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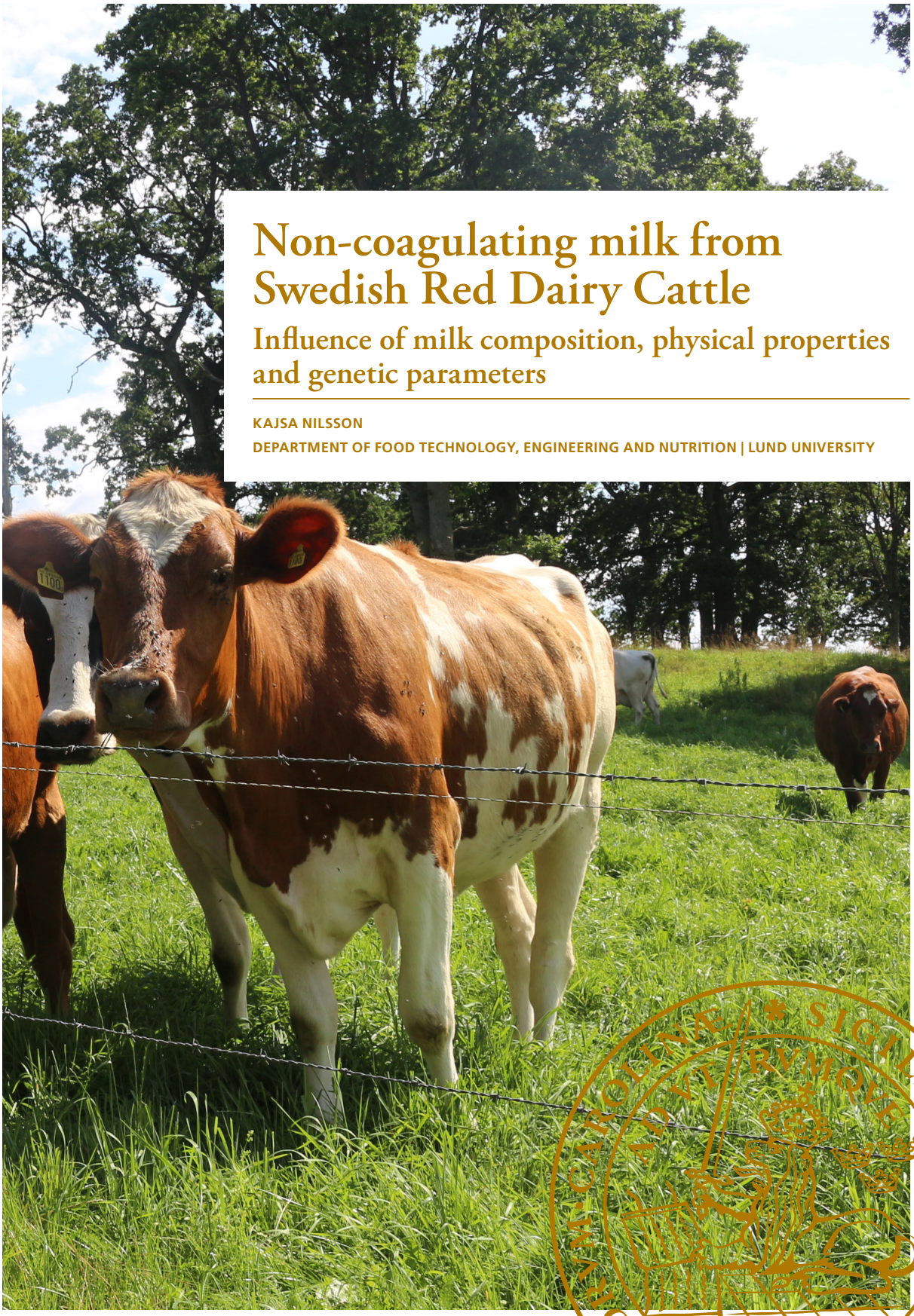

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Abstract

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Milk samples from over 700 cows from Swedish RDC was collected and rheologically evaluated. The milk samples were also used to investigate milk composition, including a detailed protein profile with genetic variants, phosphorylations and glycosylations. Additionally, the enzymatic stage of the coagulation process was studied in both coagulating and NC milk samples, in order to determine if faults in the enzymatic or in the aggregation stage causes NC milk. Further, blood samples were collected, giving possibilities to combine knowledge about milk traits with genetic parameters.

The results showed that 18.1% of the cows produced milk that did not coagulate within 40 minutes after chymosin addition and was thereby defined as NC milk samples. Additionally, 18.9% of the cows produced milk that coagulated poorly, resulting in a total frequency of 37% of the cows that produced milk which is not optimal for cheese production. NC milk was found to have a moderate estimated heritability of 0.28, suggesting possibilities for selective breeding of this trait. The study of the enzymatic stage showed cleavage of κ-casein in the NC milk samples, suggesting that NC milk is caused by issues in the aggregation stage of the coagulation process, and not the enzymatic stage.

The combined information about milk traits and genetic parameters resulted in four suggested phenotypic markers. These can be used as indirect breeding parameters to reduce the frequency of NC milk in Swedish RDC. The four markers were: κ-casein, α-lactalbumin, and calcium contents as well as genetic variants of β-casein. These showed different relations with NC milk, where NC milk contained lower κ-casein and calcium contents but higher α-lactalbumin content compared to coagulating milk. The estimated heritability of these three parameters ranged from 0.12-0.77, showing different possibilities to use them within breeding. The genetic correlations for the three parameters with NC milk were all significant and moderate to high. Further, NC milk had a higher frequency of β-CN A2 and lower frequency of β-CN A1 compared to coagulating milk, which may also be used for breeding strategies.

This thesis has thereby pointed out phenotypic markers found in NC milk that have potential to be used in selective breeding in order to reduce the frequency of NC milk in Swedish RDC. A reduced NC frequency in Swedish RDC would strengthen the competitiveness of this breed by ensuring profitable and multifunctional milk, useful for several dairy products. This leads to an increased economic gain for stakeholders and a more sustainable dairy production.

Key words Non-coagulating milk, milk composistion, protein profile, hydrolysation, heritability, genetic parameter

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Prepare for a cheesy story

Table of Contents

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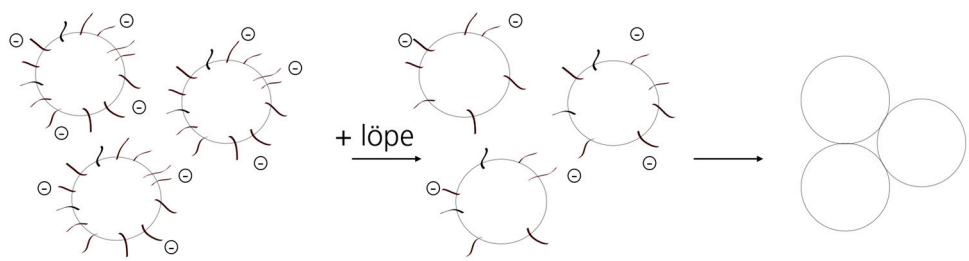
Populärvetenskaplig sammanfattning

Avla kor i en ostligare riktning

Mjölk som inte koagulerar, det vill säga icke-koagulerande mjölk, är ett stort problem i ostproduktionen eftersom det resulterar i mindre mängd producerad ost. Den till antal näst största mjölkkorasen i Sverige, Svensk röd och vit boskap, är kopplad till en hög andel icke-koagulerande mjölk. Denna avhandling är inriktad på att få mer kunskap om sammansättning, fysikaliska egenskaper och ärftliga parametrar i icke-koagulerande mjölk, för att förstå den bakomliggande orsaken och därmed på sikt kunna minska andelen icke-koagulerande mjölk genom avel.

Ost har producerats av människan i tusentals år och var ursprungligen ett sätt att konservera mjölk. Genom att tillsätta syra eller löpe kommer proteiner som heter kasein att bilda en ostgel. Förutom kasein stannar även mjölkfettet kvar i osten, medan vatten, resterande proteiner, laktos och salt pressas ut i en vätska som kallas vassle. Den minskade mängden vatten jämfört med mjölk bidrar till längre hållbarhet, eftersom bakterier och mögel föredrar fuktiga miljöer. Även om vi nuförtiden har andra metoder för att förlänga hållbarheten på mjölk (till exempel kylskåp och värmebehandling), äter vi fortfarande stora mängder ost, främst för den goda smakens skull. Det är också viktigt att komma ihåg att ost är en utmärkt källa för mineraler så som kalcium. I Sverige används runt en tredjedel av all producerad komjölk till just ostproduktion.



Schematisk bild över koaguleringsfasen vid osttillverkning. Löpe delar på κ-kasein vid micellernas yta, vilket gör att micellerna sedan kan binda in till varandra och så småningom bilda en gel.

Kasein är en grupp av olika proteiner som naturligt finns i mjölk i form av sfäriska strukturer som heter kaseinmiceller. Vissa av kaseinerna, κ-kasein, sitter på ytan av

micellerna och är negativt laddade vilket därför bidrar till att micellerna stöter bort varandra. Att ta bort micellernas förmåga att stöta bort varandra är viktigt i ostproduktionen och detta görs i det första steget i ostproduktionen som kallas för koaguleringsfasen. Det är då mjölk går från en vätska till en gel. Detta sker genom att enzymet kymosin (en av komponenterna i löpe) tillsätts till mjölken och delar på κ-kasein som sitter på ytan av kaseinmicellerna. Detta gör att både det steriska hindret och laddningarna som gjort att micellerna stött bort varandra försvinner. Därefter kommer micellerna att börja binda in till varandra med hjälp av fria kalciumjoner och en gel kommer att bildas. Om det är problem i koaguleringsfasen, påverkas resten av processen och det är därför extra viktigt att detta går rätt till. Inom ostindustrin är det önskvärt att mjölken blir till en gel inom 40 minuter, eftersom det kostar rent ekonomiskt att vänta längre. Om mjölken inte blivit en gel inom denna tid kallas mjölken för icke-koagulerande mjölk. Den icke-koagulerande mjölken lämpar sig inte särskilt bra för ostproduktion eftersom icke-koagulerande mjölk inte bildar någon ostgel. Dock blandas mjölk från flera kor och gårdar på mejerierna och därmed är resultatet inte så drastiskt att det inte blir någon ost alls. Tidigare forskning har dock visat att den icke-koagulerande mjölken påverkar koaguleringsfasen även när den är uppblandad med annan mjölk. Detta leder till att mjölken koagulerar i en långsammare hastighet vilket leder till att det blir mindre mängd ost från mjölken.

Svensk röd och vit boskap (SRB) är den till antal näst största mjölkkorasen i Sverige. Rasen är populär bland lantbrukare då korna är friska och har höga halter av fett och protein i mjölken. Tyvärr har också SRB en hög andel kor som producerar icke-koagulerande mjölk. I denna avhandling visas det att runt en femtedel av korna från denna ras producerar mjölk som inte koagulerar inom den önskvärda tiden på 40 minuter. Genom att uppskatta hur mycket mjölk från SRB kor som används i svensk ostproduktion, beräknar man att den icke-koagulerande mjölken ger en stor ekonomisk förlust varje år. Men eftersom SRB kor är friska och har hög halt av fett och protein i mjölken, är det viktigt att försöka minska mängden icke-koagulerande mjölk från denna ras för att inte lantbrukare ska sluta satsa på SRB kor. Minskar mängden icke-koagulerande mjölk från SRB, kommer det göra att mjölken passar bättre till osttillverkning.

I denna avhandling har mjölkprover från runt 700 SRB kor samlats in och undersökts för att hitta en lösning på problemet med icke-koagulerande mjölk. Mjölken har bland annat använts för att försöka hitta skillnader i innehållet mellan mjölk som koagulerar och den som inte gör det för att försöka förklara varför mjölken inte koagulerar. Resultaten visar att det finns skillnader i sammansättningen mellan icke-koagulerande mjölk och mjölk som koagulerar, speciellt i proteinsammansättningen. Icke-koagulerande mjölk kunde kopplas till mindre andel kalcium och κ-kasein i mjölken. Dessutom hade den icke-koagulerande mjölken mer av proteinet α-lactalbumin som gärna binder till kalcium, vilket

därmed ger ännu mindre mängd obundet kalcium i mjölken vid koaguleringsfasen. Förutom olika typer av proteiner i mjölken så finns det även genetiska varianter av samma protein som har uppkommit genom mutationer. Även skillnader i dessa genetiska varianter kunde ses mellan den mjölk som koagulerade och den som inte gjorde det.

I denna avhandling gjordes även undersökningar om när i koaguleringsfasen som mekanismen inte fungerar i den icke-koagulerande mjölken. Det visade sig att när löpe, eller mer specifikt kymosin, tillsätts i den icke-koagulerande mjölken klipps κ-kasein på samma sätt och i liknande hastighet som i koagulerande mjölk. Därmed är det troligtvis när micellerna ska bindas samman till en gel som problemet uppstår.

Utöver mjölkprover samlades även blodprover från korna in. I blodproven kan man uppskatta hur mycket av variansen i sammansättning som ärvs mellan kor, det vill säga arvbarheten för dessa egenskaper. Genom att kombinera kunskapen om vilka komponenter i mjölk som det finns mer eller mindre av i icke-koagulerande mjölk med dessa komponenters arvbarhet, kan denna kunskap användas i avelsindustrin i selektiv avel. Därmed kan man styra aveln mot mjölk som har större sannolikhet att koagulera. Resultat i denna avhandling visar på att det finns möjligheter att till exempel avla mot mer mängd κ-kasein i mjölk och därmed indirekt avla mot mindre sannolikhet att mjölken är icke-koagulerande.

