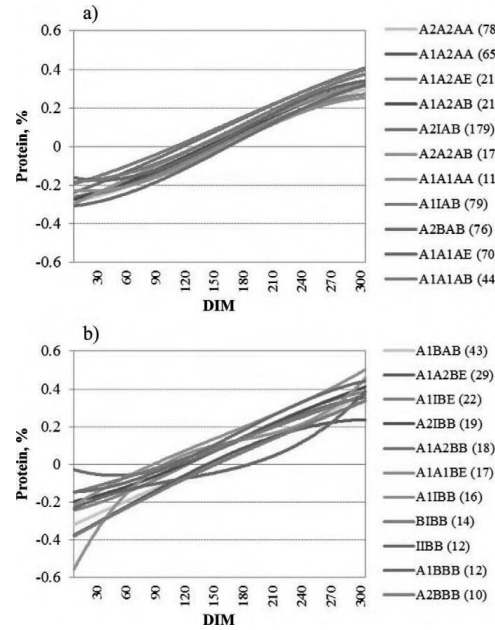

Figure 6. Genotype-specific curves for β-κ-CN genotypes with a frequency above 10 for protein percentage. The genotypes with higher frequencies are presented in panel a, and less frequent genotypes are presented in panel b. Genotype frequencies are presented in parentheses. Color version available in the online PDF.

Table 3. Lactation curve means for dispersion components of the additive genetic (σ^2_a) and permanent environmental (σ^2_{pe}) effects, estimated with random regression animal models excluding or including the β-κ-CN genotype effect (models 1 and 2, respectively)

	Mean of σ^2_a		Relative difference	Mean of σ^2_{pe}		Relative difference
Trait	Model 1	Model 2	Model 2 − model 1,[1] %	Model 1	Model 2	Model 2 − model 1,[1] %
Log(RCT),[2] min	0.0107	0.0093	−12.9	0.0031	0.0035	14.6
Curd firmness, V	7.1483	3.4949	−51.1	NE[3]	1.0809	NE
Milk yield, kg/d	10.7748	10.3217	−4.2	8.0809	8.4902	5.1
Fat, %	0.1204	0.1176	−2.4	0.0388	0.0405	4.6
Protein, %	0.0313	0.0294	−5.9	0.0028	0.0035	27.7
SCS[4]	0.2189	0.2207	0.8	0.7950	0.7968	0.2

[1][(Model 2 – model 1)/model 2] × 100.

[2]Log-transformed milk coagulation time.

[3]NE = not estimable.

