

Bovine Udder Quarter Milk in Relation to Somatic Cell Count

Focus on Milk Composition and Processing Properties

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Abstract

The dairy industry requires raw milk of high quality in order to produce milk products of high quality and quantity. Mastitis is one of the most prevalent and economically important production diseases in dairy cattle. It causes increased somatic cell count (SCC), deteriorated milk composition and consequently altered processing properties of milk. Altered milk composition due to mastitis often occurs in only one of the four udder quarters of the cow. Milk with high SCC and deteriorated milk composition can be excluded at cow level. A future option will be to detect and exclude milk at udder quarter level, which can be achieved in automatic milking (AM) systems.

This thesis examined alterations in milk composition in individual udder quarters in relation to elevated SCC. The studies were mainly carried out on Swedish Red Breed cows with low to moderate SCC, all supplying milk to a commercial dairy. It was found that around 30% of the cows with low cow composite SCC (below 100 000 cells/ml) had one or more udder quarters with elevated SCC and altered milk composition. Furthermore, individual udder quarters with deteriorated milk composition and signs of reduced blood-milk barrier were found in cows with low to moderate levels of composite SCC. In addition, cows with elevated SCC had one or more udder quarters with low SCC and unaffected milk composition. It was also observed that the relative day-to-day variation in milk components in the four udder quarters of a healthy cow is similar, which suggests that using repeated measurements of milk composition at udder quarter level could be a suitable tool for detecting alterations in milk composition and health. Milk composition was affected by duration of storage and content of bacteria, as well as SCC. Separating the milk from individual udder quarters was shown to affect the content of total protein, whey protein and lactose at cow level. The main conclusions from this thesis are that individual udder quarters with deteriorated milk composition can be found in cows with fairly low SCC, and that exclusion of the milk from these udder quarters can improve overall milk quality. These results are important when discussing whether exclusion of some milk at udder quarter level should be introduced to improve milk quality.

Keywords: dairy cow, milk composition, udder quarter, somatic cell count, processing properties, proteolysis, storage time

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Contents

List of Publications	7
Abbreviations	8
1 Introduction	9
2 Background	11
2.1 Milk Composition	11
2.1.1 Fat	12
2.1.2 Protein	12
2.1.3 Lactose	12
2.1.4 Minor Components in Milk	13
2.2 Sources of Variations in Milk Composition	13
2.2.1 Day-to-Day Variation in Milk Composition	14
2.3 Mastitis	15
2.3.1 Subclinical and Clinical Mastitis	15
2.4 Milk Composition and Milk Yield during Mastitis	16
2.4.1 Milk Yield Affected by Mastitis	16
2.4.2 Milk Fat Affected by Mastitis	16
2.4.3 Milk Protein Affected by Mastitis	17
2.4.4 Lactose Affected by Mastitis	18
2.4.5 SCC in Relation to Mastitis	18
2.4.6 Other Components in Milk during Mastitis	19
2.5 Effect on Processing Properties during Mastitis	19
2.6 Milk Quality	20
3 Aims of the Thesis	21
4 Materials and Methods	23
4.1 Animals and Housing	23
4.2 Milking Procedure and Milking Equipment	24
4.3 Milk Sampling and Sample Treatment	25
4.4 Milk Analyses	26
4.4.1 Milk Gross Composition	26
4.4.2 Somatic Cell Count and Bacteria	27
4.4.3 Lactoferrin and Lactate Dehydrogenase	27
4.4.4 Sodium and Potassium	27
4.4.5 Free Fatty Acids	27

4.4.6	Protein Profile, Proteolysis and Non-protein Nitrogen	27
4.4.7	Total Bacteria Count	28
4.4.8	Curd Yield and Coagulation Time	28
4.5	Statistical Analysis	28
5	Main Results	29
5.1	Milk Composition at Different Levels of SCC in Cow Composite and Udder Quarter Milk	29
5.2	Day-to-Day Variation in Milk Composition in Healthy Cows	32
5.3	Effect of Storage and Exclusion of Udder Quarter Milk	35
6	Discussion	39
6.1	Mastitis may go Unnoticed	39
6.1.1	Milk Composition Changes in Milk with Relatively Low SCC	39
6.1.2	Level of SCC for Healthy Cows	40
6.2	Detecting Milk with Deteriorated Milk Composition	41
6.2.1	Markers for Milk Composition	42
6.2.2	Within-Udder Comparisons	43
6.3	Day-to-Day Variation in Milk Composition at Udder Quarter Level	44
6.4	Interdependence between Udder Quarters?	44
6.5	Effect of Storage on Milk Quality	46
6.6	Yields of Milk Components in Relation to SCC	47
6.7	Economics of Separating Milk with High SCC	47
6.8	Whether to Sort Milk at Udder Quarter Level	48
7	Main Findings and Conclusions	51
8	Future Research	53
9	Populärvetenskaplig Sammanfattning	55
	References	59
	Acknowledgements	67

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I Forsbäck, L., Lindmark-Månsson, H., Andrén, A, Åkerstedt, M. & Svennersten-Sjaunja, K. (2009) Udder quarter milk composition at different levels of somatic cell count in cow composite milk. *Animal* 3(5), 710-717.
<http://journals.cambridge.org/action/displayAbstract?aid=5225772>
- II Forsbäck, L., Lindmark-Månsson, H., Andrén, A, & Svennersten-Sjaunja, K. (2010) Evaluation of quality changes in udder quarter milk from cows with low-to-moderate somatic cell counts. *Animal* 4(4), 617-626.
<http://journals.cambridge.org/action/displayAbstract?aid=7284624>
- III Forsbäck, L., Lindmark-Månsson, H., Andrén, A, Åkerstedt, M. Andrée L. & Svennersten-Sjaunja, K. (2010) Day-to-day variation in milk yield and milk composition at udder quarter level. *Journal of Dairy Science* 93(8), 3569-3577.
- IV Forsbäck, L., Lindmark-Månsson, H., Svennersten-Sjaunja, K., Bach Larsen, L. & Andrén, A. Effect of storage and separation of milk at udder quarter level on milk composition, proteolysis and coagulation properties in relation to somatic cell count (Manuscript).

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Abbreviations

AM	Automatic milking
α_{s1} -CN	α_{s1} -casein
α -la	α -lactalbumin
β -CN	β -casein
β -lg A	β -lactoglobulin A
β -lg B	β -lactoglobulin B
FFA	Free fatty acids
κ -CN	κ -casein
LDH	Lactate dehydrogenase
LPL	Lipoprotein lipase
SCC	Somatic cell count

1 Introduction

Dairy production worldwide is undergoing structural changes, with higher numbers of cows at fewer farms. In Sweden, the number of dairy farms has halved in ten years, and today there are fewer than 6000 (Swedish Dairy Association, 2010). As animal breeding and feed management of dairy cows continue to develop, milk production per cow is increasing. This creates new demands and challenges for dairy farmers. The increasing herd size requires a higher level of technology on the farm and more automation of systems. One new piece of technology that has been introduced over the past 10 years is the automatic milking (AM) system. This allows automation of the milking procedure, which is the heaviest work in dairy production. The AM system can carry out identification of the cow, cleaning of the teats, quarter milking (milking each udder quarter separately) and teat disinfection.