Sammanfattningsvis har forskningen i denna avhandling gjorts för att icke-koagulerande mjölk inte ska fortsätta att ärvas vidare utan att denna oönskade egenskap istället drastiskt ska minska. En minskning av icke-koagulerande mjölk skulle inte bara gynna ostindustrin, utan också leda till att stärka SRB rasen genom att få mjölk som passar till alla ändamål, men också genom att dessa friska kor kan fortsätta bidra till våra öppna landskap.

List of papers included in the thesis

I. **Characterisation of non-coagulating milk and effects of milk composition and physical properties on rennet-induced coagulation in Swedish Red Dairy Cattle** (2019). Nilsson K., Stålhammar H., Stenholdt Hansen M., Lindmark-Månsson H., Duchemin S., Fikse F., de Koning D.J., Paulsson M. and Glantz M. *International Dairy Journal 95:50-57*.

II. **Effects of milk proteins and post-translational modifications on non-coagulating milk from Swedish Red Dairy Cattle**. Nilsson K., Buhelt Johansen L., de Koning D.J., Duchemin S., Stenholdt Hansen M., Stålhammar H., Lindmark-Månsson H., Paulsson M., Fikse W. F. and Glantz M. *Under Review.*

III. **An investigation of the enzymatic cleavage of κ-casein in non-coagulating milk**. Nilsson K., Abdelghani A., Burleigh S., Buhelt Johansen L., Lindmark-Månsson H., Paulsson M. and Glantz M. *Under review.*

IV. **Genetic parameters for non-coagulating milk, milk coagulation properties, and detailed milk composition in Swedish Red Dairy Cattle**. Duchemin S., Nilsson K., Fikse W. F., Stålhammar H., Buhelt Johansen L., Stenholdt Hansen M., Lindmark-Månsson H., de Koning D.J., Paulsson M. and Glantz M. *Under review.*

The author's contribution to the papers

I. The author designed the study together with the co-authors, performed the experimental work, evaluated the data with some of the co-authors, wrote the paper and revised the paper together with the co-authors.

II. The author designed the study together with the co-authors, evaluated the data with some of the co-authors, wrote the paper and revised the paper together with the co-authors.

III. The author designed the study together with the co-authors, performed the experimental work together with some of the co-authors, evaluated the data with one of the co-authors, wrote the paper and revised the paper together with the co-authors.

IV. The author designed the study together with the co-authors, performed the experimental work together with some of the co-authors, wrote the paper with some of the co-authors and revised the paper together with the co-authors.

Abbreviations

General abbreviations

α-LA	α-lactalbumin
β-LG	β-lactoglobulin
σ_y	yield stress
AA	amino acid
Ca	calcium
CE	capillary electrophoresis
CFR	curd firming rate
CN	casein
CMP	caseinomacropeptide
DIM	days in milk
G’	gel strength (elastic modulus)
IMCU	international milk clotting units
LC-HRMS	liquid chromatography-high resolution mass spectrometry
NC	non-coagulating
NTM	Nordic total merit index
P	phosphate
PC	poor coagulating
PLS	partial least square
PTM	post-translational modification
RCT	rennet coagulation time
RDC	red dairy cattle
SCC	somatic cell count

Amino acid abbreviations

Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartate
Cys	Cysteine
Gln	Glutamine
Glu	Glutamate
Gly	Glycine
His	Histidine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
Phe	Phenylalanine
Pro	Proline
Ser	Serine
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine

Amino acid abbreviations will hereafter be used in all upcoming text.

Introduction

Background to present PhD thesis

Dairy products are a major food source worldwide and in 2018, the total cow milk production was 704 billion kg (International Dairy Federation, 2019). During the same year in Sweden 2,794,000 tonnes of milk were produced and from this, 82,000 tonnes of cheese were processed (International Dairy Federation, 2019). It takes about 10 kg of milk to produce 1 kg of cheese (Walstra et al., 2006), which means that about 30% of the milk in Sweden was used for cheese production. The coagulation ability of milk is crucial, as this is the first of many steps in cheese production and thereby provides the foundation for further processing. Cheese production is regulated by time, and therefore a rapid coagulation is desired as this is more likely to result in a firmer cheese gel before cutting the curd. A firmer gel leads to a higher yield, as a soft gel can lead to losses of fat and casein (CN) to the whey phase, when the curd is cut (Bynum and Olson, 1982). Arla Foods, the largest dairy company in Sweden, and the 7th largest worldwide (International Dairy Federation, 2019), has a major focus on sustainability and environmental impact. As part of this, there is a desire to minimise addition of additives during production. Thus, it is crucial to make sure that Swedish milk is of good quality for cheese production to ensure a satisfying yield and processing time.

Swedish Red Dairy Cattle (RDC) is a mixed breed of Swedish Red, Finnish Ayrshire, Norwegian Red, Danish Red and Canadian Ayrshire, and is the second largest dairy breed in Sweden, corresponding to about 35% of the total dairy cattle population. The Swedish RDC has a low frequency of stillborn calves and is associated with less udder diseases compared to other breeds (Växa Sverige, 2019), as well as less pregnancy losses compared to Swedish Holstein (Nyman et al., 2018). A recent study has shown that crossbreeding Nordic Red sires with Holstein dams improved health traits such as udder health, fertility, stillbirth and survival, compared to pure Holstein cows (Clasen et al., 2019). These positive health factors, together with high yields of protein and fat in the milk, contributes to its popularity in both the Nordic countries as well as worldwide (Växa Sverige, 2019).

The Swedish-Danish milk genomics initiative was a collaboration between Swedish and Danish universities and companies in 2009-2013. The objectives within the initiative were to combine milk properties and genetic information from three

Scandinavian breeds, Danish Holstein, Danish Jersey and Swedish Red, to identify markers that could be used in future breeding. The focus was on functionality, health properties, and milk composition where blood and milk samples were analysed from around 400 cows from each breed. Collaborating partners were Lund University, Swedish University of Agricultural Sciences, Aarhus University, VikingGenetics, Arla Foods and LRF Dairy Sweden. One of the projects within the Swedish-Danish milk genomics initiative was to determine rennet-induced coagulation ability of individual Swedish Red cows. During that project, it was found that a large fraction (18%) of the cows produced non-coagulating (NC) milk (Gustavsson et al., 2014a). If the frequency of NC milk in this breed is not decreased, there is a risk that Swedish RDC will reduce in competitiveness and that there will be a reduction in the demand for Swedish RDC semen worldwide. This will in turn have negative effects on the genetic conservation. Additionally, since Swedish RDC constitutes 35% of dairy cattle in Sweden, and 30% of the milk in Sweden is used for cheese production, it will also have economic losses for dairy companies. Therefore, it was decided by the Swedish universities together with the industry partners to start a new project that would investigate NC milk from Swedish RDC more thorough and discover possible explanations as well as future breeding goals to reduce the frequency of NC milk. The project was named "Genomic selection against non-coagulating milk" and this thesis is a part of that project.

Hypothesis

By sampling a larger set of cows from the Swedish RDC, it is possible to determine whether the frequency of cows producing NC milk is still high for this breed. Further, by analysing the composition of the milk and by comparing this with the coagulation ability, it is possible to distinguish certain compounds that are common or uncommon in NC milk, i.e. to find phenotypic markers for NC milk. The effect of these identified markers on nutrition and process properties can thereafter be theorised for possible selection strategies. Lastly, by combining the phenotypic information with genetic parameters in the milk, it is possible to give breeding suggestions to the industry that could reduce the frequency of NC milk, without having a negative effect on nutrition and processability of the milk.

Aim

The aim of this thesis has been to study the coagulation ability of milk from Swedish RDC, focusing primarily on NC milk. The overall aim is to use the obtained knowledge about milk traits in NC milk to be able to find markers that can be used in the breeding industry to reduce the frequency of cows producing NC milk. More specific aims have been to:

- determine the current frequency of cows producing NC milk within Swedish RDC (**paper I**)
- investigate the composition of NC milk to explore possible explanations of the NC milk behaviour (**paper I, II, III, IV**)
- further investigate the detailed protein composition of NC milk, including genetic variants and post-translational modifications (PTM; **paper II**)
- determine where in the coagulation process the inability of coagulation occurs, by investigation of the enzymatic cleavage of κ-CN (**paper III**)
- estimate heritability and genetic correlations for NC milk and milk composition (**paper IV**)
- identify markers in NC milk that can be used by breeding companies in order to reduce the frequency of the undesired NC trait (**paper I, II, III, IV**)

The results obtained in this thesis can be used to strengthen the competitiveness of Swedish RDC by ensuring profitable and multifunctional milk, useful for several dairy products. This contributes to an increased economic gain for stakeholders and a more sustainable dairy production.

Milk composition

Bovine milk is a nutritious fluid with an average composition of 4.0% fat, 3.3% protein, 4.6% lactose, 0.7% mineral substances and 0.2% organic acids (Walstra et al., 2006). The composition is, however, very individual and differs due to environmental, physiological and genetic factors. An example of an environmental factor is herd, where feeding practices and milking intervals differs between herds. Physiological differences are for example lactation stage and illnesses, while a genetic factor is breed. The composition of milk is important both for the nutritional value and for the processability (Walstra et al., 2006; Fox et al., 2015).

Protein composition

There are six main proteins in bovine milk, divided into whey proteins: α-lactalbumin (α-LA) and β-lactoglobulin (β-LG), and CN proteins: α_{s1}-CN, α_{s2}-CN, β-CN, and κ-CN. Within these proteins, there are variations in the amino acid (AA) sequences, resulting in different genetic variants of the same protein. All six proteins have a different number of known genetic variants, and the variants are not the same for all cow breeds (Caroli et al., 2009). Additionally, milk proteins are subjected to natural chemical processes where other molecules are bound to the protein, and these are called post-translational modifications (PTM). All CN proteins are subjected to different degree of phosphorylation and κ-CN can also have different number of glycosylation. PTM occurs on two AA, Ser and Thr (Farrell et al., 2004). Genetic variants and PTM of proteins mean that the protein profile between individual cows can have a large variation. Different genetic variants of milk proteins found in domesticated cattle (*Bos taurus*), together with average mass and UniProtID, can be seen in Table 1 (Farrell et al., 2004; Caroli et al., 2009; Martin et al., 2013; Huppertz et al., 2018).

Whey proteins

The two main whey proteins α-LA and β-LG constitutes about 14% of total milk proteins (Walstra et al., 2006). In milk, α-LA has an important part in the production of lactose by modification of the substrate specificity of the enzyme that forms lactose from glucose and galactose (Permyakov and Berliner, 2000; Farrell et al., 2004). Additionally, α-LA is known to have a strong binding site for Ca^{2+} (Hiraoka et al., 1980; Permyakov and Berliner, 2000; Walstra et al., 2006). α-LA in milk from *Bos taurus* cattle have two genetic variants, A or B, where variant B is the most common form (Caroli et al., 2009; Visker et al., 2012). The average mass for variant α-LA B is 14,186 Da (Swiss Institute of Bioinformatics, 2020a; Table 1).

Table 1. Descriptive information about the six most common milk proteins.
Differences in structure and mass between genetic variants that can be found in *Bos taurus* as well as their UniProtID.

Protein	Genetic variants	Average mass (Da)[1]	UniProtID	Abundant form
α-LA	A: Arg_{10} → Gln B: reference	14,158 14,186	P00711	α-LA B
β-LG	A: Gly_{64} → Asp; Ala_{118} → Val B: reference C: Gln_{59} → His D: Glu_{45} → Gln H: Gly_{64} → Asp; Lys_{70} → Asn; Ala_{118} → Val I: Glu_{108} → Gly J: Pro_{126} → Leu W: Ile_{56} → Leu	18,367 18,281 18,272 18,280 18,209 18,353 18,265 18,281	P02754	β-LG B
α_{s1}-CN	A: $Deletion_{14-26}$ B: reference C: Glu_{192} → Gly D: Ala_{53} → ThrP F: $SerP_{66}$ → Leu H: $Deletion_{51-58}$ I: Glu_{86} →Asp; Glu_{192} → Gly	22,055 23,614 23,542 23,664 (9P) 23,668 (7P) 22,684 23,700	P02662	α_{s1}-CN B 8P
α_{s2}-CN	A: reference B: $SerP_8$ → Phe C: Glu_{33} → Gly; Ala_{47} → Thr; Thr_{130} → Ile D: $Deletion_{51-59}$	25,228 25,208 (10P) 25,198 24,033 (8P)	P02663	α_{s2}-CN A 11P
β-CN	A^1: Pro_{67} → His; A^2: reference A^3: His_{106} → Gln B: Pro_{67} → His; Ser_{122} → Arg C: $SerP_{35}$ → Ser; Glu_{37} → Lys; Pro_{67} → His D: $SerP_{18}$ → Lys E: Glu_{36} → Lys; F: Pro_{67} → His; Pro_{152} → Leu G: Pro_{67} → His; Pro_{138} → Leu H^1: Arg_{25} → Cys; Leu_{88} →Ile H^2: Gln_{72} → Glu; Met_{93} → Leu I: Met_{93} → Leu	24,023 23,983 23,992 24,092 23,942 (4P) 24,025 (4P) 23,982 24,039 23,927 23,930 23,894 23,965	P022666	β-CN A2 5P
κ-CN	A: reference B: Thr_{136} → Ile; Asp_{148} → Ala C: Arg_{97} → His; Thr_{136} → Ile; Asp_{148} → Ala D: Arg_{97} → His E: Ser_{155} → Gly F^1: Asp_{148} → Val F^2: Arg_{10} → His; Thr_{136} → Ile; Asp_{148} → Ala G^1: Arg_{97} → Cys; Thr_{136} → Ile; Asp_{148} → Ala G^2: Asp_{148} → Ala H: Thr_{136} → Ile; I: Ser_{104} → Ala J: Thr_{136} → Ile; Asp_{148} → Ala; Ser_{155} → Arg	19,037 19,005 19,088 19,018 19,007 19,021 18,986 18,952 18,993 19,049 19,021 19,074	P022668	κ-CN A 1P

[1]The average masses are without the signal peptide and includes the most common phosphorylation form of that protein. Masses have been calculated using tools provided by Swiss Institute of Bioinformatics (Swiss Institute of Bioinformatics, 2020a; b).