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

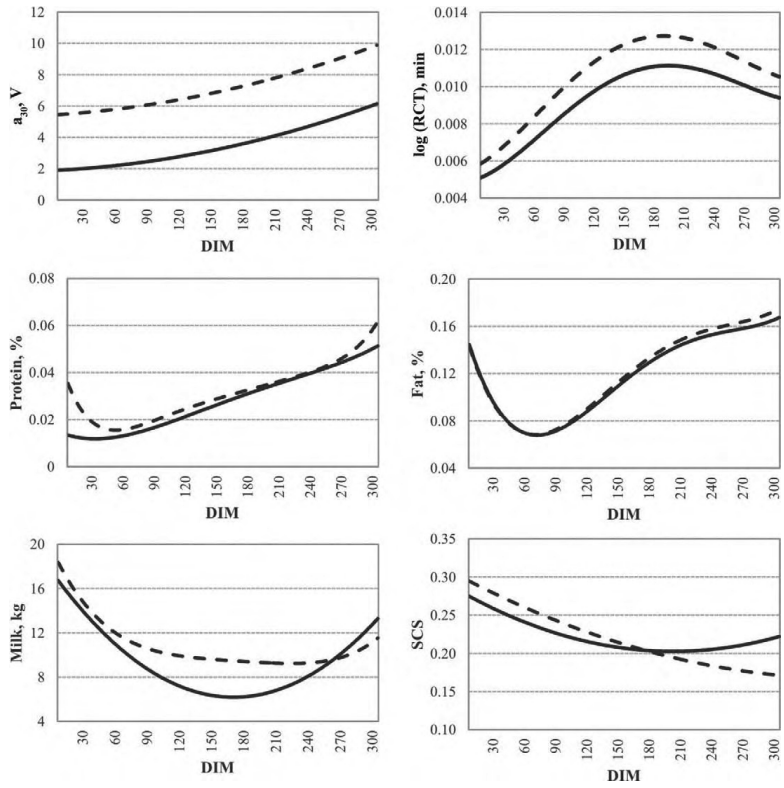

Figure 7. Dynamics of additive genetic variance of milk coagulation [log(RCT) and a_{30}] and composition traits during lactation (7 to 305 DIM) considering the β-κ-CN genotype effect (solid line) and excluding the β-κ-CN genotype effect (dashed line) as a third-order Legendre polynomial of DIM. a_{30} = curd firmness; log(RCT) = log-transformed milk coagulation time.

β-CN I allele, 343 also had at least 1 κ-CN B allele, a strong indication of the existence of a β-κ-CN IB haplotype. The B variant of κ-CN showed a favorable effect on both milk coagulation traits, whereas the IB haplotype had an increasing effect on a_{30} and protein percentage. The composite β-κ-CN genotypes influenced the additive genetic variance of milk coagulation traits, whereas changes in the additive genetic variance of milk composition traits were marginal.

Thus, considering the high frequency of unfavorable β-κ-CN genotypes for milk coagulation traits, and the positive association of the β-κ-CN IB haplotype with a_{30} and protein percentage, selection based on the β-κ-CN genotype for these traits could be a consideration. Genetic improvement is expected to be higher for milk coagulation properties because the influence of the β-κ-CN genotype on the additive genetic variation of these milk properties was stronger than its influence on the additive genetic variation of milk composition traits.

ACKNOWLEDGMENTS

The research leading to these results was co-financed by the Regional Development Fund of the European Community (Brussels, Belgium) in the framework of the Competence Centre Programme of the Enterprise Estonia (Tallinn, Estonia) under project numbers EU22868, EU27789, EU28662, and EU30002 of the Bio-Competence Centre of Healthy Dairy Products (Tervisliku Piima Biotehnoloogiate Arenduskeskus OÜ, Tartu, Estonia), and by Targeted Finance Projects (Tartu, Estonia) 1080045s07 and 1080022s07. The staff from the Estonian Animal Recording Centre (Tartu), the Animal Breeders' Association of Estonia (Keava), and the Institute of Veterinary Medicine and Animal Sciences of the Estonian University of Life Sciences (Tartu) are warmly thanked for their contributions to the collection and laboratory analysis of data for the databases COAGEN and COACAS.

REFERENCES

Aleandri, R., L. G. Buttazzoni, J. C. Schneider, A. Caroli, and R. Davoli. 1990. The effects of milk protein polymorphisms on milk components and cheese-producing ability. J. Dairy Sci. 73:241–255.

Bhandari, V., and H. Singh. 2002. Physical methods. Pages 93–101 in Encyclopedia of Dairy Sciences. Vol. 1. H. Roginski, J. W. Fuquay, and P. F. Fox, ed. Academic Press, San Diego, CA.

Boettcher, P. J., A. Caroli, A. Stella, S. Chessa, E. Budelli, F. Canavesi, S. Ghiroldi, and G. Pagnacco. 2004. Effects of casein haplotypes on milk production traits in Italian Holstein and Brown Swiss cattle. J. Dairy Sci. 87:4311–4317.

Bonfatti, V., G. Di Martino, A. Cecchinato, L. Degano, and P. Carnier. 2010a. Effects of β-κ-casein (*CSN2-CSN3*) haplotypes and β-lactoglobulin (*BLG*) genotypes, and detailed protein composition on coagulation properties of individual milk of Simmental cows. J. Dairy Sci. 93:3809–3817.

Bonfatti, V., G. Di Martino, A. Cecchinato, D. Vicario, and P. Carnier. 2010b. Effects of CSN2-CSN3 haplotypes and BLG genotypes on milk production traits, protein fractions contents and protein composition of individual milk of Simmental cows. J. Dairy Sci. 93:3797–3808.

Bovenhuis, H., J. A. M. van Arendonk, and S. Korver. 1992. Associations between milk protein polymorphism and milk production traits. J. Dairy Sci. 75:2549–2559.