This automation of the milking procedure could allow the detection and exclusion of milk with lower quality during milking. Today, this is mainly done manually by the farmer. Since it is time-consuming and sometimes difficult to detect and separate out milk with deteriorated quality, new techniques and knowledge are needed on the market. Today there are some systems where milk is analysed automatically in-line in conventional milking systems, for example Herd Navigator®, Afimilk® and Sentsortec. The AM systems are able to measure parameters such as milk somatic cell count (SCC), conductivity, milk colour and content of blood in the milk. Herd Navigator® can now also be incorporated into the Voluntary Milking System (VMS™), provided by DeLaval International AB, Tumba, Sweden. These systems enable analysis of different milk components, and at least in the AM system also automatic separation of milk at cow level. Most of these systems specialise in detecting udder health problems, but there is a lack of solutions for evaluating milk in terms of compositional quality.

Dairy products are very important for the food supply and have been so for thousands of years. Worldwide the consumption of all milk products is increasing, especially yoghurt and cheese products (IDF, 2009). In Sweden the majority (43%) of milk is used for drinking, fermented milk products and cream. However, the milk used for cheese production (35%) is an important part of the total milk produced (Swedish Dairy Association, 2010). In dairy production, especially cheese production, raw milk composition has a significant influence on the yield and quality of the final product (Wedholm *et al.*, 2006; Marino *et al.*, 2005; Le Roux *et al.*, 2003; Auldust & Hubble, 1998; Auldust *et al.*, 1996). The production of milk products of high quality and quantity requires high quality raw milk. Milk of low quality originating from individual cows cannot today be eliminated by processing at the commercial dairy (Harding, 1995). Therefore dairy farms need to produce a large quantity of high quality milk.

Altered milk quality due to subclinical mastitis often occurs in only one of the four udder quarters, since it is very seldom that all quarters are affected (Barkema *et al.*, 1997). Due to the anatomical construction of the udder, milk cannot pass from one udder quarter to another. Furthermore, milk with deteriorated quality from one quarter cannot pass to another. Therefore exclusion of milk from individual udder quarters with deteriorated milk composition could be an option in achieving better milk quality. Since the AM system allows quarter milking, it provides possibilities to separate milk at udder quarter level already during milking. However, this is not practised to date. If milk were to be separated based on quality assessment already at milking, there might have to be two different bulk tanks at the farm, one containing excellent milk quality from healthy and high producing cows and one bulk tank with ordinary quality milk. This would make it possible to deliver high quality milk for processing, *i.e.* cheese and yoghurt production, and milk for consumption milk separately. In addition, most of the milk could be delivered and the amount of milk discarded could be minimised.

In order to decide whether milk should be separated at udder quarter level, more knowledge is needed about milk composition in relation to SCC at udder quarter level. In addition, the ways in which common milk quality changes occur in separate udder quarters need to be investigated, as well as the normal variation in milk composition at udder quarter level.

2 Background

2.1 Milk Composition

The biological reason for mammals producing milk is to supply the offspring with nutrients. In order to meet the nutrient requirements of their particular offspring, the composition of milk varies between different mammals. Table 1 shows the average composition of milk from different species that is used in human consumption.

Table 1. *Average composition of milk from different species*

Species	Percentage by weight					
	Water	Fat	Casein	Whey protein	Carbohydrates	Ash
Human	87.1	4.5	0.4	0.5	7.1	0.2
Cow (<i>Bos taurus</i>)	87.3	3.9	2.6	0.6	4.6	0.7
Zebu (<i>Bos indicus</i>)	86.5	4.7	2.6	0.6	4.9	0.7
Sheep	82.0	7.2	3.9	0.7	4.8	0.9
Goat	86.7	4.5	2.6	0.6	4.3	0.8
Camel	86.6	4.5	2.7	0.9	4.5	0.8
Buffalo (<i>Bubalus bubalis</i>)	82.5	7.5	3.6	0.7	4.8	0.8
Horse	88.8	1.9	1.3	1.2	6.2	0.5

Adapted from Akers (2002) and Walstra *et al.* (1999).

In dairy production there are some differences in milk composition and production between cow breeds. In Sweden there are two main cow breeds, Swedish Red Breed and Swedish Holstein. Swedish Red Breed cows make up 43.1% of the Swedish dairy herd and Swedish Holstein cows 50.4% (Swedish Dairy Association, 2010). The Swedish Holstein is known to produce more milk with lower dry matter content than the Swedish Red Breed, as indicated in Table 2.

Table 2. Average milk production, fat content and protein content of the two main dairy cow breeds used in Sweden, Swedish Red Breed and Swedish Holstein

Breed	Milk production (kg)	Fat (%)	Protein (%)
Swedish Red Breed	8730	4.30	3.49
Swedish Holstein	9648	4.01	3.32

Adapted from Swedish Dairy Association (2010).

2.1.1 Fat

The main component in milk fat is triglycerides, which consist of three fatty acids attached with ester bonds to a glycerol molecule. There are many different fatty acids, more than 440 different types having been identified in milk. These fatty acids either originate from the cow's diet or from *de novo* synthesis in the epithelial cells. The shorter fatty acids, C₄-C₁₄, are generally derived from *de novo* synthesis, while the longer fatty acid, C₁₈ originates from the diet and C₁₆ is derived 50% from the diet and 50% from *de novo* synthesis. The fatty acid composition and fat content are highly dependent on the cow's diet. The fat content in milk usually ranges between 3.8 and 4.9%. Milk fat exists primarily as fat globules, with a size of 0.1–15 µm. Each fat globule is surrounded by a membrane originating from the apical plasma membrane of the epithelial cells (for review see Akers, 2002; Larson, 1985; Walstra & Jenness, 1984).

2.1.2 Protein

Milk proteins are synthesised from amino acids derived either from blood or synthesis in the epithelial cells. The mammary specific proteins consist mainly of different caseins, α_{s1}-, α_{s2}-, β- and κ-casein, and of two whey proteins, α-lactalbumin and β-lactoglobulin. Approximately 80% of the proteins are caseins and 20% are whey proteins. The total protein content ranges between 3.0 and 3.6%. Other components that may be present in the protein fraction of milk are proteins derived from blood and γ-casein derived from proteolysis of β-casein. The caseins are associated to form casein micelles with a size of 10–300 nm. The micelles mainly contain caseins, but calcium, phosphorus, citric acid and magnesium are also included. The proportions of α_s-, β- and κ-casein in milk are 49, 35 and 10%, respectively (for review see Akers, 2002; Larson, 1985; Walstra & Jenness, 1984).

2.1.3 Lactose

The most common carbohydrate in milk is lactose, which is specific to mammalian milk. The lactose content of milk is usually in the range 4.6–4.8%. Lactose is synthesised in the Golgi apparatus and consists of galactose

and glucose linked together. The enzyme lactose synthase, which is essential for lactose synthesis, is derived from galactosyl-transferase and α -lactalbumin. The osmotic balance of milk is to a high extent derived from lactose. As the content of lactose increases in the Golgi vesicles, water diffuses from the cytosol by osmosis into the vesicles. The membrane of the vesicles is impermeable to lactose but highly permeable to water. Lactose and water, together with other milk compounds, are moved into the alveoli across the epithelium by exocytosis of the Golgi vesicles (for review see Sjaastad *et al.*, 2003; Akers, 2002; Larson, 1985; Walstra & Jenness, 1984).

2.1.4 Minor Components in Milk

In addition to the main components of milk (water, fat, protein and lactose), there are many other compounds present in milk. Milk contains numerous different components with low molecular weight such as calcium, potassium, sodium, chloride, magnesium, zinc, phosphates and citric acid. Milk is a major source of vitamin A and vitamin B in particular, but all vitamins are present in milk, although some in small concentrations e.g. vitamin C (for review see Walstra *et al.*, 1999; Walstra & Jenness, 1984).