β-LG is the most abundant whey protein (Farrell et al., 2004), and its AA composition has high nutritional value. Further, β-LG also has the ability to bind several molecules, such as vitamin D, fatty acids and retinoids (Farrell et al., 2004; Sawyer, 2013). For β-LG, at least eight different genetic variants have been found

in *Bos taurus* cattle (Table 1), where the two main variants are β-LG A and B (Farrell et al., 2004; Caroli et al., 2009). The average mass is 18,281 Da for β-LG B (Swiss Institute of Bioinformatics, 2020a) and 18,367 for β-LG A (Swiss Institute of Bioinformatics, 2020b; Table 1).

Casein proteins

The CN proteins have a role as ion carriers and sources of bioactive peptides in milk (Berry et al., 2014). With the proper amount of amorphous CaP nanoclusters, the CN proteins will aggregate into CN micelles (Holt et al., 2013), which will further be discussed in the next section. α_{s1}-CN constitutes about 40% of total CN content in milk (Huppertz et al., 2018) and have several genetic variants that can be found in *Bos taurus* cattle (A, B , C, D, F, H, and I) (Caroli et al., 2009). Additionally, α_{s1}-CN has two common phosphorylation isoforms 8 and 9 phosphate (**P**) (Farrell et al., 2004), and a more uncommon 7P isoform (Léonil et al., 1995). The reference protein is α_{s1}-CN B 8P (Farrell et al., 2004), that has an average mass of 23,614 Da (Swiss Institute of Bioinformatics, 2020a). About 10% of CN are α_{s2}-CN (Huppertz et al., 2018) and this is the most phosphorylated CN protein with isoforms of 9P-15P (Fang et al., 2016), and is also the most hydrophilic CN protein (Farrell et al., 2004). Additionally, four different genetic variants (A, B, C, and D) are known (Caroli et al., 2009) and the reference protein is α_{s2}-CN A 11P (Huppertz et al., 2018) with an average mass of 25,228 Da (Swiss Institute of Bioinformatics, 2020a).

β-CN is the second most abundant CN protein, and constitutes approximately 35% of CN (Huppertz et al., 2018). β-CN have several genetic variants in *Bos taurus* cattle which can be seen in Table 1. The two most common variants are A^1 and A^2, where β-CN A^2 5P is the reference (Huppertz et al., 2018), which has a mass of 23,983 Da (Swiss Institute of Bioinformatics, 2020a). The difference between A^1 and A^2 has been said to be one AA where A^1 has a His instead of Pro on position 67 (Farrell et al., 2004). However, there is currently a discussion weather also Glu on position 117 has switch place with Gln on position 175 and that Leu on position 137 has switched place with Pro on position 138, which is reported in the UniProtKB database (UniProtKB, 2020). However, as the AA have switched positions, this does not affect the mass and thereby not the quantification made in this thesis but may affect the structure of the protein. β-CN is the most hydrophobic of the CN proteins, affecting its positioning in the CN micelle (Farrell et al., 2004).

κ-CN differs from the other three by having a lower mass, being less phosphorylated and the only CN protein that can be glycosylated (Huppertz et al., 2018). Three phosphorylation forms are known, 1P to 3P and according to literature, κ-CN can be glycosylated with up to 6 glycans (Huppertz et al., 2018), where about 50% of the κ-CN molecules are glycosylated (Vreeman et al., 1986). There are five known glycans that can attach to κ-CN (Saito and Itoh, 1992; Farrell et al., 2004; Huppertz et al., 2018) and these can be seen in Table 2, together with structure, mass, and

reported relative percentage based on Saito and Itoh (1992). The labels in Table 2 will hereafter be used in this thesis to describe the glycans. Like β-CN, there are several genetic variants for κ-CN (Table 1), were the two most common ones are A and B that have a mass of 19,037 and 19,005 Da, respectively (Swiss Institute of Bioinformatics, 2020a; b).

Table 2. Glycans that can attach to κ-casein.
The labels for which the glycans will be referred to, the name, structure, mass, as well as reported percentage.

Label	Glycan	Structure	Mass (Da)	Reported %
a	GalNAc	Monosaccharide	221.2	0.8
b	Galβ(1-3)GalNAc	Disaccharide	383.3	6.3
c	NeuAcα(2-3)Galβ(1-3)GalNAc	Trisaccaride (linear)	674.6	18.4
d	Galβ(1-3)[NeuAcα(2-6)]GalNAc	Trisaccaride (branched)	674.6	18.5
e	NeuAcα(2-3)Galβ(1-3)[NeuAcα(2-6)]GalNAc	Tetrasaccaride	965.6	56.0

Abbreviations used: galactose (Gal), N-acetylglucosamine (GalNAc), and neuraminic acid (NeuAc).

Casein micelles

The CN proteins self-assembles into colloidal structures called CN micelles, which are roughly spherical with a diameter between 50-600 nm (Holt et al., 2003; Walstra et al., 2006; O'Mahony and Fox, 2014). The structure of the CN micelle has been studied for many years and several models have been suggested (e.g. Horne, 2006; Walstra et al., 2006; Dalgleish and Corredig, 2012; Holt et al., 2013; Huppertz et al., 2017). However, common knowledge of the micelles is that α_{s1}-CN, α_{s2}-CN, and β-CN are distributed in the micelle together with nanoclusters of amorphous CaP, while mainly κ-CN is on the surface of the micelle with hydrophilic parts dispersed into the serum phase. This layer of κ-CN result in steric and electrostatic repulsion between the micelles, preventing them from aggregating (Walstra et al., 2006; Huppertz et al., 2018). Further, CN micelles have a dynamic structure and composition that changes with external conditions such as temperature and pH, where the micelle and the serum phase exchange compounds (Walstra et al., 2006). Also, the CN micelle size differs with factors such as individual cow, protein content, genetic variants of proteins, and glycosylation of κ-CN (Devold et al., 2000; Bijl et al., 2014; Huppertz et al., 2018).

CN micelle size distribution can be measured using static light scattering, as was done in this thesis (**Paper I**). The advantages with the method are that it is fast, requires small volumes and the only pre-treatment needed is de-fatting of the milk. However, the drawback with the method is that large particles, for example remaining fat globules, may overlap smaller particles as a result of the static measurement, and thereby block the signal. As static light scattering measures the average intensity (which is proportional to the diameter) during the measurement, a large particle will affect the average intensity (Wishard and Gibb, 2019).

Analytical techniques for protein determination

Protein composition can be determined in several ways. In this thesis, liquid chromatography-high resolution mass spectrometry (LC-HRMS; **Paper II, III, IV**) and capillary electrophoresis (CE; **Paper III**) were used, and both methods have previously been used for investigation of milk proteins and peptides (Otte et al., 1997; Rauh et al., 2015; Jørgensen et al., 2016). The LC-HRMS was used to determine the content of different proteins in the milk samples as can be seen in Figure 1. LC separates proteins based on their affinity with the stationary phase of the column (Harris, 2010). Even though some gentic variants can be determined in the chromatograms from the LC (Figure 1), the LC was coupled to a MS were multiple variants and PTM can be detemined using the masses of each protein. A MS transformes molecules into gas-phase ions that are accelerated by an electric field and thereafter separates them based on mass-to-charge ratio. The mass-to-charge ratio is then processed in to a mass spectra (Harris, 2010). The mass spectras can thereafter be compared to databases with known protein masses (Steen and Mann, 2004). In this thesis, the database UniProtKB, which is a collaboration between European Bioinformatics Institute, Swiss Institute of Bioinformatics and Protein Information Resource, was used (UniProt Consortium, 2019).

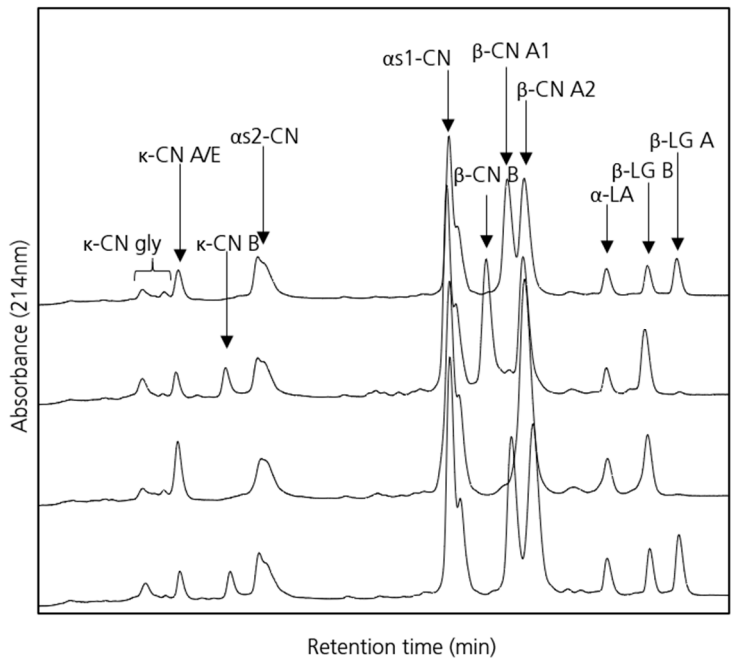


Figure 1 Chromatogram of proteins and genetic variants that can be detected using liquid chromatography. Four different samples are presented. Abbreviations used: casein (CN), glycosylated (gly), α-lactalbumin (α-LA), and β-lactoglobulin (β-LG).

Genetic variants can be determined in both milk and blood samples. By determining the genetic variants in milk, as was done in this thesis (**Paper II**), it is possible to determine the expressed amount of protein, instead of only determining the coding genes. This gives a more accurate result of genetic variants in each individual cow.

The CE was used to detect peptide products after chymosin cleavage in both coaulating and NC milk, as can be seen in Figure 2. In CE, proteins are separated by applying voltage and since different ions have different mobilites, the proteins will migrate through the capillary at different speeds (Harris, 2010). The protein is then separated based on differences in charge-to-mass ratio (Otte et al., 1997).

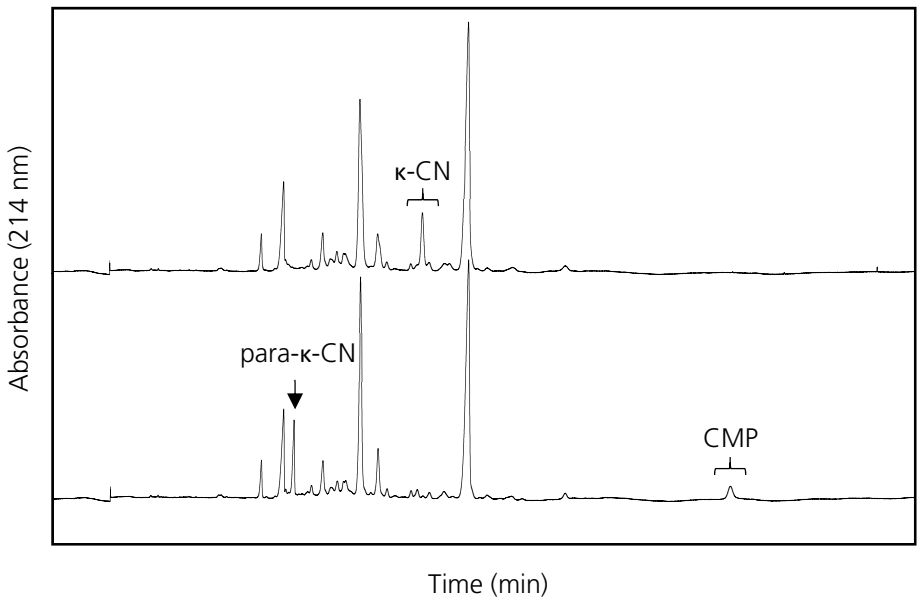


Figure 2 Electropherogram of the same milk sample without added chymosin (on the top) and with added chymosin (on the bottom).
The electropherogram is from Paper III. Abbreviations used: casein (CN) and caseinomacropeptide (CMP).

Cheese production

Cheese has been produced for thousands of years by either letting milk become sour or by adding rennet. Both these techniques will result in the aggregation of CN micelles together with fat, while water, lactose, and whey proteins are expelled in the whey (Walstra et al., 2006). Until the 19th century, cheese production was farm-based and is still produced at an artisanal level today. However, most cheeses are produced in large scales using highly developed techniques (Fox et al., 2017b). Even if there are many cheese varieties, several of the processing steps are general. The production of an industrial hard cheese is initiated by standardisation of the milk to control the

fat content. After this, the milk is pasteurised, in order to reduce microorganisms. Thereafter, the milk is acidified by addition of a starter culture, and rennet is added which initiate proteolysis. This will result in coagulation into a cheese gel that is eventually cut into cheese grains. The grains are thereafter moulded and often pressed, resulting in a fresh cheese, while the serum phase (whey) is expelled. The fresh cheese is usually salted and left for ripening (maturation). During ripening, the cheese develops its characteristic flavour and texture due to decomposition of milk fat, lactose and proteins. After ripening, the final product of a mature cheese is obtained (Fox et al., 2017b). This thesis has been focused on the first part of cheese production: the coagulation of milk into a cheese gel. This is the most critical production step as it provides a foundation for further processing.