Caroli, A. M., S. Chessa, and G. J. Erhardt. 2009. Invited review: Milk protein polymorphisms in cattle: Effect on animal breeding and human nutrition. J. Dairy Sci. 92:5335–5352.

Comin, A., M. Cassandro, S. Chessa, M. Ojala, R. Dal Zotto, M. De Marchi, P. Carnier, L. Gallo, G. Pagnacco, and G. Bittante. 2008. Effects of β- and κ-casein genotypes on milk coagulation, quality, and yield traits in Italian Holstein cows. J. Dairy Sci. 91:4022–4027.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, S. J. Welham, and R. Thompson. 2002. ASReml User Guide Release 2.0. VSN International, Hemel Hempstead, UK.

Hallén, E., T. Allmere, J. Näslund, A. Andrén, and A. Lundén. 2007. Effect of genetic polymorphism of milk proteins on rheology of chymosin-induced milk gels. Int. Dairy J. 17:791–799.

Hallén, E., A. Wedholm, A. Andrén, and A. Lundén. 2008. Effect of β-casein, κ-casein and β-lactoglobulin genotypes on concentration of milk protein variants. J. Anim. Breed. Genet. 125:119–129.

Heck, J. M. L., A. Schennink, H. J. F. van Valenberg, H. Bovenhuis, M. H. P. W. Visker, J. A. M. van Arendonk, and A. C. M. van Hooijdonk. 2009. Effects of milk protein variants on the protein composition of bovine milk. J. Dairy Sci. 92:1192–1202.

Ikonen, T., K. Ahlfors, R. Kempe, M. Ojala, and O. Ruottinen. 1999a. Genetic parameters for the milk coagulation properties and prevalence of noncoagulating milk in Finnish dairy cows. J. Dairy Sci. 82:205–214.

Ikonen, T., M. Ojala, and O. Ruottinen. 1999b. Associations between milk protein polymorphism and first lactation milk production traits in Finnish Ayrshire cows. J. Dairy Sci. 82:1026–1033.

Ikonen, T., M. Ojala, and E. L. Syvaoja. 1997. Effects of composite casein and β-lactoglobulin genotypes on renneting properties and composition of bovine milk by assuming an animal model. Agric. Food Sci. Finl. 6:283–294.

ICAR (International Committee for Animal Recording). 2009. International agreement of recording practices. Guidelines approved by the General Assembly held in Niagara Falls, USA, June 18, 2008. Accessed August 4, 2011. http://www.icar.org/Documents/Rules%20and%20regulations/Guidelines/Guidelines_2009.pdf.

Lundén, A., M. Nilsson, and L. Janson. 1997. Marked effect of β-lactoglobulin polymorphism on the ratio of casein to total protein in milk. J. Dairy Sci. 80:2996–3005.

Macheboeuf, D., J.-B. Coulon, and P. D'Hour. 1993. Effect of breed, protein genetic variants and feeding on cow's milk coagulation properties. J. Dairy Res. 60:43–54.

Mayer, H. K., M. Ortner, E. Tschager, and W. Ginzinger. 1997. Composite milk protein phenotypes in relation to composition and cheesemaking properties of milk. Int. Dairy J. 7:305–310.

McLean, D. M., E. R. B. Graham, R. W. Ponzoni, and H. A. McKenzie. 1984. Effects of milk protein genetic variants on milk yield and composition. J. Dairy Res. 51:531–546.

Ng-Kwai-Hang, K. F., J. F. Hayes, J. E. Moxley, and H. G. Monardes. 1984. Association of genetic variants of casein and milk serum proteins with milk, fat, and protein production by dairy cattle. J. Dairy Sci. 67:835–840.

Ojala, M., T. R. Famula, and J. F. Medrano. 1997. Effects of milk protein genotypes on the variation for milk production traits of Holstein and Jersey cows in California. J. Dairy Sci. 80:1776–1785.

Ojala, M., A.-M. Tyrisevä, and T. Ikonen. 2005. Genetic improvement of milk quality traits for cheese production. Pages 307–311 in Indicators of Milk and Beef Quality. Eur. Assoc. Anim. Prod. Publ. 112. J. F. Hocquette and S. Gigli, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.