Milk contains many different types of enzymes, both native enzymes excreted by the mammary gland and enzymes of microbiological origin. The function of many of the enzymes in milk is not yet fully known, but some have antimicrobial function. Lactoperoxidase and lysozyme are examples of enzymes acting against bacteria (Walstra *et al.*, 1999). Some enzymes found in milk are also used as markers for mastitis, e.g. N-acetyl- β -D-glucosaminidase (NAGase) and more recently lactate dehydrogenase (LDH) (Chagunda *et al.*, 2006). NAGase is an intracellular enzyme that is released from the polymorphonuclear leucocytes during phagocytosis and cell lysis. LDH is a cytoplasmic enzyme and part of the glycolytic pathway.

2.2 Sources of Variations in Milk Composition

Milk composition can vary for different reasons. Factors affecting milk composition can be categorised into genetic, physiological, environmental and pathological. Most of the components in milk are affected by pathological changes, mastitis in particular (Walstra *et al.*, 1999). The ways in which mastitis affects the milk composition are described in section 2.4.

Variations in fat content are highly dependent on milking and feeding. The residual milk is high in fat content and during the milking procedure the fat content increases. Therefore the fat content in milk is affected by udder emptying during milking (Tancin *et al.*, 2007; Nielsen *et al.*, 2005;

Svennersten *et al.*, 1991; Johansson *et al.*, 1952). The degree of udder filling also contributes to the level of fat in milk (Weiss *et al.*, 2002). Fat content is the milk component most sensitive to dietary influences. The amount of roughage or fibre, forage:concentrate ratio, soluble carbohydrates and lipids in the diet, dietary intake and frequency of feeding are examples of dietary factors affecting the fat content in milk (Sutton, 1989).

The genetic capacity of the cow contributes strongly to the content and composition of proteins in milk. In addition, physiological factors such as stage of lactation contribute to variations in content and composition of milk proteins (Walstra *et al.*, 1999). The protein content of milk can also be affected by feeding, but to a much smaller extent than fat (Sutton, 1989).

Since lactose has the major osmotic regulatory function in milk, it is a very stable component of milk (Ling *et al.*, 1961). It is generally considered that content of lactose cannot be changed by dietary factors (Sutton, 1989). Decreased lactose content is often correlated to disturbances in the secretion process.

2.2.1 Day-to-Day Variation in Milk Composition

Relative day-to-day variation is the random variation in a parameter that is not explained by any known variance component. Factors that contribute to the day-to-day variation are the random biological variation, errors in the analytical methods used, the sampling technique and variation due to pathological and physiological changes. Čandek-Potokar *et al.* (2006) suggested that the main variation in milk components can be related to the unexplained part associated with different biological and uncontrolled factors, *i.e.* day-to-day variation. In practice, knowledge about day-to-day variation is essential when deciding how often different parameters should be analysed for different purposes and in determining the normal variation in each parameter.

It has been observed that the day-to-day variation at cow composite level in fat content and milk yield is often higher than that in protein and lactose content (for review see Quist *et al.*, 2008; Čandek-Potokar *et al.*, 2006; Svennersten-Sjaunja *et al.*, 1997; Sjaunja, 1986; Syrstad, 1977).

Day-to-day variations in milk composition have been described in several studies at cow composite level (Millogo *et al.*, 2009; Quist *et al.*, 2008; Čandek-Potokar *et al.*, 2006; Rook *et al.*, 1992; Sjaunja, 1986; Syrstad, 1977; Gilbert *et al.*, 1973) but few studies have been carried out at udder quarter level (Linzell & Peaker, 1972). In order to detect abnormalities in milk composition, it is important to have knowledge about the normal composition in healthy cows. Hence, there is a need for

knowledge about the day-to-day variations in milk components at udder quarter level.

2.3 Mastitis

Mastitis is one of the most prevalent and economically severe production disease in dairy cattle. It causes decreased milk production, deteriorated milk quality, lower animal welfare, increased antibiotic treatments, increased labour costs and increased need for culling of cows (Halasa *et al.*, 2007; Seegers *et al.*, 2003; Bradley, 2002). Despite decades of research, mastitis continues to be a widespread problem in dairy production.

Mastitis (from the Greek *mastos* = breast and *itis* = inflammation) is an intramammary inflammation caused by infection by micro-organisms, primarily bacteria. However, other micro-organisms such as yeast, moulds and fungi can also cause mastitis, as can traumas to the udder. The udder quarter becomes infected when bacteria penetrate the teat canal and multiply in the udder. The cow's reaction to the bacterial infection results in an inflammatory response characterised by redness, swelling, heat, pain and loss of function. The main function of the inflammation is to destroy the infectious agent and heal the udder. The influx of somatic cells in milk is a primary feature of inflammation (for review see Harding, 1995; Sandholm *et al.*, 1995; Larson, 1985).

2.3.1 Subclinical and Clinical Mastitis

Mastitis can be divided into two forms, subclinical and clinical. Subclinical mastitis, which is the most prevalent form, displays no visible signs in the cow or the milk. However, the SCC is increased, the milk composition is altered, milk yield is decreased and pathogens are present in the udder. The clinical form of mastitis is characterised by hot, painful and swollen udder quarters, fever and loss of appetite in the cow, as well as the effects seen in the subclinical form. In clinical mastitis, the milk may contain flakes, clots and blood. These visible symptoms of clinical mastitis make detection easy and the milk can be separated out and not delivered to the dairy. In the case of subclinical mastitis, detection is more difficult and laboratory tests are needed (for review see Akers, 2002; Sandholm *et al.*, 1995). Subclinical mastitis causes problems, since it can go unnoticed and generate milk with lower quality that is allowed to enter the bulk tank. In addition, the affected cows remain contagious. The effects of exclusion of milk from affected udder quarters with subclinical mastitis on cow composite milk have not been fully evaluated.

2.4 Milk Composition and Milk Yield during Mastitis

During mastitis the permeability of the blood–milk barrier increases, allowing the somatic cells to pass from blood to milk. This increased permeability also results in other components moving from blood to milk and *vice versa*. The epithelial cells can be destroyed due to bacteria toxins and hence the secretion capacity is impaired (Sandholm *et al.*, 1995). The increased permeability and reduced milk synthesis result in decreased milk production and altered milk composition, which have been thoroughly described in the literature (Hamann, 2002; Auldism & Hubble, 1998; Hortet & Seegers, 1998; Munro *et al.*, 1984; Dohoo & Meek, 1982; Kitchen, 1981; Tolle *et al.*, 1971). However, most of these studies were performed at cow level and there is a lack of investigations performed at udder quarter level. In addition, most investigations of composition in relation to SCC have been performed at high SCC and fewer studies have focused on alterations in milk with low to moderate SCC increase.

2.4.1 Milk Yield Affected by Mastitis

One of the major symptoms of mastitis is the reduction in milk yield. The reduction in milk production at udder quarter level can be in the range 3–50% of the potential milk yield, depending on the severity of the inflammation. There is a strong relationship between production losses and increased SCC, with the greatest reduction observed at the highest SCC (Harding, 1995). Miller *et al.* (1983) found that milk production decreased with increasing SCC, irrespective of the bacteriological status of the cow. Another study observed that the lower the quarter SCC, the greater the milk production. Milk production at udder quarter level started to decline already at quarter SCC > 100 000 cells/ml, which has been suggested to be a fairly low SCC (Fox *et al.*, 1985). Tolle *et al.* (1971) observed a reduction in milk production already at SCC just above 50 000 cells/ml in udder quarter milk. It has also been questioned whether the consequences of inflammation, including elevated SCC, lead to lower milk yield or whether high milk yield leads to lower SCC. Previous results indicate that there is a dilution effect of milk yield on SCC. This should be kept in mind when evaluating the impact of high SCC on milk production losses (Green *et al.*, 2006).