Rennet-induced coagulation

Rennet is an enzyme mixture found in stomachs of ruminant animals and constitutes of two enzymes, chymosin and pepsin, in different ratios. Rennet is used to produce cheese from milk, were the first step is to add rennet and thereby start the rennet-induced coagulation process. The coagulation can be divided into two major stages, the enzymatic stage and the aggregation stage. In the enzymatic stage, chymosin cleaves κ-CN primarily at the bond between Phe_{105}-Met_{106}. Thereby, κ-CN is divided into two peptides, para-κ-CN (residues 1-105) and caseinomacropeptide (CMP; residues 106-169), where the CMP will diffuse away from the micelle to the serum phase (Walstra et al., 2006; Lucey, 2009; Fox et al., 2017b). This leads to a reduction in the zeta potential of the CN micelles and removal of the hydrophilic CMP, thus eliminating both the electrostatic and steric micelle-stabilizing factors. When approximately 85% of the κ-CN has been hydrolysed, the reduction in stability will lead to collisions between micelles (Fox et al., 2017b) and the aggregation stage starts, were the micelles start interacting with each other (Walstra et al., 2006; Lucey, 2009; Fox et al., 2017b). A basic drawing of the two stages can be seen in Figure 3.

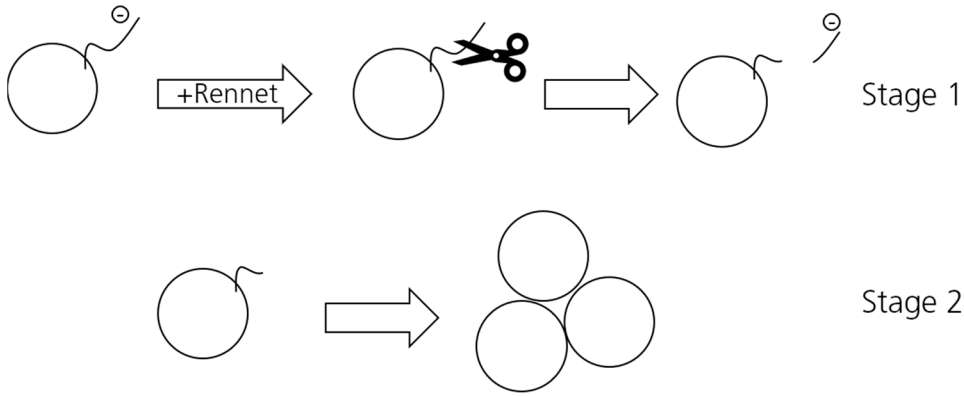


Figure 3. Basic drawing of the coagulation process.
The two stages of the coagulation process, the enzymatic stage (stage 1) and the aggregation stage (stage 2). In stage 1, κ-casein is cleaved into para-κ-casein and caseinomacropeptide by chymosin, causing the caseinomacropeptide to diffuse away in to the whey phase. Thereafter, the casein miceles will aggregate in to a gel in stage 2.

The two stages are affected by different physico-chemical properties. The enzymatic stage is affected by pH, ionic strength, temperature, rennet concentration, CN content and heat treatment of milk (Lucey, 2009; Fox et al., 2017b). The aggregation stage is also affected by temperature, pH, and content of CN, as well as by contents of ionic Ca and denatured whey proteins (Lucey, 2009).

Similar to milk composition, milk coagulation properties are influenced by both genetic and environmental factors such as breed, somatic cell count (SCC), protein and CN composition as well as stage of lactation (Okigbo et al., 1985; Tyrisevä et al., 2004; Cassandro et al., 2008; Bittante et al., 2012). Milk coagulation properties are also heritable and can be improved by selective breeding (Ikonen et al., 2004). Milk coagulation properties can be determined using several different techniques and can be either mechanical or optical (Troch et al., 2017). In this thesis, two different mechanical rheometer systems were used (**Paper I**). All samples were analysed in duplicates using free oscillation and half of the samples were randomly selected for further analyses using low amplitude oscillation. The advantage of using the free oscillation rheometer on all milk samples was the four sample chambers that could be analysed simultaneously. Thus, this instrument provided a time saving screening procedure where duplicates could be measured, yielding more reliable results. Another benefit with the free oscillation rheometer is the small sample requirement of only 1 mL. However, a drawback is that this instrument could not measure the stability of the obtained gel. This was instead done in the low-amplitude rheometer. By using two methods, the obtained results can also be compared to several other studies where one of the instruments have been used.

However, exact comparisons can rarely be made with rennet-induced coagulation studies due to different procedures.

From the free oscillation rheometer, the following parameters were obtained: rennet coagulation time (RCT), curd firming rate (CFR), and gel strength (G'). RCT can be determined using different approaches, such as the time after rennet addition to the onset of increase in viscosity (Amenu and Deeth, 2007). Another way is by measuring the elastic and viscous moduli obtained by measuring rheological properties. The elastic modulus describes milk behaviour as an elastic solid and can thereby be translated into gel strength, whereas the viscous modulus describes a viscous liquid. The relationship between these phases is described by the phase angle (δ), where the liquid phase dominates when $\delta > 45°$ and the solid phase when $\delta < 45°$. Therefore, RCT can be expressed as the time when milk goes from a viscous liquid to an elastic solid at $\delta = 45°$ (Horne and Lucey, 2017), and this definition was used from the free oscillation rheometer. CFR was defined as the rate of gel formation during the measuring time. An example curve from the free oscillation rheometer and the obtained parameters from the measurement can be seen in Figure 4.

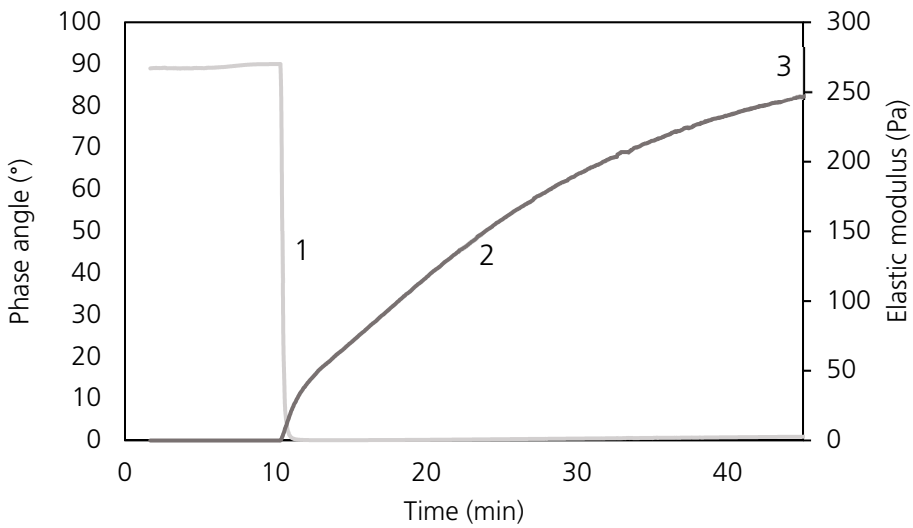


Figure 4. Example curve from the free oscillation rheometer.
Phase angle is light grey while elastic modulus is dark grey. Parameters obtained from the free oscillation rheometer are the following: 1. Rennet coagulation time = the time when the phase angle is 45°, 2. Curd firming rate = the rate of gel formation, 3. G'_{max} = maximum gel strength.

From the low-amplitude rheometer, the following parameters were obtained: RCT, G' and yield stress (σ_y). From this measurement, RCT was defined as when G' started to increase continuously. To investigate the firmness of the obtained gel, a stress sweep was preformed after the coagulation in the low-amplitude rheometer.

During the stress sweep, the shear stress was increased until the gel no longer remains solid and the gel thereby becomes liquid again. σ_y was defined as the shear stress when the viscosity reached 90% of the maximum recorded viscosity in the performed stress sweep. The obtained parameters will thereby explain different parts of the rennet-induced coagulation and contribute to an overall understanding of the process. Example curves from the gel formation and stress sweep in the low-amplitude rheometer can be seen in Figure 5. To summarise, by using two instruments and by measuring different parameters in the two systems, the coagulation study (**Paper I**) provided valuable information regarding the coagulation ability of milk from Swedish RDC.

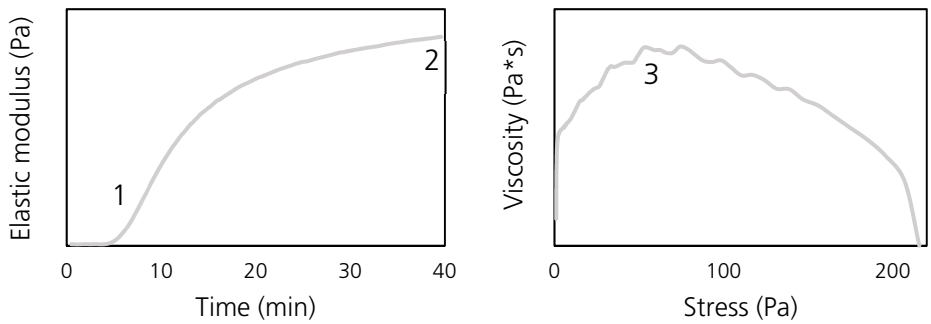


Figure 5. Example curves from the low-amplitude rheometer.
Coagulation curve to the left and stress sweep to the right. Parameters obtained from the low-amplitude rheometer are: 1. Rennet coagulation time = the time when elastic modulus starts to increase continously, 2. G'_{40} = gel strength after 40 minutes, 3. σ_y = the stress when 90% of maximum viscosity is obtained.

Non-coagulating milk

Even if rennet-induced coagulation is a result of bio-chemical processes in stomachs of ruminants, there is milk that does not coagulate after rennet addition, which is defined as NC milk. There is no universal definition of NC milk, and therefore studies differ when determining this trait and Table 3 shows some different definitions used for NC milk. It is not only definitions that varies between studies, but also the pre-treatment of the samples prior to analysis, as well as the processing parameters. For example, NC milk has been found in both skim (Frederiksen et al., 2011; Jensen et al., 2012; Gustavsson et al., 2014b) and whole milk (Ikonen et al., 2004; Cassandro et al., 2008; Malacarne et al., 2014), pH adjusted samples (Frederiksen et al., 2011; Jensen et al., 2012), and using preservatives (Gustavsson et al., 2014b). Further, the temperature during coagulation has varied between 32-35°C (Ikonen et al., 2004; Frederiksen et al., 2011; Gustavsson et al., 2014b) and both pure chymosin (Frederiksen et al., 2011; Jensen et al., 2012; Gustavsson et al., 2014b) and a mixture of chymosin and pepsin (Ikonen et al., 2004; Cassandro et al.,

2008) has been used. This makes it difficult to compare studies of NC milk and to determine an exact frequency of NC milk. However, since NC milk is found in several different studies, it proves that NC milk is a complex issue and that there should be a focus on minimising it.

Table 3. Definitions of non-coagulating (NC) milk samples used in different studies.
Definitions as well as techniques used.

Definition of NC samples	Technique used	Reference
RCT ≥ 40	Rheometry (ReoRox4)	Paper I
CFR = 0; G'_{max} = 0	Rheometry (ReoRox4)	(Jensen et al., 2012)
RCT > 40; CFR < 10; No curd	Rheometry (ReoRox4)	(Frederiksen et al., 2011)
RCT > 40; G'_{40} = 0	Rheometry (Stresstech)	(Gustavsson et al., 2014b)
RCT > 40; G'_{15} = 0; No curd after 30 min	Rheometry (Bohlin)	(Wedholm et al., 2006)
E30 = 0	Milk coagulation meter (Polotrade)	(Ikonen et al., 2004)
RCT ≥ 29.45, < 100; a_{30} < 100	Lactodynamograph (Formagraph)	(Malacarne et al., 2014)
RCT > 31	Computerised renneting meter (Polo Trade)	(Cassandro et al., 2008)

Abbreviations and units used: RCT = Rennet coagulation time (min); CFR = curd firming time (Pa/min); G' = gel strength (Pa); a = curd firmness (mm), where the subscript number means time after rennet addition in min. The studies that used "No curd" used oculary evaluation.

A common difference regarding the definition of NC milk is whether 30 or 40 min should be used as measuring time (Table 3). From an industrial perspective, 30 minutes is of course more beneficial as the process is optimised by time. However, by extending the measuring time and thereby the definition to 40 minutes, more definite NC samples will be detected, and the results will therefore be more reliable.

Using rheometry, as was done in this thesis (**Paper I**), NC milk samples can be distinguished by the lack of increase in gel strength during the time of the recording. Figure 6 shows examples of one coagulating sample and one NC milk sample, both recorded from the low-amplitude rheometer.

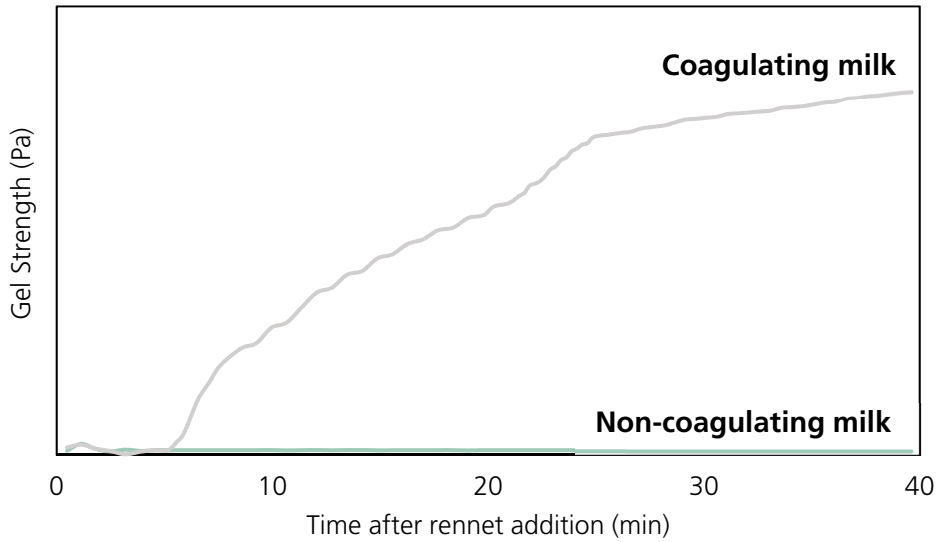


Figure 6. Coagulation curves from coagulating milk sample (grey) and non-coagulating milk sample (green). Gel strength was measured over time in a low-amplitude rheometer. Rennet coagulation time is determined as when the gel strength starts to increase continously, meaning that non-coagulating milk does not fulfill the requirment during the measuring time.