Penasa, M., M. Cassandro, D. Pretto, M. De Marchi, A. Comin, S. Chessa, R. Dal Zotto, and G. Bittante. 2010. Short communication: Influence of composite casein genotypes on additive variation of milk production traits and coagulation properties in Holstein-Friesian cows. J. Dairy Sci. 93:3346–3349.

SAS Institute. 2008. SAS/STAT 9.2 User's Guide. SAS Inst. Inc., Cary, NC.

Vallas, M., H. Bovenhuis, T. Kaart, K. Pärna, H. Kiiman, and E. Pärna. 2010. Genetic parameters for milk coagulation properties in Estonian Holstein cows. J. Dairy Sci. 93:3789–3796.

Velmala, R., E. A. Mäntysaari, and A. Mäki-Tanila. 1993. Molecular genetic polymorphism at the κ-casein and β-lactoglobulin loci in Finnish dairy bulls. Agric. Food Sci. Finl. 2:431–435.

Velmala, R., J. Vilkki, K. Elo, and A. Mäki-Tanila. 1995. Casein haplotypes and their association with milk production traits in the Finnish Ayrshire cattle. Anim. Genet. 26:419–425.

Visker, M. H. P. W., B. W. Dibbits, S. M. Kinders, H. J. F. van Valenberg, J. A. M. van Arendonk, and H. Bovenhuis. 2010. Short communication: Association of bovine β-casein protein variant I with milk production and milk protein composition. Anim. Genet. 42:212–218.

CURRICULUM VITAE

First name:	Mirjam
Surname:	Vallas
Citizenship:	Estonian
Date of birth:	17.05.1980
Address:	Institute of Veterinary Medicine and Animal Sciences, Department of Animal Genetics and Breeding, Estonian University of Life Sciences, Kreutzwaldi 62, Tartu 51014, Estonia
E-mail:	mirjam.vallas@emu.ee

Education:

2005–2011	Estonian University of Life Sciences, *PhD* studies in Animal Science
1999–2005	University of Tartu, Faculty of Mathematics and Computer Science, *BSc* and *MSc* studies in mathematical statistics
1987–1999	Toila Gymnasium

Professional employment:

2010–…	Estonian University of Life Sciences (EMÜ), Institute of Veterinary Medicine and Animal Sciences, researcher (0.5)
2008–…	Bio-Competence Centre of Healthy Dairy Products LLC, researcher (0.5)
2008–2009	EMÜ, Institute of Veterinary Medicine and Animal Sciences, statistician (0.5)
2003–2008	Estonian Agricultural University/EMÜ, Institute of Veterinary Medicine and Animal Sciences, statistician

Research projects:

2013–2018	Fertility and health in dairy cattle (IUT8-1)
2008–2013	Sustainable breeding strategies to produce compositionally designed milk to promote biotechnological properties of milk, foster human health, and increase profitability of milk production (8-2/T9083VLVL)
2007–2012	Physiology, pathology and biotechnology of animal reproduction (SF1080045s07)
2005–2008	Improvement of raw milk coagulation properties (8-2/T5015VLVL)

ELULOOKIRJELDUS

Eesnimi:	Mirjam
Perekonnanimi:	Vallas
Kodakondsus:	Eesti
Sünniaeg:	17.05.1980
Töökoht:	Veterinaarmeditsiini ja loomakasvatuse instituut, loomageneetika ja tõuaretuse osakond, Eesti Maaülikool, Kreutzwaldi 62, Tartu 51014
E-mail:	mirjam.vallas@emu.ee
Hariduskäik:	
2005–2011	Eesti Maaülikool, veterinaarmeditsiini ja loomakasvatuse instituut, loomakasvatus, doktoriõpe
1999–2005	Tartu Ülikool, matemaatika-informaatika teaduskond, matemaatiline statistika, bakalaureuse ja magistriõpe
1987–1999	Toila Gümnaasium
Teenistuskäik:	
2010–…	Eesti Maaülikool, veterinaarmeditsiini ja loomakasvatuse instituut, teadur (0,5)
2008–…	Tervisliku Piima Biotehnoloogiate Arenduskeskus OÜ, teadur (0,5)
2008–2009	Eesti Maaülikool, veterinaarmeditsiini ja loomakasvatuse instituut, statistik (0,5)
2003–2008	Eesti Põllumajandusülikool/Eesti Maaülikool, loomakasvatuse instituut/veterinaarmeditsiini ja loomakasvatuse instituut, statistik
Projektid:	
2013–2018	Piimalehmade sigimine ja tervis (IUT8-1)
2008–2013	Jätkusuutlikud aretusstrateegiad disainitud koostisega tervisliku ning tehnoloogiliselt sobiva piima tootmiseks (8-2/T9083VLVL)
2007–2012	Loomade reproduktsiooni füsioloogia, patoloogia ja biotehnoloogia (SF1080045s07)
2005–2008	Piima valgulise koostise kujundamine (8-2/T5015VLVL)