2.4.2 Milk Fat Affected by Mastitis

The results from studies investigating fat content in cows with mastitis are not in agreement. Some studies report higher fat content during mastitis, while others report lower (Ma *et al.*, 2000; Munro *et al.*, 1984; Kitchen,

1981; Ali *et al.*, 1980). Declining fat content during mastitis is primarily the result of reduced synthetic and secretory capacity of the mammary gland. Increased fat content is probably due to milk yield being reduced to a greater extent than fat synthesis. Irrespective of changes in fat content, the physical properties and composition of fat are changed during mastitis (Santos *et al.*, 2003; Ma *et al.*, 2000; Munro *et al.*, 1984; Kitchen, 1981). Mastitis may result in higher levels of free fatty acids (FFA) during the secretion of milk as a consequence of the inflammation, which causes altered milk synthesis and higher level of unesterified fatty acids. Higher levels of FFA in milk result in off-flavours such as rancid and butyric flavour. The functionality is also impaired, with depressed ability to produce foam when injected with steam (used for cappuccino). The enzyme lipoprotein lipase (LPL) accounts for the lipolytic activity in milk and is responsible for the lipolysis of fat triglycerides (for review see Deeth, 2006; Walstra *et al.*, 1999). The LPL activity has been found to be higher in milk from udder quarters with subclinical mastitis (Azzara & Dimick, 1985). LPL is unstable to heat and therefore it has minor effects on lipolysis in pasteurised milk. However, LPL is active during cold storage and can therefore affect the milk before pasteurisation. LPL is not responsible for all the lipolysis in milk, since some psychrotrophic bacteria can produce bacterial lipase that is stable to heat treatment (for review see Deeth, 2006).

2.4.3 Milk Protein Affected by Mastitis

Changes in total protein content due to mastitis reflect the changes that occur in the different protein components, casein and whey proteins. As the casein content declines and the whey proteins increase due to the inflammation, the total protein content depends on these changes. Some studies report higher and others lower or unchanged total protein content during mastitis (Nielsen *et al.*, 2005; Urech *et al.*, 1999; Auldish & Hubble, 1998; Harmon, 1994; Munro *et al.*, 1984; Kitchen, 1981). The increased permeability of the blood–milk barrier allows serum proteins to leak into the milk, which results in higher whey protein content. The casein content can be affected by both proteolysis and reduced synthesis (Auldish & Hubble, 1998; Munro *et al.*, 1984). The major proteolytic enzyme in milk during mastitis is plasmin. Plasmin increases during mastitis due to the increased transport of plasminogen (the inactive form of plasmin) from blood to milk and the elevated conversion of plasminogen to plasmin. Other factors such as stage of lactation, lactation number and breed also influence the plasmin activity. The biological function of plasmin in blood is to break down blood clots. In milk, there is increased activation of plasminogen during the

involution of the mammary gland. Plasmin is associated with the casein micelles in milk and in particular is responsible for the degradation of β -casein, but also α_{s1} - and α_{s2} -casein. The optimum temperature for plasmin is 37 °C, which is close to the body temperature of the cow. However, proteolysis can continue during cold storage and after heat treatment of milk. Thus plasmin also contributes to proteolysis in processed products such as cheese (Leitner *et al.*, 2008; Le Roux *et al.*, 2003; Santos *et al.*, 2003; Athie *et al.*, 1997; Bastian & Brown, 1996; Bastian *et al.*, 1991; Politis *et al.*, 1989; Grufferty & Fox, 1988; Schaar & Funke, 1986; Schaar, 1985). This makes proteolysis a problem that starts in the udder and continues during cold storage of milk in the farm bulk tank and dairy silos, as well as in the final milk products. However, proteolytic processes are essential in obtaining the special characteristics of ripened cheese. Therefore plasmin has a negative impact before processing but a positive effect during ripening of cheese (Walstra *et al.*, 1999). Other enzymes such as cathepsins and elastase also contribute to proteolysis in milk (Kelly *et al.*, 2006; McSweeney *et al.*, 1995). In addition, enzymes from psychrotrophic bacteria have a proteolytic action in milk, especially during cold storage (Kelly *et al.*, 2006; Sørhaug & Stepaniak, 1997).

2.4.4 Lactose Affected by Mastitis

It is well established that lactose content decreases in milk from cows and individual udder quarters during mastitis. The lower lactose content is a consequence of reduced synthesis and losses to the circulation due to the lower blood-milk barrier caused by increased permeability of tight junctions and damaged epithelial cells (Auldish & Hubble, 1998; Munro *et al.*, 1984; Kitchen, 1981; Linzell & Peaker, 1972). The balance between lactose and soluble minerals maintains the osmotic pressure of the milk. Therefore influx of minerals from blood results in lower lactose content to maintain the osmotic balance (Stelwagen *et al.*, 1999; Ling *et al.*, 1961). Lactose has been suggested as a mastitis marker, especially at udder quarter level, since reduction of lactose is observed already during moderate SCC increase (Berglund *et al.*, 2007).

2.4.5 SCC in Relation to Mastitis

The increase in SCC during mastitis is part of the immune defence system of the cow. SCC consists of different cell types, including neutrophils, macrophages, lymphocytes and some epithelial cells. The neutrophils are the major cell type found in milk from cows with mastitis, while macrophages are the dominant cell type in milk from healthy cows. Consequently not

only does the number of cells increase during mastitis, but the proportion of the different cell types also changes (Sordillo *et al.*, 1997; Saad & Östensson, 1990; Paape & Tucker, 1966). SCC is the predominant marker for mastitis used worldwide. The level of SCC indicating mastitis has been debated, with varying results.

However, it has been shown that elevated SCC is not always associated with mastitis. Elevated SCC has been found after a single prolonged milking interval (Lacic *et al.*, 2009; Fox & Schultz, 1985). Wredle *et al.* (2008) and Coulon *et al.* (1998) found elevated SCC in relation to pasture turnout.

2.4.6 Other Components in Milk during Mastitis

The composition of ions in milk changes due to mastitis. The increased permeability of the blood-milk barrier results in increased levels of sodium and chloride and decreased levels of potassium. As mentioned above, the enzyme activity in milk increases due to high SCC, with proteolytic, lipolytic and bacteriostatic enzymes all being elevated (for review see Munro *et al.*, 1984; Kitchen, 1981).

2.5 Effect on Processing Properties during Mastitis

Mastitis not only affects milk composition, but also the processing properties of the milk. Many studies have confirmed negative effects on cheese-making properties of milk with high SCC, such as lower cheese yield, casein loss into whey and slower curd formation (Leitner *et al.*, 2008; Le Roux *et al.*, 2003; Auldist *et al.*, 1996; Barbano *et al.*, 1991; Claesson, 1965). Barbano *et al.* (1991) found that the relationship between SCC and cheese yield was not linear, as cheese yield was lower when SCC exceeded 100 000 cells/ml in milk commingled from different cows. However, the change was not as great when the SCC increased from 127 000 cells/ml to 1 300 000 cells/ml in milk commingled from different cows. Another study found that the changes in milk composition related to high SCC influenced the quality and yield of cheddar cheese (Auldist *et al.*, 1996). Leitner *et al.* (2006) found the influence on curd formation in subclinical udder quarters to be bacteria-specific. O'Brien *et al.* (2001) found the coagulation time to increase in udder quarter milk with SCC > 1 000 000 cells/ml, and the curd firmness 60 minutes after rennet addition to decrease. When udder quarter milk with different SCC was commingled and stored for 0, 72 and 144 h, the coagulation time decreased and the curd firmness 60 minutes after rennet addition was reduced with increasing storage duration. The curd firmness was numerically lower in udder quarter milk with SCC > 1 000 000

cells/ml compared with commingled milk with various SCC stored for 144 h.