Most studies have investigated NC milk from individual cows, which makes it hard to know the consequences of NC milk in an industrial cheese production. However, in Malacarne et al. (2014) coagulation ability of herd bulk milk samples were investigated. They found that 6% of the herds, where SCC was < 400,000 cells/mL, produced NC milk, and as much as 35% of the herds when SCC was > 400,000 cells/mL (Malacarne et al., 2014). SCC is used as an indication for infections in cows and is often implemented as a factor in milk price at the dairies. Arla Foods will reduce the payment to the farmers when the SCC is > 301,000 cells/mL on farm level (Arla Foods, 2019). The results from Malacarne et al. (2014) show that some NC milk may be due to infections in the cows, but not all NC milk. Further, Frederiksen et al. (2011) investigated RCT and CFR of NC milk combined with well coagulating milk. With only 5% NC milk, the CFR started to decrease and with 25% NC milk, the RCT started to increase (Frederiksen et al., 2011). Thus, the two studies by Malacarne et al. (2014) and Frederiksen et al. (2011) indicate that NC milk is an issue in bulk milk and thereby also an issue in an industrial cheese production. However, no study has followed NC milk in blended bulk milk to determine where the CN from NC milk ends up, trapped in the cheese coagulum or disposed in the whey.

Cow genetics

Breeding and heritability

Breeding of dairy cattle is an essential factor for ensuring technological and nutritional quality of milk. In Sweden, Denmark, and Finland, there is a joint calculation of breeding values, known as Nordic total merit index (NTM). NTM describes the economic potential determined by genes, where cows with high NTM means a larger economic profit for the farmer. Some of the traits in NTM are fertility, udder health, and young stock survival (Nordic Cattle Genetic Evaluation, 2020). By breeding for certain phenotypes, there is a possibility to add value in the milk by for example breed for proteins with a high nutritional value for humans or breed for certain antibodies that can protect calves from colonisation of unwanted gut microbiota (Berry et al., 2014). However, the main focus in this thesis is processability of milk which will therefore be the main consideration.

Heritability can be defined as the strength of the relationship between genetic merit and observed performance (Berry et al., 2019). Heritability is calculated as the genetic variance divided by the phenotypic variance. However, as the phenotypic variance is also dependent on genetics, it is often expressed as the genetic variance plus the residual variance (**Paper IV**). Heritability impacts selection accuracy but a low heritability does not mean that the trait cannot be used in breeding, but suggests that more information about the trait is needed (Berry et al., 2019). By estimating the heritability of different phenotypic traits in milk, it gives an indication about how that phenotype can be used in breeding. Heritability of numerous compositional and productional traits have been estimated (e.g. Ikonen et al., 2004; Cecchinato et al., 2011; Sanchez et al., 2018), and an estimated heritability of 0.45 for NC milk has previously been reported (Gustavsson et al., 2014c). Thereby, it is suggested that NC milk is inherited and may therefore increase between generations if no actions are implemented.

Milk genomics

Sequencing of the bovine genome was finished in 2005. The sequencing allows for the field of milk genomics, where bovine genes are correlated to milk traits and the genes can thereby be used in genomic selection (Berry et al., 2014). Genomic selection enables to add more phenotypic traits in the breeding strategies, without having to determine the phenotype in each animal (Boichard and Brochard, 2012). Instead, genes coding for a specific phenotype can be identified and screened in dams or sires to determine which individuals to use in order to breed for a specific trait. The bovine genome consists of 29 autosomes plus the X and Y chromosomes, and over 26,000 genes are predicted (Berry et al., 2014).

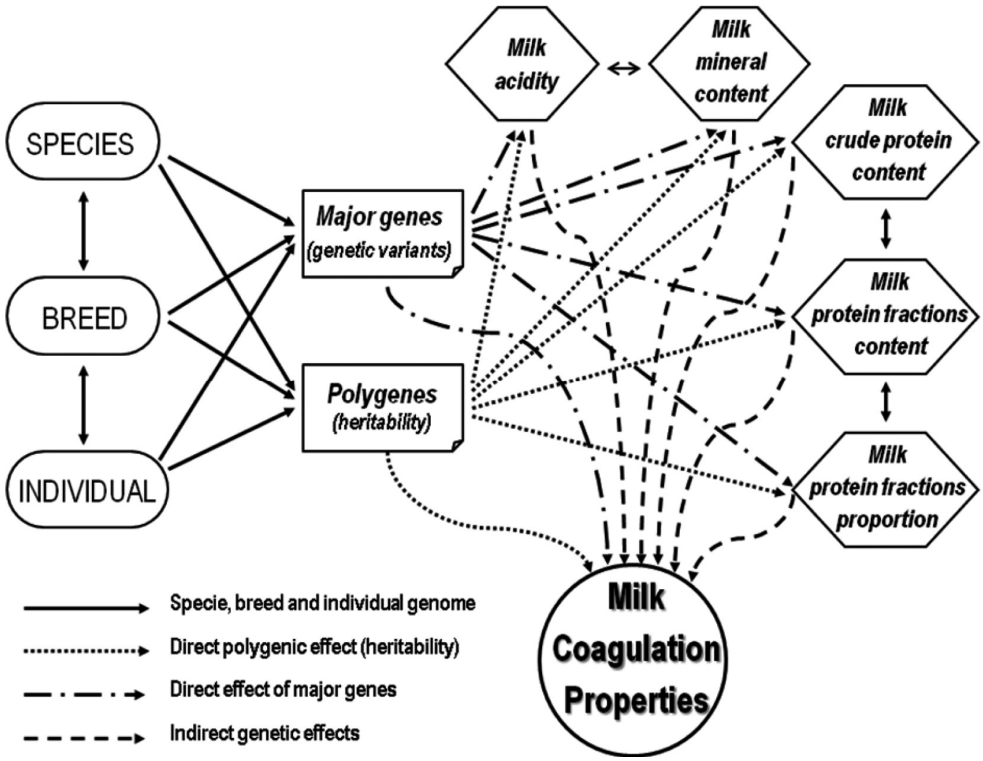


Figure 7. Direct and indirect genetic effects on milk coagulation properties.
The figure is from Bittante et al. (2012).

In order to modify milk protein composition in milk, detailed knowledge in both structure and genes is needed (Berry et al., 2014). The genes coding for the four CN proteins are closely linked in a 250-kilobasepair cluster on chromosome 6, while the genes coding for α-LA and β-LG are found on chromosome 5 and 11, respectively (Caroli et al., 2009; Martin et al., 2013). Figure 7 shows that protein composition, as well as other milk traits have both direct and indirect genetic effects on milk coagulation properties (Bittante et al., 2012), which can be used in breeding.

Prevalence of non-coagulating milk

Non-coagulating milk in Swedish Red Dairy Cattle

In this thesis, milk was collected from 724 cows from the Swedish RDC breed. In order to fully determine the frequency of NC milk samples, certain experimental conditions were applied. Prior to addition of chymosin, the milk samples were pH adjusted to 6.5 to standardise the pH-value, but also to chemically imitate the acidification of a starter culture (**Paper I**). A lower pH value has previously been linked with faster coagulation (Nájera et al., 2003), which means that the NC milk samples obtained would not be due to a too high pH. Additionally, the concentration of added chymosin (CHY-MAX Plus, Chr Hansen A/S, Hørsholm, Denmark) was 0.09 international milk clotting units (IMCU)/mL milk sample, which is higher than normally used in cheese production. The recommended dosage of CHY-MAX Plus is between 0.03-0.06 IMCU/mL milk (Chr Hansen, 2013). A higher concentration of rennet reduces coagulation time (Bittante et al., 2012). By this, the high frequency of NC milk samples can also be discarded as being an effect of low rennet concentration.

Even when these conditions were applied, 18.1% of the milk samples could still be defined as NC and 18.9% as poor coagulating (PC). That results in a total of 37% of the milk being unsuitable for cheese production (**Paper I**). As explained earlier, definition of NC milk often varies between 30 or 40 min. In this thesis, 40 min was used, which again was to determine samples that had extreme NC behaviour. If the definition would have been lowered to 30 min, 21.2% instead of 18.1% would have be classified as NC milk. The high values of NC and PC milk are concerning for cheese production and shows that Swedish RDC is still a breed that has large amounts of milk that does not coagulate. However, it is hard to conclude the real effect of this high frequency in an industrial cheese production, since this has not been studied. Nevertheless, Frederiksen et al. (2011) showed that blending NC milk with well coagulating milk in different proportions results in impaired coagulation properties of bulk milk (Frederiksen et al., 2011). The high frequency of NC milk should therefore be treated as a serious issue and both dairies and breeding companies should focus on minimisation of NC milk.

Frequencies of NC milk found in other breeds are 18% in Red Danish 1970 (Poulsen et al., 2016), 16% in crossbreed of Swedish Red and Holstein (Malchiodi et al., 2014), 13% in Finnish Ayrshire (Ikonen et al., 2004), 6% in Alpine Grey, 4% in

Simmental (Stocco et al., 2017), 10% in Italian Holstein-Friesian and 4% in Brown swiss (Cecchinato et al., 2011), to name a few. However, the differences in frequencies cannot be compared directly as there are too many differences in the studies but provides an indication of the differences. Even so, Nordic red breeds, such as Swedish RDC, Danish Red 1970 and Finnish Ayrshire, seems to have higher frequencies than other breeds. Swedish RDC and Finnish Ayrshire are also related as they are both part of the VikingRed breeding program. Tyrisevä et al. (2003) reported that most of the cows that produced NC milk in their study, had common ancestors from Finnish Ayrshire, Norwegian Red and Canadian Ayrshire (Tyrisevä et al., 2003), which also shows the relationship between the breeds. To strengthen the competitiveness of the Nordic reds breeds in global breeding, it is important to reduce the high NC frequencies. Other studies have found both genetic and phenotypic correlations with NC milk and milk composition (Hallén et al., 2010; Bittante et al., 2012; Gustavsson et al., 2014c; Poulsen et al., 2016), suggesting that NC milk is caused by both genetic and environmental factors. Further, there have also been studies investigating chromosomes connected to NC milk, showing that a few chromosomes have association with NC milk (Tyrisevä et al., 2008; Duchemin et al., 2016). This suggests that there is potential in breeding against NC milk and this thesis has shown that the estimated heritability is 0.28 for NC milk (**Paper IV**).

Farm distribution

Even if NC milk has been a studied subject in different breeds, investigation of herd distribution is scarce. 31 farms were sampled in this project and the frequency of NC milk differed between them, which can be seen in Figure 8 (**Paper I**). As explained before, the current project is a continuation of the Swedish-Danish Milk Genomics Initiative where the coagulation ability in milk from Swedish Red cows was investigated. Even if the same breed was analysed, there was no overlap between farms in the two studies.

From Figure 8, it is clear that the distribution varies a lot between the farms, from 0% on five farms, up to almost 40% NC milk samples on one farm. The cows in this thesis had similar genetic background, as they all use sires from VikingGenetics in their breeding, which was one of the reasons they were chosen. The difference between farms despite similar genetic background is an indicator that the NC milk trait is a combination of both environmental and genetic factors. Tyrisevä et al. (2004) presented the frequency of cows producing NC milk from 20 of their 125 investigated herds. The results from those 20 herds showed that the frequency varied from 0-29% (Tyrisevä et al., 2004). Although Tyrisevä et al. (2004) had different breed distributions in the herds, it corresponds well with the theory that NC milk can be caused by a combination of genes and environment.

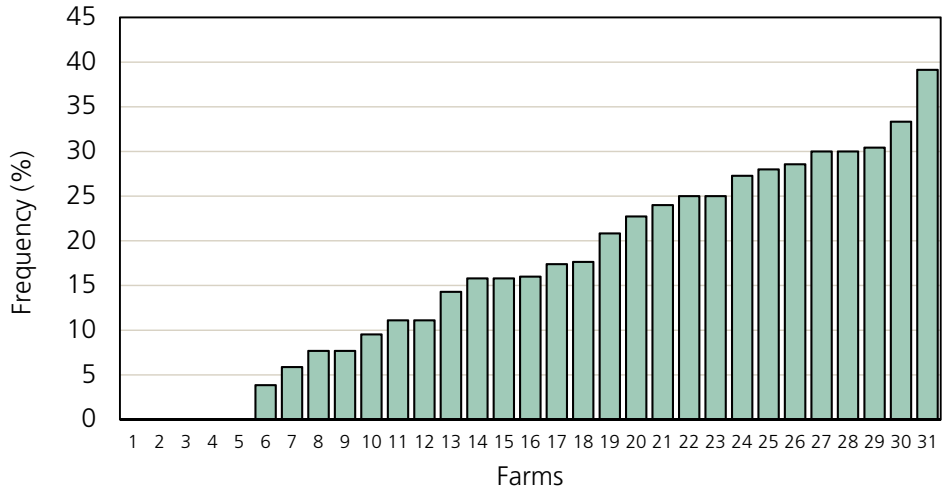


Figure 8. Distribution of cows producing non coagulating milk over the studied farms.
The graph is addapted from Paper I.

Lactation and parity distribution

Other studies have suggested that NC milk is connected to parity and lactation of the cow (Ikonen et al., 2004; Wedholm et al., 2006; Malchiodi et al., 2014). Malchiodi et al. (2014) showed that the number of cows producing NC milk increased during lactation until day 240, when the frequency started to decrease again. Ikonen et al. (2004) also reported that the frequency of NC milk increased during lactation, but they observed a decrease after 210 days in milk (DIM). Additionally, the frequency of NC milk was higher than the average frequency between 91-210 DIM (Ikonen et al., 2004). Wedholm et al. (2006) found an association with less NC and PC samples in very late lactation, i.e. after 316 DIM. The distribution of NC milk over lactation in this thesis shows that the highest frequency was found with cows between 181 and 210 DIM (Figure 9). The frequency was also higher than the average of 18% between 91 and 210 DIM, agreeing with Ikonen et al (2004).