LIST OF PUBLICATIONS

1.1 – Articles indexed by Thomson Reuters Web of Science

Vallas, M., Kaart, T., Värv, S., Pärna, K., Jõudu, I., Viinalass, H., Pärna, E. 2012. Composite β-κ-casein genotypes and their effect on composition and coagulation of milk from Estonian Holstein cows. – Journal of Dairy Science, 95(11), 6760–6769.

Pretto, D., Kaart, T., **Vallas, M.**, Jõudu, I., Henno, M., Ancilotto, L., Cassandro, M., Pärna, E. 2011. Relationship between milk coagulation property traits analyzed with different methodologies. – Journal of Dairy Science, 94(9), 4336–4346.

Vallas, M., Bovenhuis, H., Kaart, T., Pärna, K., Kiiman, H., Pärna, E. 2010. Genetic parameters for milk coagulation properties in Estonian Holstein cows. – Journal of Dairy Science, 93(8), 3789–3796.

Pärna, E., Kiiman, H., **Vallas, M.**, Viinalass, H., Saveli, O., Pärna, K. 2007. Development of a breeding objective for Estonian Holstein cattle. – Agricultural and Food Science, 16, 212–221.

1.2 – Peer-reviewed articles in other international research journals with an ISSN code and international editorial board

Piirsalu, P., Samarütel, J., Tölp, S., Nutt, I., **Vallas, M.** 2012. Mahelammaste söötmine, uttede toitumus ning jõudlus sigimistsükli erinevatel perioodidel. – Agraarteadus, 23(2), 27–35.

Pärna, E., Kiiman, H., Viinalass, H., **Vallas, M.** 2005. Bio-economical model application in cattle breeding. – Acta Agriculturae Slovenica, 2, 117–124.

3.2 – Articles in books or proceedings published by Estonian or other publishers not listed by Thomson Reuters Conference Proceedings Citation Index

Kaart, T., **Vallas, M.**, Waldmann, A., Kiiman, H., Pärna, K., Pärna, E. 2010. Associations between milk quality traits and coagulation properties and fertility in Estonian Holstein heifers and first lactation cows. – Abstract 0567 in Proceedings of the 9th World Congress on Genetics Applied to Livestock Production, Leipzig, Germany, August 1-6, 2010.

Vallas, M., Kaart, T., Värv, S., Viinalass, H., Kiiman, H., Pärna, K., Pärna, E. 2010. Associations between milk protein genotypes and milk coagulation properties of Estonian Holstein cows. – Abstract 0507 in Proceedings of the 9th World Congress on Genetics Applied to Livestock Production, Leipzig, Germany, August 1-6, 2010.

3.4 – Articles and presentations published in the conference proceedings not listed by Thomson Reuters Conference Proceedings Citation Index

Poikalainen, V., Praks, J., Veermäe, I., Aland, A., **Vallas, M.** 2011. Automatic lameness detection of dairy cows at the feed barrier by leg weight distribution. – Proceedings of the 15th International Congress of the ISAH: Animal Hygiene and Sustainable Livestock Production, Vienna, Austria, July 3-7, 2011, 317–319.

Ling, K., Kaart, T., **Vallas, M.**, Samarütel, J., Ots, M., Jaakson, H., Henno, M., Kärt, O. 2010. Negative energy balance of primiparous dairy cows reared on different farms. – Proceedings of the 14th International Conference on Production Diseases in Farm Animals, Ghent, Belgium, June 20-24, 2010, 143–144.