2.6 Milk Quality

Milk quality can be defined in different ways; hygiene, composition or consumer-specific aspects. Taste, smell, appearance, content of different bacteria and milk composition are all examples of parameters that can be related to milk quality. However, in this thesis the main focus is in milk quality described as gross compositional quality. Milk quality is highly dependent on the intended end-use of the milk. In general, the compositional milk quality is more important in milk intended for processing. High milk quality in the perspective of milk composition is milk with a high quantity and quality of the major milk components, protein and fat.

3 Aims of the Thesis

The overall aim of the thesis was to obtain further knowledge about alterations in milk composition in individual udder quarters owing to elevated SCC.

Specific aims were to:

- Determine the prevalence of elevated SCC and deteriorated milk composition at udder quarter level.
- Evaluate how the milk composition is altered in udder quarters with different levels of elevated SCC, when cow composite milk exhibits low to moderate SCC levels.
- Define the normal variation in milk composition and milk yield from healthy cows at udder quarter and cow composite level.
- Examine how storage duration affects milk composition, coagulation properties and proteolysis in udder quarter milk and cow composite milk with elevated SCC.
- Determine whether exclusion of milk from udder quarters with elevated SCC and deteriorated milk composition improves milk quality at cow level.

4 Materials and Methods

All studies were performed at the Kungsängen Research Centre, Swedish University of Agricultural Sciences and at Jälla Experimental Farm in Uppsala, Sweden. The studies were approved by the Uppsala Ethical Committee.

4.1 Animals and Housing

All cows included in Papers I-IV were fed with grass silage and concentrate according to Swedish recommendations (Spörndly, 2003). They were all delivering milk to the processing dairy on the sampling occasion and none of them was being treated for mastitis. All cows were clinically healthy, with no clinical signs either in the milk or at udder and cow level. All studies included cows of the Swedish Red Breed except Paper I, where cows from the Swedish Holstein Breed were also included. The cows were kept in two different housing systems, two stanchion barns and a loose house barn equipped with AM system. The cows in the stanchion barns were milked twice daily with a milking interval of 9 h during the day and 15 h during the night. The cows in the AM barn were milked on average 2.4 (Paper I), 2.3 (Paper II) and 2.3 (Paper IV) times per day, with an average milking interval of 10.2, 10.4 and 10.3 h, respectively.

Paper I included 90 cows with average lactation number and lactation week \pm standard deviation of 2.1 ± 1.4 and 31 ± 17 , respectively. The cows were kept in two stanchion barns and one loose house barn equipped with AM.

Paper II included 17 cows with average lactation number and lactation week \pm standard deviation of 1.2 ± 0.4 and 31 ± 21 , respectively. The cows were kept in one stanchion barn and one loose house barn equipped with AM. Before the experiment started, 42 cows were sampled at udder quarter

level, but only 17 of these fulfilled the pre-set criteria for inclusion in the experiment. The selected cows were divided into three groups according to their SCC at udder quarter level. Each group contained five or six cows. The three groups, were defined as follows: Group 1 (n = 6): SCC < 50 000 cells/ml for all udder quarters; group 2 (n = 5): 101 000 cells/ml < SCC < 600 000 cells/ml for one udder quarter and SCC < 100 000 cells/ml for the other three udder quarters; group 3 (n = 6): SCC > 700 000 cells/ml for one udder quarter and SCC < 100 000 cells/ml for the other three udder quarters.

Paper III included 10 cows with average lactation number and lactation week \pm standard deviation of 2.2 ± 1.8 and 27 ± 2 , respectively. One week before the experiment started these cows were tested for SCC at cow composite level and bacteriologically for each udder quarter. All 10 cows met the pre-set criteria, which were SCC level < 100 000 cells/ml in cow composite milk and negative tests for bacteria in all four udder quarters. Despite this, early in the trial period one cow (cow A) showed high SCC and bacteria (*Enterobacter cloacae*) but no clinical signs in one udder quarter, which persisted during the whole trial. The remaining nine cows were tested bacteriologically negative throughout the trial period. The cows were kept in a stanchion barn.

Paper IV included 13 cows with mean lactation number and lactation week \pm standard deviation of 3.1 ± 1.6 and 19 ± 15 , respectively. The cows were kept in one stanchion barn and one loose house barn equipped with AM. Before the experiment started, milk at udder quarter level was analysed for SCC in 34 cows, but only 13 of these fulfilled the pre-set criteria for inclusion in the experiment. To be included in the experiment, the cows needed to have one or two udder quarters with SCC > 100 000 cells/ml and the others < 100 000 cells/ml; and the udder quarters with elevated SCC had to have at least three times higher SCC than the healthy contralateral udder quarter.

4.2 Milking Procedure and Milking Equipment

The cows in the AM barn were milked with a VMSTM (Voluntary Milking System, provided by DeLaval International AB, Tumba, Sweden) with monovac, pulsation ratio 70/30, pulsation rate 60 cycles/minute and system vacuum 42 kPa. The cows in the stanchion barns were milked with a special quarter milking machine (provided by DeLaval International AB, Tumba, Sweden) with monovac, pulsation ratio 70/30, pulsation rate 60 cycles/minute and system vacuum 42 kPa. Before milking the cows in the

stanchion barns, each teat was wiped with an udder towel and the first beams of milk were rejected. In the AM system, the cleaning of the teats and removal of the first milk were performed by the robot.

4.3 Milk Sampling and Sample Treatment

In Papers I, II and IV, cow composite and udder quarter milk samples were collected on one milking occasion from each cow. In Paper III, udder quarter and cow composite milk samples were collected during morning and evening milkings for three weeks, *i.e.* for 42 consecutive milkings. During sampling, all milk from the entire milking and each separate udder quarter was collected in special containers. Quarter milk and representative cow composite milk were sampled for analyses. Milk yield from each quarter was recorded. The following sampling routine was used: after gentle stirring, milk samples were collected from each quarter container, then all milk from separate quarters was mixed, and after gentle stirring a cow composite sample was taken. In Paper IV, additional milk samples were created by commingling milk from healthy ($< 100\,000$ cells/ml) udder quarters from each cow to one sample. These samples were designated cow separated milk samples.

Milk samples for milk gross composition and SCC analyses in Papers I-III were treated with Bronopol, 2-bromo-2-nitropropane-1,3-diol (VWR International AB, Stockholm, Sweden). Samples for fat, protein, lactose, citric acid, SCC analyses in Papers I-III and whey protein samples in Papers I-II were stored at $+4^{\circ}\text{C}$ and analysed on the day of sampling or the following day. Samples for whey protein analysis in Paper III and the remaining samples in Paper II were frozen and stored at -80°C until analysis. In Paper IV, gross milk composition and coagulation properties were analysed in fresh milk samples on the day of sampling and in stored milk ($+4^{\circ}\text{C}$) two and five days after sampling. Milk samples for proteolysis analysis and total bacteria plate count were collected on the sampling day and in milk stored for two and five days after sampling. These samples were stored at -80°C until analysis.