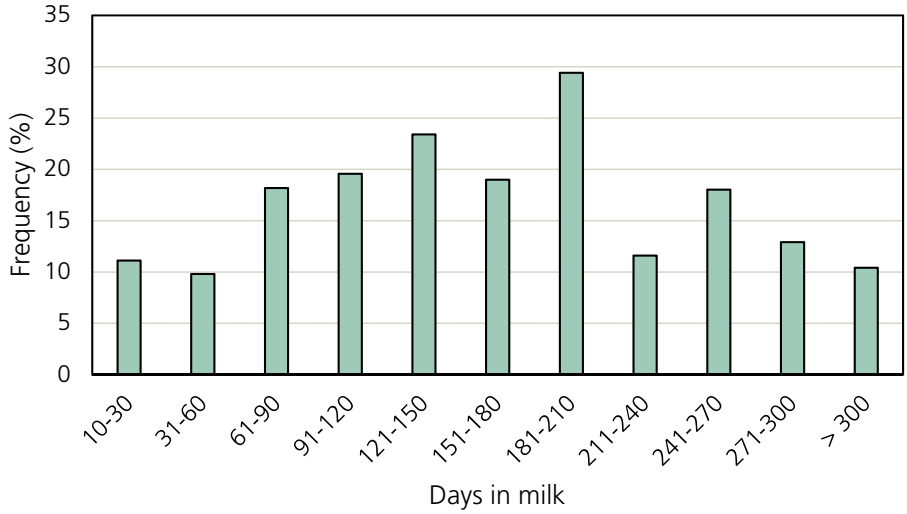


Figure 9. Frequency of non-coagulating samples distributed over lactation.

Tyrisevä et al. (2003) re-sampled cows multiple times during lactation. They observed that cows producing NC milk could be divided into two groups, with half of the cows in each group: cows that produced NC milk a few times and cows that almost always produced NC milk through lactation. The cows that only produced NC milk occasionally was in peak- or mid-lactation (Tyrisevä et al., 2003). This thesis was focused on individual cows and therefore each cow was sampled once. However, two cows producing NC milk were re-sampled and both cows produced NC milk again at the second sampling. On the first sampling they were 74 respectively 71 DIM and on the second occasion 129 respectively 127 DIM.

Regarding parity, Malchiodi et al. (2014) showed that there was a higher frequency of cows producing NC milk in parity 2 compared to parity 1 and ≥ 3. Wedholm et al. (2006) found an association with more NC and PC milk samples in parity 1. Figure 10 shows the distribution of NC milk samples in different parity in this thesis. The results agree with Malchiodi et al. (2014), however, the frequency in parity 1 and 2 are almost the same.

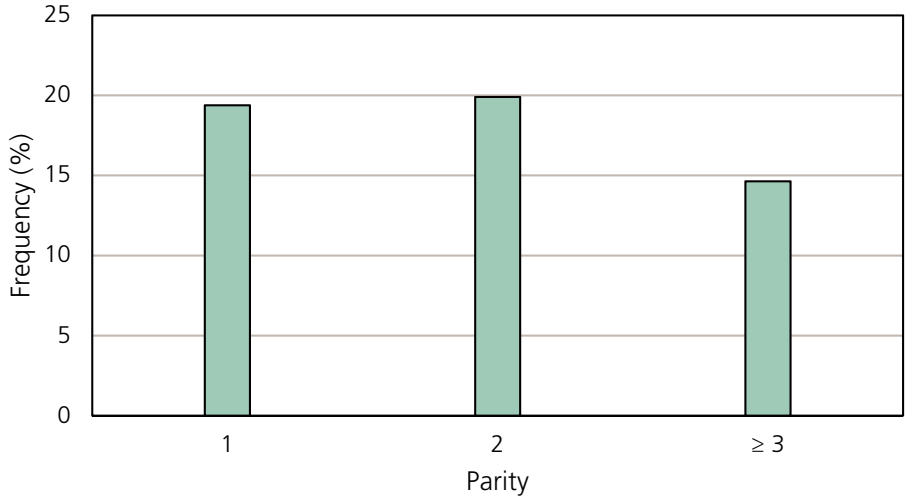


Figure 10. Frequency of non-coagulating milk samples distributed over parity.

Even if there are some visual differences in the NC frequency between farm, lactation and parity in Figures 8, 9, and 10, no significant effect from herd, DIM or parity was detected in the generalised linear model in **Paper II**, where instead strong effects were found from protein composition. The strong effect from protein composition might be dominating in the model, and therefore there might still be effects from herd, DIM, and parity, but a smaller effect. As previously mentioned, herd and lactation stage has an impact on milk composition and thus protein content (Walstra et al., 2006), and will therefore have indirect effects on coagulation as well.

Composition of non-coagulating milk

In this thesis, several compositional parameters were measured and their relation to NC milk was evaluated in different ways. In **Paper I**, milk composition was compared between NC, PC and coagulating milk using a general linear model, where coagulation group was added as x-variable and the compositional traits were the y-variables. Additionally, the Pearson correlations with a binary NC milk trait was calculated. In **Paper II**, protein profile was determined and its impact on NC milk was evaluated using a generalised linear model where a binary NC trait was the y-variable and the protein amounts were x-variables. **Paper III** was focused on differences between extreme samples, i.e. samples that did not coagulate and samples with the shortest coagulation time. In **Paper III**, mean values between these two extreme groups were compared. In **Paper IV**, both genetic and phenotypic correlations with a binary NC trait were estimated. A summary of compositional parameters measured in the different papers can be seen in Table 4.

From Table 4, it becomes clear that NC milk is a complex issue that is influenced by several parameters. Most of the parameters investigated could be linked with NC milk in at least one of the papers. However, milk composition is complex and components in the milk are dependent on each other and some parameters might therefore only have indirect effects. During the work of this thesis, some of the parameters showed more prominent linkage with NC milk (Table 4) and will therefore be further discussed. Those parameters are: κ-CN, α-LA, as well as total and ionic Ca. Additionally, genetic variants of proteins that were determined in **Paper II** will also be addressed.

Table 4. Summary of different compositional parameters.
Parameters measured in this thesis, in which paper they were investigated, as well as eventual relation of parameter with non-coagulating (NC) milk.

Parameter	Paper	Relation with non-coagulating milk
Milk yield	I, III	Significant effect of coagulation group (Paper I) Significant difference between extreme coagulation samples (Paper III)
SCC	I, IV	No
Fat content	I, III, IV	Significant phenotypic correlation (Paper I & Paper IV)
Lactose content	I, III, IV	Significant phenotypic correlation (Paper I) Significant difference between extreme coagulation samples (Paper III)
Protein content	I, III, IV	Significant difference between extreme coagulation samples (Paper III)
CN content	I, III, IV	Significant difference between extreme coagulation samples (Paper III)
Ratio CN to protein	I, III	Significant phenotypic correlation (Paper I) Significant difference between extreme coagulation samples (Paper III)
α_{s1}-CN content	II, III, IV	Significant effect from α_{s1}-CN 8P on NC milk (Paper II) Significant difference between extreme coagulation samples (Paper III)
α_{s2}-CN content	II, III, IV	Significant genetic correlation (Paper IV)
β-CN content	II, III, IV	Significant effect on NC milk (Paper II) Significant difference between extreme coagulation samples (Paper III) Significant phenotypic correlation (Paper IV)
κ-CN content	II, III, IV	Significant effect from total κ-CN and κ-CN 1P U.G. on NC milk (Paper II) Significant difference between extreme coagulation samples (Paper III) Significant phenotypic correlation (Paper IV) Significant genetic correlation (Paper IV)
para-κ-CN content	III	Significant difference between extreme coagulation samples (Paper III)
CMP content	III	No
Sum of whey proteins	IV	No
α-LA content	II, III, IV	Significant effect on NC milk (Paper II) Significant difference between extreme coagulation samples (Paper III) Significant phenotypic correlation (Paper IV) Significant genetic correlation (Paper IV)
β-LG content	II, III, IV	Significant effect on NC milk (Paper II) Significant phenotypic correlation (Paper IV)
Total calcium content	I, III, IV	Significant effect of coagulation group (Paper I) Significant phenotypic correlation (Paper I & Paper IV) Significant difference between extreme coagulation samples (Paper III) Significant genetic correlation (Paper IV)
Ionic calcium content	I, III, IV	Significant effect of coagulation group (Paper I) Significant phenotypic correlation (Paper I & Paper IV) Significant difference between extreme coagulation samples (Paper III)
Citric acid content	I, III, IV	No
pH	I, III, IV	No
CN micelle size	I, III, IV	No
Titratable acidity	I	Significant phenotypic correlation (Paper I)

Abbreviations used: somatic cell count (SCC), casein (CN), caseinomacropeptide (CMP), un-glycosylated (U. G.) α-lactalbumin (α-LA), β-lactoglobulin (β-LG).

Protein profile

Since the ratio of CN to protein could be correlated to NC milk, but not total protein or CN content (**Paper I**), it suggested that a more detailed protein composition would reveal valuable information, and the protein profile of the milk samples was therefore measured (**Paper II**). Several genetic variants and PTM were found in the milk samples, even if the results were limited to the most abundant proteins (**Paper II**). Figure 11 and Figure 12 shows which proteins that were detected in coagulating and NC samples, respectively.

Comparisons between Figure 11 and Figure 12 shows that less genetic variants and PTM could be detected in the NC milk samples, compared to coagulating samples. For the genetic variants, it was only β-CN B and I that were not found in the NC milk samples. Regarding both α_{s2}-CN and κ-CN, NC milk did not contain the highest phosphorylation isoforms, 14P and 3P respectively. Additionally, some of the glycosylation isoforms of κ-CN could also only be detected in coagulating milk samples. These were variant A 1P 1G (c/d), variant E 1P 2G (c/d, e), variant E 1P 2G (c/d, e), and variant E 2P 1G (e). However, the proteins that were not found in NC samples were only found in a small amount (five samples or less) of the coagulating samples (**Paper II**).

The investigation of protein profile using a generalised linear model showed that α-LA, β-LG, α_{s1}-CN 8P, β-CN, and κ-CN contents had significant impact on a binary NC milk trait. Odds ratios revealed that higher α-LA and β-CN contents in the milk increased the odds of having NC milk, while higher β-LG, α_{s1}-CN 8P, and κ-CN contents had the opposite effect (**Paper II**). Further, comparison of protein contents in a subset of extreme samples showed that there were significant differences in α_{s1}-CN, β-CN, κ-CN, and α-LA contents (**Paper III**). Additionally, α-LA and κ-CN contents had both phenotypic and genetic significant correlations with NC milk, while β-LG and β-CN contents only had phenotypic correlations and α_{s2}-CN only had a significant genetic correlation with NC milk (**Paper IV**). This suggests that the different proteins are contributing in different ways in NC milk, but α-LA and κ-CN seems to be more prominent than the other proteins. Linkage between higher α-LA and lower κ-CN contents with NC milk agrees with other studies (Hallén et al., 2010; Jensen et al., 2012; Gustavsson et al., 2014c; Poulsen et al., 2017). As mentioned earlier, α-LA has a strong binding site for Ca ions and a higher α-LA content could therefore inhibit the Ca ions to participate in the aggregation stage. However, only a significant phenotypic negative correlation between total Ca and α-LA, and no correlations between ionic Ca and α-LA was found in this thesis (**Paper IV**).

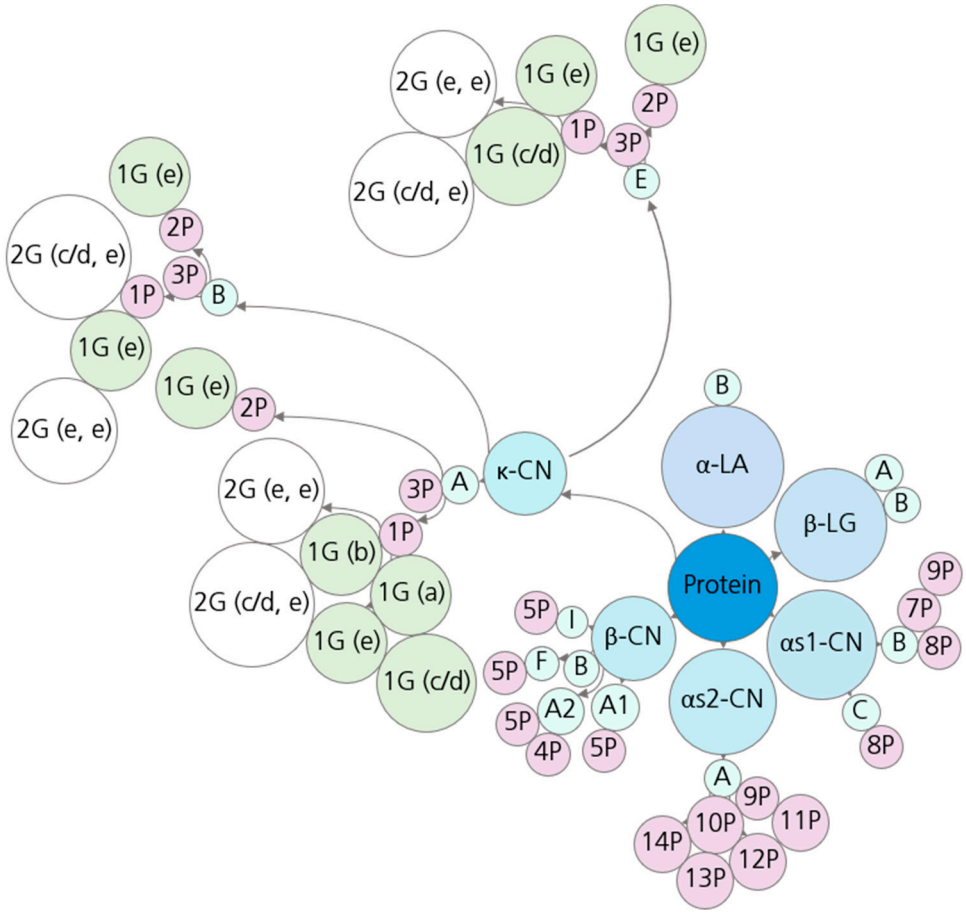


Figure 11. Proteins detected in the coagulating milk samples.
Abbreviations used: casein (CN), α-lactalbumin (α-LA), β-lactoglubulin (β-LG), phosphorylation (P), and glycosylation (G). The letter in the light blue circles shows genetic variants while the letter in the bracket in the green circles refers to the label of each glucan, according to Table 2.