Kass, M., Kaldmäe, H., Ots, M., **Vallas, M.**, Tölp, S., Kärt, O., Arney, D. 2008. Predicting rumen protein degradability of rapeseed cake. – Proceedings of the International Scientific Conference: Implication of Different Production Technologies on Animal Health and Food Products Quality Indices, Sigulda, Latvia, December 4-5, 2008, 68–76.

Pärna, E., **Vallas, M.**, Kaart, T., Kübarsepp, I., Kiiman, H., Pärna, K. 2006. Genetic improvement of milk coagulation properties. – Communication No 01–55 in Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, Brazil, August 13-18, 2006.

Pärna, E., **Vallas, M.**, Saveli, O., Pärna, K. 2006. Genetic variants of κ-casein and its association with rennet coagulation of Estonian Holstein cows. – Proceedings of the 12th Baltic Animal Breeding Conference, Jurmala, Latvia, April 27-28, 2006, 211–216.

Kärt, O., Rihma, E., Tölp, S., **Vallas, M.** 2004. Efficiency of converting ration dry matter, energy and protein to milk different cattle breeds. – Proceedings of the 10th Baltic Animal Breeding Conference, Tartu, Estonia, May 13-14, 2004, 244–249.

3.5 – Articles published in the proceedings of Estonian conferences

Kaart, T., **Vallas, M.**, Värv, S., Jõudu, I., Pärna, E., Viinalass, H. 2013. Piima laapumisomaduste parandamise võimalusi selektsiooni teel. – Kogumikus: Terve loom ja tervislik toit 2013. Jaakma, Ü., Henno, M., Kass, M., Jaakson, H., Jalakas, M., Orro, T., Roasto, M., Praakle, K. (Toim.). Tartu, 21.-22. märts, 2013, 54–59.

Vallas, M., Bovenhuis, H., Kaart, T., Pärna, K., Kiiman, H., Pärna, E. 2011. Geneetilised parameetrid piima laapumis- ja koostisnäitajatele. – Kogumikus: Terve loom ja tervislik toit 2011. Jaakma, Ü. (Toim.). Tartu, 17.-18. märts, 2011, 80–84.

Vallas, M. 2011. Geneetiliste parameetrite hindamine piima laapumis- ja koostisnäitajatele. – Kogumikus: Eesti statistikaseltsi teabevihik 21: Statistika ja eluteadused. Narusk, E., Heinapuu, O. (Toim.). Tartu, 13.-14. aprill, 2010, 40–45.

5.2 – Conference abstracts not indexed by Thomson Reuters Web of Science

Jõudu, I., Henno, M., Ots, M., Harzia, H., Värv, S., **Vallas, M.** 2012. Review of studies concerning milk rennet coagulation properties in Estonia during last decade. – Proceedings of 7th Baltic Conference on Food Science and Technology Foodbalt-2012: Innovative and Healthy Food for Consumers, Kaunas, Lithuania, May 17-18, 2012, 77.

Lepik, T., Peterson, H., **Vallas, M.** 2011. The types of higher nervous activity and preliminary results in local horse breeds. – Book of Abstracts of the 23rd Nordic Symposium of the ISAE: The Environmental Effects on the Behaviour and Welfare of Animals, Tartu, Estonia, January 19-21, 2011, 19.

Vallas, M., Pretto, D., Kaart, T., Cassandro, M., Ancilotto, L., Pärna, E. 2011. Genetic trends for milk yield, milk components and milk coagulation properties in Italian Holstein Friesian and Estonian Holstein dairy cattle population. – Book of Abstracts of the 62nd Annual Meeting of the European Association for Animal Production, Stavanger, Norway, August 29-September 2, 2011, 413.

Vallas, M., Pärna, E., Kaart, T., Kiiman, H. 2009. Genetic parameters for milk coagulation properties in the first lactation Estonian Holstein cows. – Book of Abstracts of the 60th Annual Meeting of the European Association for Animal Production, Barcelona, Spain, August 24-27, 2009, 204.