Bacteriological milk samples for determination of mastitis pathogens were collected directly after milking. In Paper I milk samples for bacteriological analysis were collected once, on the day after the sampling from udder quarters with milk SCC $> 300\,000$ cells/ml and from quarters with five-fold higher SCC than the quarter with the lowest SCC within an udder. In Papers II and IV, milk samples for bacteriological analysis were collected from all udder quarters on the day of sampling and the following day. In

Paper III, milk samples for bacteriological analysis were collected at every morning milking throughout the trial period. Before collecting milk for bacteriological analysis in sterile tubes, the teats were wiped with an udder towel, the first beams of milk rejected and the teats disinfected with 70% alcohol and allowed to dry.

4.4 Milk Analyses

The different milk parameters that were analysed and recorded in the Papers I-IV are described in Table 3.

Table 3. *Milk parameters analysed and recorded in Papers I-IV of this thesis*

	Paper I	Paper II	Paper III	Paper IV
Milk yield	X	X	X	X
Fat	X	X	X	X
Total protein	X	X	X	X
Whey protein	X	X	X	X
Casein	X	X	X	X
Lactose	X	X	X	X
Citric acid	X	X		
SCC	X	X	X	X
Bacteriology				
- Mastitis pathogens	X	X	X	X
- Total bacteria count				X
Lactoferrin		X		
Na/K		X		
FFA		X		
NPN		X		
LDH		X		
Proteolysis		X		X
Protein profile		X		
Coagulation time				X
Curd yield				X

4.4.1 Milk Gross Composition

To estimate the content of milk fat, total protein, lactose, citric acid and whey protein, the mid-infrared spectroscopy method (Fourier Transform Instrument, FT 120, Foss, Hillerød, Denmark) was used. The content of casein was determined from the whey protein and total protein proportions, using an indirect method. The whey fraction was determined by mid-

infrared spectroscopy after rennet coagulation of the caseins. The casein number was calculated as the proportion of casein in relation to total protein.

4.4.2 Somatic Cell Count and Bacteria

The milk samples were analysed for SCC using electronic fluorescence based cell counting (Fossomatic 5000, A/S N. Foss). Bacteriological analyses for determination of mastitis pathogens in the milk samples in Papers I-III were performed by the National Veterinary Institute, Uppsala, Sweden, according to quality assurance protocol SS-EN ISO/IEC 17025. In Paper IV bacteriological analyses were performed using the PathoProof Mastitis PCR Assay (Finnzymes Oy) according to Koskinen *et al.* (2009).

4.4.3 Lactoferrin and Lactate Dehydrogenase

Lactoferrin was determined quantitatively, according to the manufacturer's instructions, using a commercial enzyme-linked immunosorbent assay (Bovine Lactoferrin ELISA Quantification Kit, Bethyl Laboratories, Inc., Montgomery, TX, USA). LDH was analysed by a fluorometric, kinetic method according to Larsen (2005).

4.4.4 Sodium and Potassium

Sodium and potassium contents were determined by flame photometry (Flame Photometer FF-IL 943, ILS Instrumentation Laboratory S.p.A., Milano, Italy).

4.4.5 Free Fatty Acids

The FFA content was determined using the Autoanalyzer II method (Lindqvist *et al.*, 1975).

4.4.6 Protein Profile, Proteolysis and Non-protein Nitrogen

The composition of individual milk proteins (protein profile), α_{s1} -casein (α_{s1} -CN), β -casein (β -CN), κ -casein (κ -CN), α -lactalbumin (α -la), β -lactoglobulin A (β -lg A) and β -lactoglobulin B (β -lg B), in skimmed milk samples was determined using the RP-HPLC-method of Bordin *et al.* (2001) and modified according to Hallén *et al.* (2008).

The presence of proteolysis products in the milk samples in Paper II was evaluated in skimmed milk samples using the fluorescamine method of Wiking *et al.* (2002). In Paper II one modification of the method was made, which comprised a second centrifugation $14\ 000 \times g$ for 30 minutes with a filter (10 kDa cut-off), in order to obtain a pure supernatant. In Paper IV

the fluorescamine method of Wiking *et al.* (2002) was used to evaluate the presence of proteolysis products in whey prepared from skimmed milk samples.

Non-protein nitrogen (NPN) content was analysed according to International IDF Standard 20B:1993, determination of non-protein nitrogen content (IDF, 1993).

4.4.7 Total Bacteria Count

Milk samples were analysed for total bacteria using standard plate counting.

4.4.8 Curd Yield and Coagulation Time

A cheese-making model similar to that of Hallén *et al.* (2010) was used to measure curd yield and dry matter of the curd. In contrast to Hallén *et al.* (2010), whole milk samples were used and the dry matter of the curd was determined as follows: After weighing and decanting of the whey, the remaining coagulum was removed and weighed before incubation at 105 °C overnight. The dried coagulum was weighed warm the next day.

The coagulation time was estimated by recording the time taken for visible flocculation of the milk samples after rennet addition. The milk samples (10 ml) were pre-heated in a water bath at 30 °C. A 25 µl portion of chymosin solution (Chymax Plus, 190 international milk clotting units, Chr. Hansen A/S, Hørsholm, Denmark) was added and mixed with the milk sample. The milk sample was stirred slowly until the milk coagulated and the time was recorded.

4.5 Statistical Analysis

For Papers I and II, statistical analyses were performed using the GLM procedure in SAS 9.1 (SAS Institute, 2004). In Papers I and II, LSmeans were calculated to estimate differences between groups. Paired two-sided *t*-tests were used in Papers I, II and IV to calculate the difference between affected and contralateral healthy udder quarters within cows. In Paper III the GLM and VARCOMP procedure in SAS 9.1 were used to calculate descriptive statistics and coefficient of variation. In Paper IV the MIXED procedure in SAS 9.1 was used to test the effects of different variables on parameters measured in all milk samples. In all papers, SCC was used for assigning cows to different groups and logarithmic values of SCC were used in all statistical analysis. Logarithmic values for total bacteria count (log cfu) were used in Paper IV.

5 Main Results

This chapter presents a summary of the results from Papers I-IV. More detailed information is given in each individual paper.

5.1 Milk Composition at Different Levels of SCC in Cow Composite and Udder Quarter Milk

In the first study, where 90 cows were screened (Paper I), the cows were divided into three groups based on their SCC at cow composite level. Group 1, which consisted of cows with cow composite milk SCC < 100 000 cells/ml, represented 54% of the cows. Group 2 comprised cows with cow composite milk SCC between 100 000 cells/ml and 300 000 cells/ml and represented 26% of the cows. The remaining 20% of the cows belonged to group 3, which consisted of cows with cow composite milk SCC > 300 000 cells/ml. Differences between these three groups at cow composite level were found in milk yield, casein number, content of lactose and SCC (Table 4). It was found that 29% of the cows in group 1 had one or more udder quarters with elevated SCC (> 100 000 cells/ml). When these udder quarters were compared with the healthy contralateral udder quarter within the same cow, it was observed that the casein number and lactose content were lower and contents of total protein and whey protein higher (Table 5). In groups 2 and 3, 91% and 72%, respectively, of the cows had one or more udder quarters with SCC below 100 000 cells/ml.