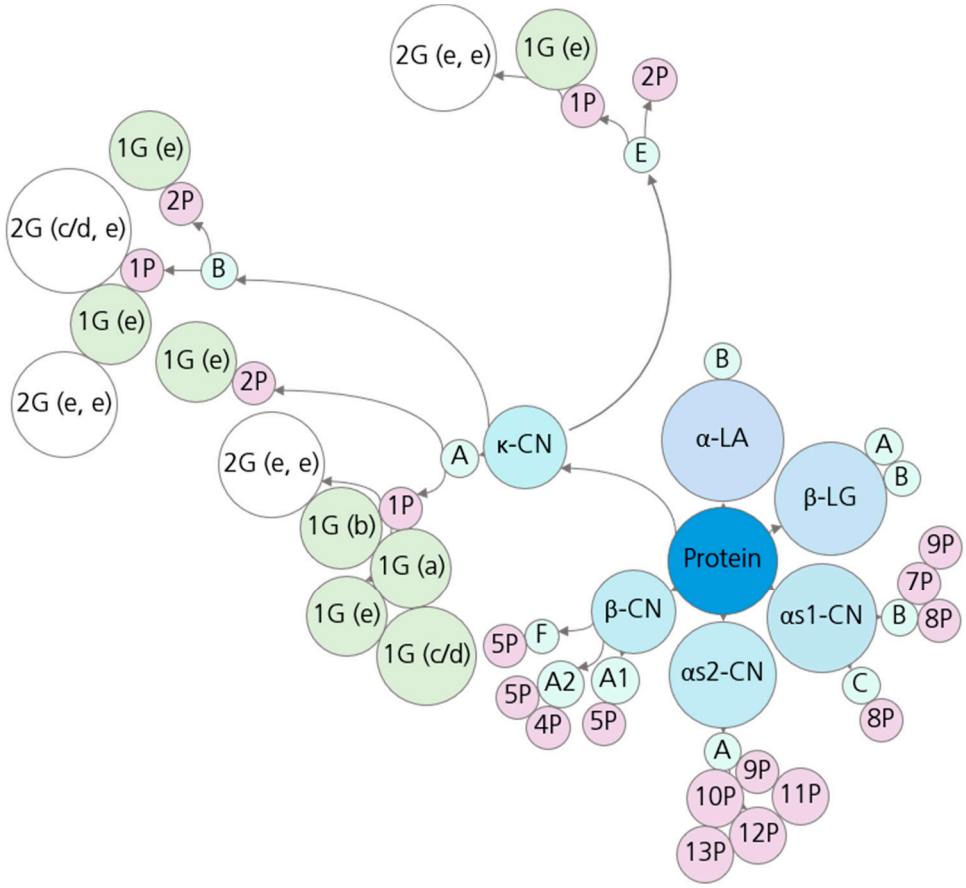


Figure 12. Proteins detected in the non-coagulating milk samples.
Abbreviations used: casein (CN), α-lactalbumin (α-LA), β-lactoglubulin (β-LG), phosphorylation (P), and glycosylation (G). The letter in the light blue circles shows genetic variants while the letter in the bracket in the green circles refers to the label of each glucan, according to Table 2.

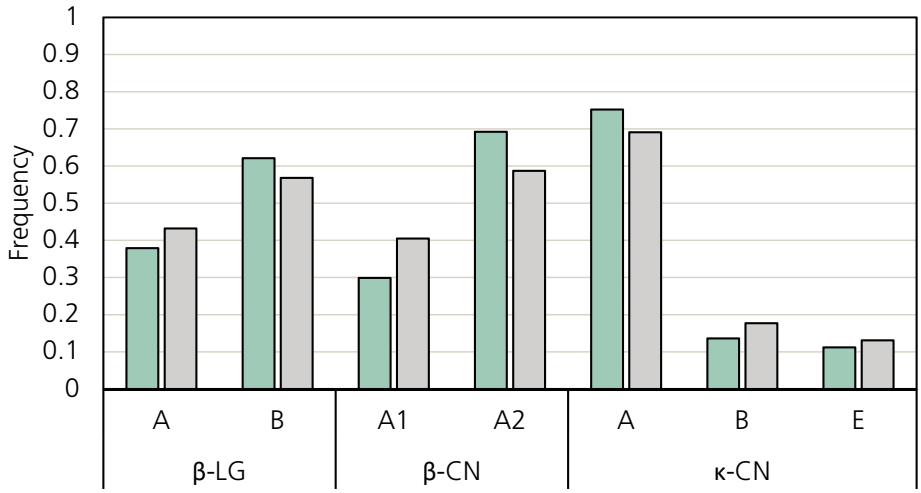


Figure 13. Allele frequencies of the most abundant alleles found.
Frequencies divided into coagulating (grey) and non-coagulating milk (green). Abbreviations used: casein (CN) and β-lactoglubulin (β-LG). The graph is adapted from data in Paper II.

Alleles and genotypes in the milk samples were also investigated and compared between NC and coagulating milk (**Paper II**), as well as a subgroup of extreme NC and coagulating samples (**Paper III**). Figures 13 and 14 shows the allele and genotype distribution, respectively, in NC and coagulating samples, where only alleles and genotypes found in >4% of the samples are presented.

Largest differences for the alleles can be seen for β-CN A^1 and β-CN A^2, where NC milk had higher A^2 and lower A^1 frequencies (Figure 13). Higher frequency of β-CN A^2 in NC milk agrees with other publications (Jensen et al., 2012; Gustavsson et al., 2014a; Poulsen et al., 2017), and investigation of the structural differences between A^1 and A^2 suggests that they have different behaviour as they may self-assemble differently (Raynes et al., 2015). When the alleles were added to a partial least square (PLS) plot, β-CN A^2 were plotted on the same side as the NC milk samples, but not very close. Instead, the coagulation group was connected to component 1 and the β-CN alleles with component 2 (**Paper III**).

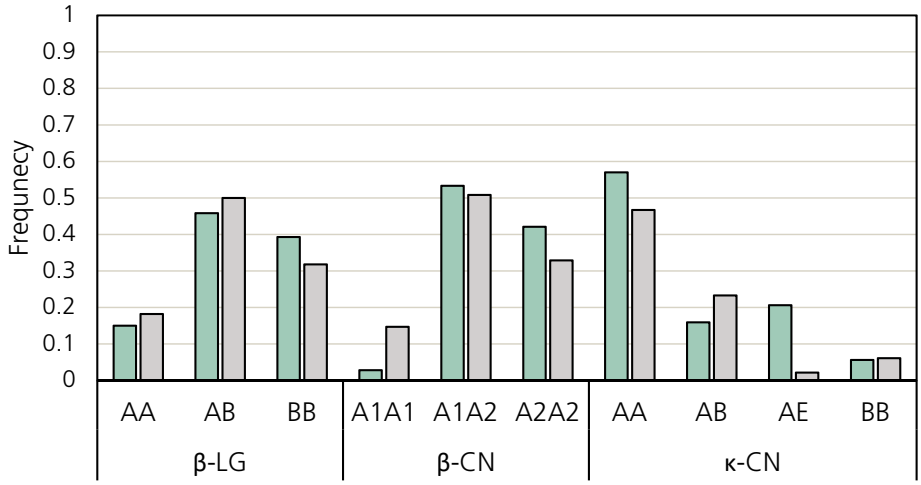


Figure 14. Genotype frequencies of the most abundant genotypes found.
Frequencies divided into coagulating (grey) and non-coagulating milk (green). Abbreviations used: casein (CN) and β-lactoglubulin (β-LG). The graph is adapted from data in Paper II.

There seems to be larger differences between the genotypes of the two groups, then for the alleles (Figure 13 and Figure 14). The largest difference between NC and coagulating milk samples regarding genotypes can been seen for β-CN A^1A^1 and κ-CN AE, where NC milk has less β-CN A^1A^1 and more κ-CN AE than coagulating milk (Figure 14). However, genotype had no significant impact on NC milk, but had a significant impact on protein amount (**Paper II**). It is therefore believed that genotype has an indirect effect on NC milk by determining the amount of expressed protein, but not a direct effect. From the generalised linear model in **Paper II** and the PLS in **Paper III**, it was concluded that genetic variants are important for NC milk, but the influence of them are not as strong as other parameters in milk and might mostly be influencing indirectly. However, β-CN variants could still be used in selective breeding as a strategy to reduce the frequency of NC milk, due to the indirect effects.

Calcium content

NC milk had less total and ionic Ca contents compared to milk that coagulated (**Paper I**). This is in agreement with other studies of NC milk (van Hooydonk et al., 1986; Gustavsson et al., 2014b). Due to the finding of less Ca in NC milk, it has previously been suggested that $CaCl_2$ addition to milk prior to coagulation, could be the solution to reduce NC milk. An addition of 0.02% $CaCl_2$ is sometimes added, which corresponds to about 40 mg Ca/L milk (Fox et al., 2017a). The difference of mean values of total Ca content in NC and coagulating milk was 135 mg/kg (**Paper I**) which is roughly 139 mg/L, with a milk density of 1.029 kg/L (Walstra et al., 2006). Thus, the addition of 0.02% $CaCl_2$ would not result in the same amount of total Ca in NC milk as in coagulating milk.

Ca content, among other minerals in milk, has been suggested to be caused by both environmental and genetic factors. Environmental factors are for example lactation, parity, and feeding (Tsioulpas et al., 2007; van Hulzen et al., 2009; Gaignon et al., 2018), while genetic factors are for example breed (Buitenhuis et al., 2015; Gaignon et al., 2018). A study by van Hulzen et al. (2009) showed that the intraherd estimated heritability was much higher than the herd variance (0.57 compared to 0.14). They therefore concluded that Ca content is mostly affected by genetic factors (van Hulzen et al., 2009). In this thesis, herd had a significant impact on total and ionic Ca, while total Ca also was impacted by parity and DIM (**Paper I**). Additionally, a strong estimated heritability of ionic Ca ($h^2 = 0.60$), but a non-significant heritability for total Ca was found (**Paper IV**). van Hulzen et al. (2009) speculated that the genetic variation of Ca secretion could be linked with the number of phosphorylated CN (van Hulzen et al., 2009). However, this has not been confirmed. Buitenhuis et al. (2015) has identified a quantitative trait locus for Ca on chromosome 14 (Buitenhuis et al., 2015), which could be used in breeding programs.

Coagulation process

To understand the mechanisms causing NC milk, it is of interest to know in which step the coagulation process is faulty. To determine this, the enzymatic cleavage of κ-CN to para-κ-CN and CMP was investigated in milk samples with extreme good and bad coagulation abilities (**Paper III**).

Previous studies have also investigated the enzymatic cleavage of NC milk samples but in a lower number of samples. They have also only investigated if either para-κ-CN or CMP could be detected and did not investigate the amounts detected. However, the studies implied that the enzymatic stage is occurring in NC milk (Hallén et al., 2010; Frederiksen et al., 2011). In this thesis, both para-κ-CN and CMP were detected after chymosin addition, while κ-CN was notably reduced (**Paper III**). Thereby, it is strongly believed that the enzymatic stage is not the cause of NC milk, but more likely, the aggregation stage. Figure 15 shows an example of the detection of para-κ-CN and CMP peaks in two coagulating and two NC milk samples.

As mentioned, the aggregation stage is affected by temperature, pH, as well as contents of CN, ionic Ca and denatured whey proteins (Lucey, 2009). In this thesis, coagulation experiments were always conducted at the same temperature, 32°C, and the same pH of 6.5. Therefore, the effect of these two parameters is neglected. Further, both total and different CN contents have been investigated as well as total and ionic Ca, which has been discussed before. The likeliness that NC milk is cleaved enzymatically but does not aggregate therefore strengthens the hypothesis that CN proteins and Ca plays a major role in the inability of the milk to coagulate. Ca content has a significant contribution in rennet-coagulation, as it links the hydrolysed CN micelles with each other (Corredig and Salvatore, 2016). However, ionic Ca content is influenced by pH (Walstra et al., 2006), and ionic Ca was in this thesis measured prior to pH adjustment, which can thereby influence the actual amount of ionic Ca during the coagulation measurements. At the same time, NC milk had lower amount of total Ca (**Paper I, III**), and it is therefore reasonable to assume that NC still will have less ionic Ca after pH adjustment.