Pärna, E., Kiiman, H., **Vallas, M.**, Viinalass, H., Saveli, O., Pärna, K. 2008. Development of a breeding objective for Estonian Holstein cattle. – Book of Abstracts of the 59th Annual Meeting of the European Association for Animal Production, Vilnius, Lithuania, August 24-27, 2008, 205.

Ling, K., Kaart, T., **Vallas, M.**, Samarütel, J., Jaakson, H., Kärt, O. 2007. Energy balance of primiparous Estonian Holstein, Estonian Red Holstein and Estonian Red cows. – Proceedings of 13th International Conference on Production Diseases in Farm Animals, Leipzig, Germany, July 29-August 4, 2007, 185.

Inventions

Method for identifying of cows, sires and bull dams that produce milk with modified coagulation properties; Owner: Bio-Competence Centre of Healthy Dairy Products LLC; Authors: Pärna, E., **Vallas, M.**, Pärna, K., Kaart, T.; Priority: 10.08.2006 EE P200600029.

Способ идентификации крупного рогатого скота, производящего молоко с модифицированными коагуляционными свойствами; Патентообладатель: ОЮ Тервислику Пиима Биотехнолоогиате Арендускескус (Биокомпетентс Сентр оф Хеалси Дэйри продуктс); Авторы: Пярна, Э., **Валлас**, **М.**, Пярна, К., Каарт, Т.; Конвенционный приоритет: 10.08.2006 EE P200600029.

VIIS VIIMAST KAITSMIST

AIVE LIIBUSK

PRECISE HYDRODYNAMIC LEVELLING USING PRESSURE GAUGES WITH APPLICATION TO IMPROVEMENT OF THE ESTONIAN NATIONAL LEVELLING NETWORK
RÕHUANDURITEL PÕHINEV TÄPNE HÜDRODÜNAAMILINE LOODIMINE RAKENDATUNA EESTI RIIKLIKU KÕRGUSVÕRGU REKONSTRUEERIMISEL
Dots. **Harli Jürgenson**, Prof. **Artu Ellmann** (Tallinna Tehnikaülikool)
22. aprill 2013

LIINA TALGRE

BIOMASS PRODUCTION OF DIFFERENT GREEN MANURE CROPS AND THEIR EFFECT ON THE SUCCEEDING CROPS YIELD
ERINEVATE HALJASVÄETISKULTUURIDE BIOPRODUKTSIOON JA MÕJU JÄRGNEVATE KULTUURIDE SAAGILE
Dots. **Enn Lauringson**, Prof. *emer*. **Hugo Roostalu**
22. aprill 2013

TEA TULLUS

UNDERSTOREY VEGETATION AND FACTORS AFFECTING IT IN YOUNG DECIDUOUS FOREST PLANTATIONS ON FORMER AGRICULTURAL LAND
ALUSTAIMESTIK JA SEDA MÕJUTAVAD TEGURID ENDISTEL PÕLLUMAJANDUSMAADEL KASVAVATES NOORTES LEHTPUUISTANDIKES
Prof. **Hardi Tullus**, *PhD* **Elle Roosaluste** (Tartu Ülikool)
22. mai 2013

MIGUEL PORTILLO ESTRADA

ON THE RELATIONSHIPS BETWEEN PLANT LITTER AND THE CARBON AND NITROGEN CYCLES IN EUROPEAN FOREST ECOSYSTEMS
EUROOPA METSAÖKOSÜSTEEMIDE SÜSINIKU- JA LÄMMASTIKURINGE SEOSED TAIMSE VARISEGA
Prof. **Ülo Niinemets**, Teadur **Steffen M. Noe**
30. mai 2013

ZHIHONG SUN

ISOPRENE EMISSION FROM ASPEN (*Populus sp.*) IN RELATION TO ENVIRONMENTAL DRIVERS
KESKKONNATEGURITE MÕJU HAAVA (*Populus sp.*) ISOPREENI EMISSIOONILE
Prof. **Ülo Niinemets**
5. juuni 2013

ISBN 978-9949-484-87-4 (trükis)
ISBN 978-9949-484-88-1 (pdf)

roheline trükis

Trükitud taastoodetud paberile looduslike trükivärvidega © Ecoprint