Table 4. Parameters in cow composite milk that differed significantly between the three groups in Paper I. Results presented as LSmeans

Parameter	Group 1 ¹	Group 2	Group 3
Milk yield/milking (kg)	11.16 ^a	10.92 ^{ab}	9.37 ^b
Casein number	0.74 ^a	0.73 ^{ab}	0.72 ^b
Lactose (%)	4.55 ^A	4.51 ^{ab}	4.39 ^c
SCC ² (cells/ml)	32 000 ^A	160 000 ^B	784 000 ^C

¹The groups were: 1) cow composite SCC < 100 000 cells/ml; 2) cow composite SCC 100 000 - 300 000 cells/ml; 3) cow composite SCC > 300 000 cells/ml

² Displayed as antilogarithmic values

^{a-c} Significant difference ($P < 0.05$)

^{A-C} Significant difference ($P < 0.01$)

Table 5. Milk components in group 1 (cow composite SCC < 100 000 cells/ml) that differed significantly between healthy (SCC < 100 000 cells/ml) and affected (SCC > 100 000 cells/ml) quarters within cows in Paper I. Results presented as mean values from healthy and affected udder quarters and significance from paired t-tests, $n=15$

Parameter	Mean values of healthy quarters	Mean values of affected quarters	Significance ³
Total protein (%)	3.65	3.69	*
Whey protein (%)	1.01	1.04	**
Casein number	0.73	0.72	**
Lactose (%)	4.48	4.44	*
SCC ¹ (cells/ml)	27 000	175 000	***

¹ Displayed as antilogarithmic values

³ * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

When yields of milk components in group 1 in Paper I were compared between affected and healthy udder quarters, it was found that the fat yield was significantly lower, and lactose and citric acid showed a tendency to decrease in the affected udder quarters. In group 2, yields of total protein, casein, lactose and citric acid were significantly lower in affected udder quarters, while fat yield showed a tendency to decrease. Lactose yield showed a tendency to decrease in group 3.

In Paper I, the composition of theoretical bulk tank milk was calculated from the values at udder quarter level. The theoretical bulk tank contained 904 kg milk and had a SCC value of approximately 274 000 cells/ml. By a theoretical separation of four udder quarters and 9.6 kg of milk the bulk tank SCC was reduced to approximately 135 000 cells/ml. The SCC of the four separated udder quarters was 18 433 000, 14 388 000, 11 976 000 and 5 113 000 cells/ml, respectively. At this level of theoretical separation, no influence was observed on mean values of milk composition.

In Paper II, 17 cows were selected to participate in the study based on their SCC at udder quarter level. The cows were grouped according to the SCC at udder quarter level. Group 1 contained cows with SCC < 50 000 cells/ml in all four udder quarters. Group 2 and 3 comprised cows with one udder quarter with SCC 101 000 – 600 000 cells/ml and > 700 000 cells/ml, respectively. The remaining three udder quarters in the cows in groups 2 and 3 had SCC < 100 000 cells/ml. The SCC at cow composite level for groups 1, 2 and 3 was 20 000, 100 000 and 214 000 cells/ml, respectively. When milk composition at cow composite level was compared between the three groups, it was found that casein number, LDH and SCC differed significantly. Higher values of sodium, LDH and SCC and lower values of casein number and α -la were found in affected udder quarters of group 3 cows compared with affected udder quarters of group 2 cows. The level of FFA showed a tendency to be higher in the affected udder quarters of group 3 cows. Figure 1 summarises the significant changes observed in mean values in the affected udder quarters of groups 2 and 3 cows in Paper II compared with the contralateral healthy udder quarters.

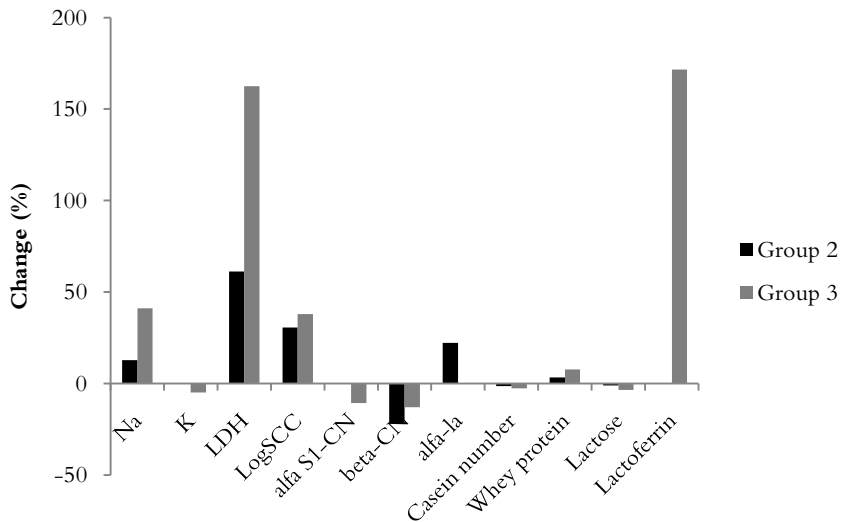


Figure 1. Significant percentage changes in mean values of parameters in the affected udder quarters of group 2 and 3 cows compared with the healthy contralateral udder quarter (Paper II).

Table 6 lists the changes detected in affected udder quarters (SCC > 100 000 cells/ml) compared with the contralateral healthy quarters (SCC < 100 000 cells/ml) in Papers I, II and IV. In all three papers there were significant changes in content of whey protein and lactose and SCC.

Table 6. *Changes in milk composition and milk yield in affected udder quarters (> 100 000 cells/ml) compared with the contralateral healthy quarters (< 100 000 cells/ml) in Papers I, II and IV. Arrows indicate significant changes (P<0.05)*

	Paper I	Paper II	Paper IV
Milk yield (kg)	↓	NS ¹	↓
Fat (%)	NS	NS	NS
Total protein (%)	↑	NS	↑
Whey protein (%)	↑	↑	↑
Casein (%)	↓	NS	NS
Lactose (%)	↓	↓	↓
Citric acid (%)	NS	NS	- ²
SCC (cells/ml)	↑	↑	↑

¹ NS = non significant

² Not measured

5.2 Day-to-Day Variation in Milk Composition in Healthy Cows

Paper III studied day-to-day variation in milk composition in healthy cows. The highest day-to-day variation was found in content of fat and the lowest in lactose content at both udder quarter and cow composite level. Day-to-day variation in fat content for udder quarter and cow composite samples was 7.7% and 7.2%, respectively. For lactose content the day-to-day variation was 0.9% for both udder quarter and cow composite samples. When day-to-day variation was studied within individual cows, it was found that this variation was equal for the four separate udder quarters in healthy cows. Figures 2 and 3 show the content of lactose and Figures 4 and 5 the content of fat during the study period in two different cows: Cow A, with subclinical mastitis in one udder quarter, and Cow I, a healthy cow.

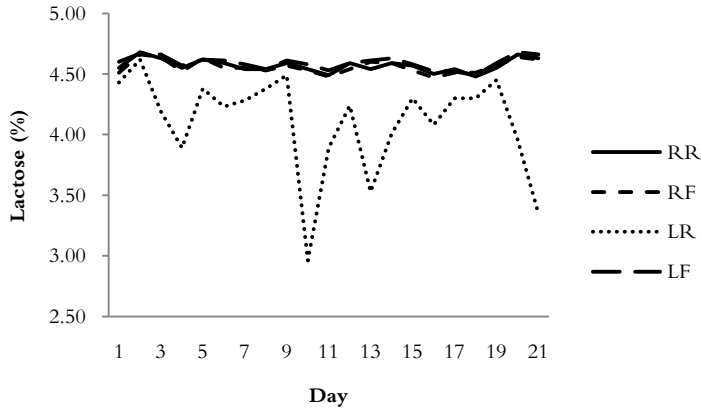


Figure 2. Concentration of lactose in all udder quarters of Cow A (which had subclinical mastitis in her left rear udder quarter with mean SCC of 6 300 000 cells/ml) at evening milking for the whole study period. RR = right rear udder quarter, RF = right front udder quarter, LR = left rear udder quarter and LF = left front udder quarter.