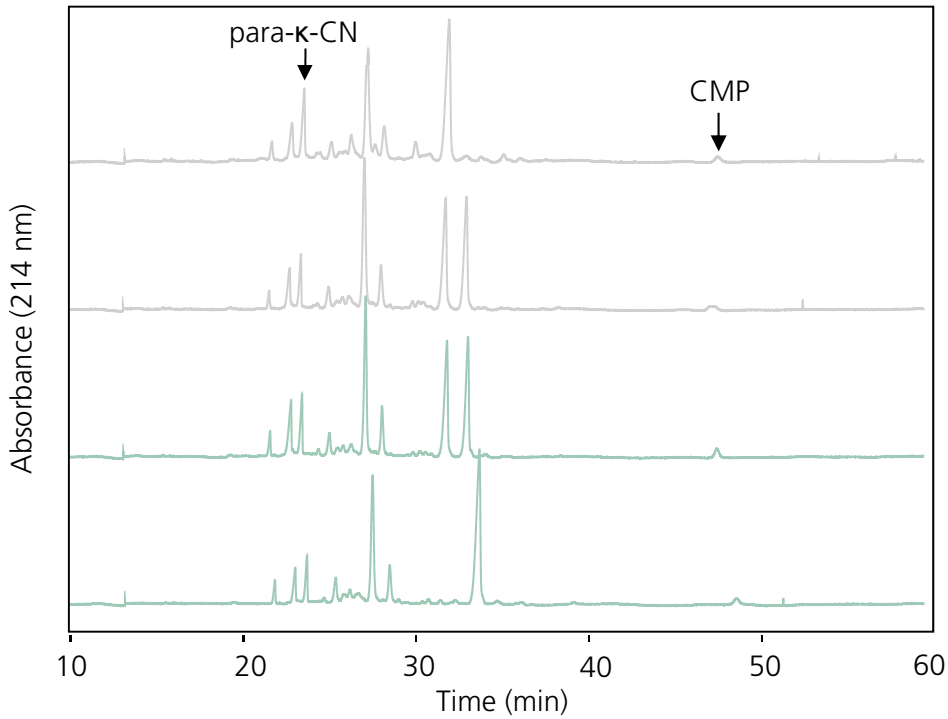


Figure 15. Example of the detection of para-κ-casein (CN) and caseinomacropeptide (CMP) peaks after chymosin cleavage of κ-CN.
The two electopherograms on the top (grey) are coagulating samples while the two in the bottom (green) are non-coagulating milk samples. The graph is adapted from Paper III.

A study by Gamlath et al. (2018) has also investigated the role of native whey proteins in the coagulation process. Their result suggests that higher whey protein content can impair the aggregation stage (Gamlath et al., 2018). This agrees with the results in this thesis that NC milk can be associated with higher α-LA content but does not agree with the association between NC milk and less β-LG (**Paper II**).

Breeding perspectives to reduce non-coagulating milk

From the results obtained in this thesis, four possible markers for NC milk is suggested: κ-CN content, α-LA content, calcium content and β-CN variant. These markers may be used in the breeding industry to reduce the frequency of NC milk. In this thesis, genetic parameters, such as genetic correlations and estimated heritability for the different milk traits, were determined (**Paper IV**), and Figure 16 shows the estimated heritability for the traits.

The genetic correlation of -0.65 between NC milk and κ-CN and the high estimated heritability of 0.77 for κ-CN suggests that κ-CN is a good marker for the reduction of NC milk (**Paper IV**). Further, the genetic correlations also suggest that breeding for milk with higher κ-CN content would reduce the CN micelle size (based on genetic correlation found in **Paper IV**), and a smaller CN micelle size has previously been linked with better coagulation properties (Glantz et al., 2010; Gustavsson et al., 2014b; Ketto et al., 2017).

Regarding Ca content, both total and ionic Ca was linked to NC milk in different ways. From the genetic correlations, it is suggested that total Ca would be a better marker for NC milk as the correlation was very strong (-0.96). At the same time, the heritability estimate was not significant (Figure 16). However, other studies have found stronger heritability estimates of 0.57 (van Hulzen et al., 2009) and of 0.50 (Sanchez et al., 2018) for total Ca. Therefore, total Ca content should still be considered as a breeding marker for NC milk. Breeding for an increased total Ca content to reduce NC milk suggests that protein, CN and citric acid content would increase (based on genetic correlations found in **Paper IV**). Higher protein and CN contents in milk is beneficial for cheese production, as higher amounts improve coagulation ability (Jõudu et al., 2008). Protein content is often used as a factor when determining milk price and a higher content would therefore also benefit farmers. The role of citric acid in rennet coagulation is unclear and has been shown to be inconsistent. Higher citric acid content has been reported to have a significant phenotypic correlation with coagulation ability in Danish Jersey where higher citric acid increased CFR and decreased RCT. However, in the same study, no significant phenotypic correlation with coagulation ability in Danish Holstein was found, and the genetic correlations suggested that coagulation ability was impaired with higher

citric acid content (Poulsen et al., 2015). Other studies have also shown that citrate impairs coagulation ability (Udabage et al., 2001; Sundekilde et al., 2011). Further, breeding for an increased Ca content would increase the nutritional value, as milk is an important source of this mineral in human diets.

The genetic correlation between α-LA content and the binary NC trait was 0.39 (**Paper IV**). The correlation is therefore weaker than NC correlations with κ-CN and Ca contents. The estimated heritability was 0.57 (Figure 16) and was therefore in between heritability of κ-CN and Ca. Breeding for a lower α-LA content to reduce NC would, based on the other significant correlations found, increase fat and CN contents (**Paper IV**). An increased fat content is also beneficial for farmers, as this is often also used when determining milk prices.

Genetic variants of β-CN were not evaluated using genetic parameters. However, due to findings in **Paper II**, where there were differences in the frequencies of allele A^1 and A^2 between NC and coagulating samples (Figure 13), there is potential with the genetic variants. Therefore, breeding for more β-CN A^1 or A^1A^1 could be a way of minimising NC milk. A study by Heck et al. (2009) showed that variant A^1 had significant lower protein yield in the milk, compared to variant A^2, resulting from a lower milk yield. Regarding different proteins, variant A^1 had higher relative frequencies of α_{s1}-CN and κ-CN but lower relative frequencies of α_{s2}-CN and β-CN compared to variant A^2 (Heck et al., 2009). However, another study by McLean et al. (1984) showed that the only significant difference in composition between A^1A^1 and A^2A^2 was that the later had less whey protein amount (McLean et al., 1984). Breeding for more β-CN A^1 to reduce NC milk is therefore likely to affect protein amount or protein composition, but it is hard to conclude to what extent.

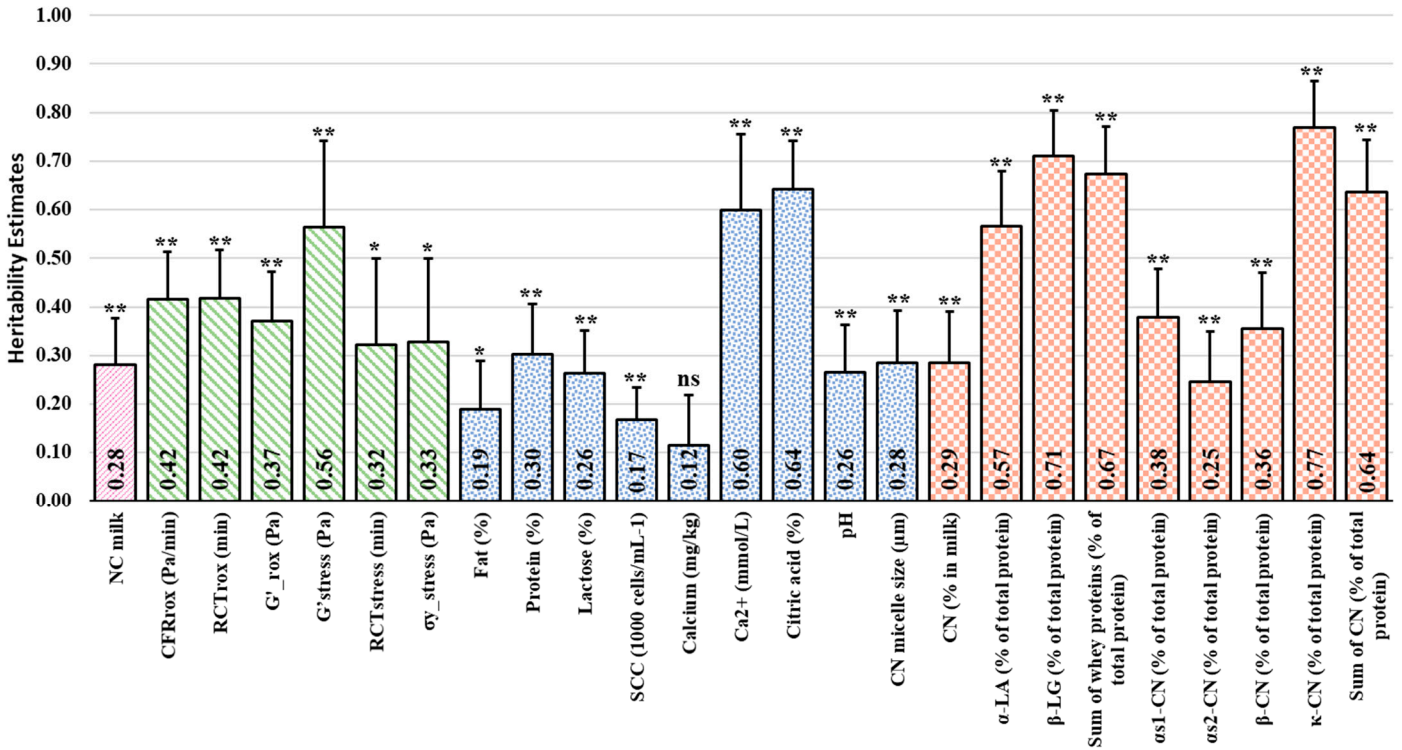


Figure 16. Estimated heritability of milk traits.

The graph is from Paper IV. Heritability estimates for non-coagulating (NC) milk, milk coagulation properties, milk composition and physical traits as well as milk protein composition. Error bars indicate standard errors (SE). Significance levels at: * $P < 0.05$; ** $P < 0.025$ and ns non-significant ($P > 0.05$). CN = casein; α-LA = α-lactalbumin; β-LG = β-lactoglobulin; CFR = curd firming rate; RCT = rennet coagulation time; G' = gel strength; σy_stress = yield stress. Rox or stress refers to the rheometric system used, free oscillation or low amplitude oscillation, respectively. Ca2+ = ionic calcium. Measurements for SCC are log-transformed, and measurements for CFRrox and G'rox are BoxCox-transformed. Four outliers were removed from calcium content

Conclusions

This thesis has shown that 18.1% of cows within Swedish RDC produces NC milk, and additional 18.9% produces PC milk (**Paper I**). Thereby, it can be concluded that a large frequency of Swedish RDC produces milk that is not suited for cheese production. NC milk was found to have a moderate estimated heritability of 0.28 (**Paper IV**).

By analysing peptides after addition of chymosin to NC and well coagulating milk, it was possible to determine that the enzymatic stage is occurring in NC milk (**Paper III**). Therefore, it is strongly believed that the impaired coagulation in NC milk is caused by issues in the aggregation stage of the coagulation process.

Investigation of milk composition in NC milk (**Paper I, II, III, IV**) resulted in four possible phenotypic markers to be used in selective breeding.

- First, NC milk can be linked with a lower Ca content, both total and ionic Ca (**Paper I, III**). Ca has a large role in the aggregation of CN micelles, agreeing with a faulty aggregation stage in NC milk. Ionic Ca showed a high estimated heritability of 0.60, while total Ca had a strong genetic correlation of -0.96 with NC milk (**Paper IV**).
- The second phenotypic marker was κ-CN as it was shown that NC milk had a lower κ-CN content (**Paper III**), and κ-CN content had a significant impact on a binary NC trait (**Paper II**). For κ-CN, both estimated heritability and genetic correlation with NC milk was high, 0.77 and -0.65 respectively (**Paper IV**).
- The third marker was α-LA, since NC milk could be linked with a higher content of α-LA (**Paper III**), and α-LA content had a significant impact on a binary NC trait (**Paper II**). The estimated heritability was high (0.57), while the genetic correlation was moderate at 0.39 (**Paper IV**).
- Lastly, when investigating genetic variants in NC milk, it was revealed that NC milk had lower frequency of β-CN A^1 and higher frequency of β-CN A^2 (**Paper II, III**), which could also be used in breeding strategies.

By combing information about the suggested effect from the phenotypic markers on milk composition and processability with the genetic parameters, κ-CN is suggested to be the most prominent phenotypic marker trait. This thesis has thereby pointed out phenotypic markers found in NC milk that has potential to be used in selective breeding in order to reduce the frequency of NC milk in Swedish RDC.

Future outlook

The aim of the project "Genomic selection against non-coagulating milk", which this thesis is a part of, is to reduce the frequency of NC milk in the Swedish RDC breed by selective breeding. This thesis has provided suggestions on phenotypic markers that could be used in breeding strategies to reduce the frequency of NC milk while still maintaining a functional milk. However, the exact effects can only be determined by incorporating the markers in a breeding strategy and thereafter investigate the milk of the offspring. In order to fully develop a breeding program with genomic selection against NC milk, future research on finding the genes connected to NC milk would be highly recommended.

Even if NC milk has been studied for several years, the exact underlying mechanism or the full impact in cheese production has not been determined. Therefore, there is still a need for research within these areas. Results in this study suggest that the aggregation stage is the faulty step, which has added to the information about the mechanism. Investigation of NC milk in a cheese production line would determine if the CN proteins in NC milk ends up in the whey, or if they are somehow incorporated in the cheese gel thanks to the CN that aggregates from other individual cows. This would make it possible to determine the economic loss from NC milk as well as its environmental impact.

The first step in future research should however be focused on determining a standardised method for defining NC milk. This would provide better comparisons between studies and researches could combine their knowledge, making the research more efficient. Another topic that has been discussed for some time is to develop a field test, allowing NC milk to be determined directly at farms instead of rheological measurements in laboratories. This could provide a time and cost-efficient tool that could enable larger screening.

A reduced frequency of NC milk would not only benefit Swedish cheese dairies but also dairies worldwide as this breed is both exported and used in crossbreeding. As there is a desire to minimise additives in dairy processing, it would also ensure a processable milk without these additives.

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