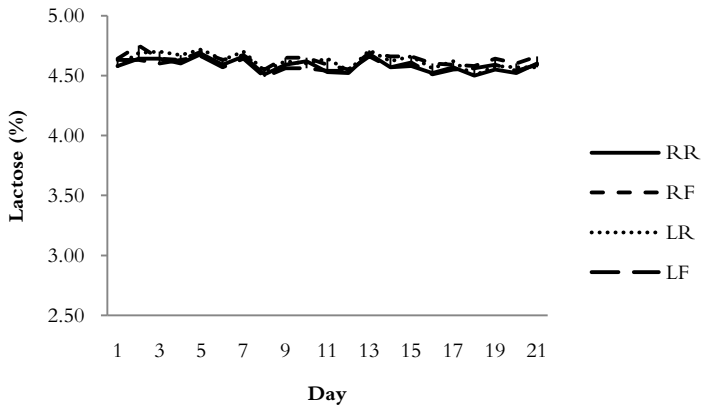


Figure 3. Concentration of lactose in all udder quarters of Cow I (all quarters tested bacteriologically negative during the trial period) at evening milking for the whole study period. RR = right rear udder quarter, RF = right front udder quarter, LR = left rear udder quarter and LF = left front udder quarter.

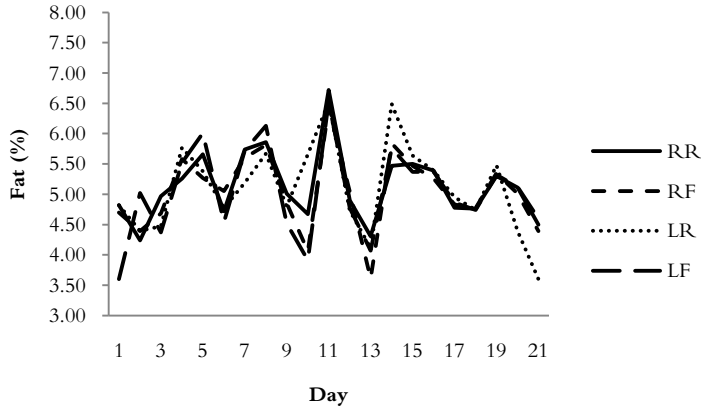


Figure 4. Concentration of fat in all udder quarters of Cow A (which had subclinical mastitis in her left rear udder quarter with mean SCC of 6 300 000 cells/ml) at evening milking for the whole study period. RR = right rear udder quarter, RF = right front udder quarter, LR = left rear udder quarter and LF = left front udder quarter.

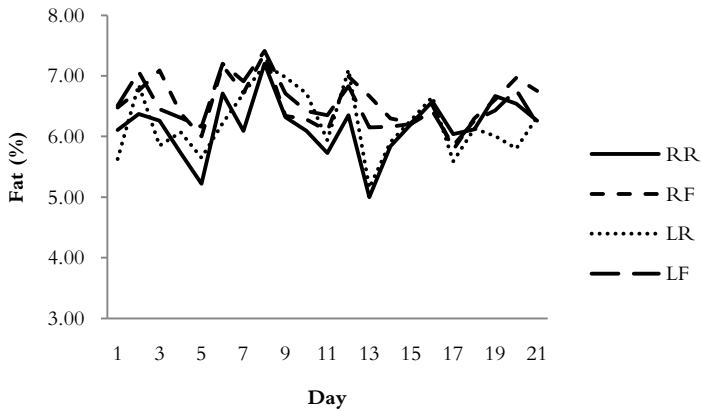


Figure 5. Concentration of fat in all udder quarters of Cow I (all quarters tested bacteriologically negative during the trial period) at evening milking for the whole study period. RR = right rear udder quarter, RF = right front udder quarter, LR = left rear udder quarter and LF = left front udder quarter.

5.3 Effect of Storage and Exclusion of Udder Quarter Milk

In all udder quarter samples in Paper IV, the level of SCC significantly affected the whey fraction, lactose, proteolysis and curd yield. The whey fraction, proteolysis and curd yield increased with elevated SCC, while the lactose content decreased. When affected udder quarters were compared with the contralateral healthy udder quarters, whey fraction, casein number and lactose differed significantly on all days of storage. Casein number and lactose content were higher and whey fraction content lower in healthy udder quarters. The content of total protein and proteolysis were significantly lower in milk stored for zero and two days and showed a tendency to decline after five days of storage in healthy udder quarters compared with affected. The content of casein showed a tendency to be lower in affected udder quarters compared with healthy on day two and five of storage. On day five of storage the curd yield showed a tendency to be higher in affected udder quarters compared with the contralateral healthy udder quarter. Figure 6 illustrates the mean values for all cows of whey fraction content in healthy and affected udder quarters for the different storage days. The mean SCC of affected and healthy udder quarters was 1 385 900 cells/ml and 17 900 cells/ml, respectively.

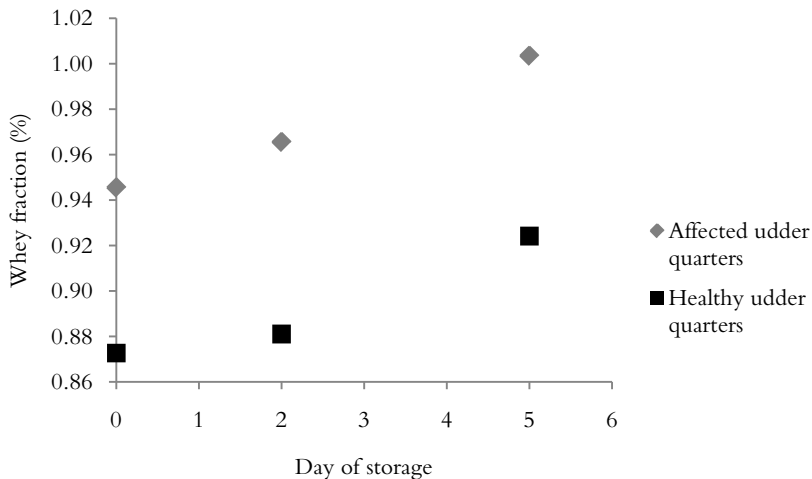


Figure 6. Mean values of whey fraction in affected udder quarters and contralateral healthy udder quarters of all cows on different days of storage.

When milk from individual udder quarters with elevated SCC was excluded, this significantly affected the content of total protein, whey fraction and lactose in milk at cow level. The total protein and whey fraction contents were lower in cow separated milk samples, while the content of lactose was higher. The mean values of milk composition, proteolysis and coagulation properties for the different storage days in cow composite and cow separated milk samples are presented in Table 7. No significant effects of the interaction between storage duration and exclusion of udder quarter milk could be seen in cow composite and cow separated milk samples.

Table 7. Mean values of milk composition, proteolysis and coagulation properties of cow composite and cow separated milk samples on different days of storage

	Storage day 0		Storage day 2		Storage day 5	
	Cow composite	Cow separated	Cow composite	Cow separated	Cow composite	Cow separated
Total protein (%)	3.28	3.27	3.29	3.28	3.32	3.29
Casein (%)	2.39	2.40	2.39	2.40	2.37	2.34
Whey fraction (%)	0.89	0.86	0.90	0.88	0.98	0.94
Casein number	0.73	0.74	0.74	0.74	0.71	0.71
Lactose (%)	4.76	4.82	4.77	4.83	4.77	4.84
Fat (%)	3.69	3.61	3.52	3.50	3.60	3.50
Proteolysis (eq Leu ¹)	0.54	0.58	0.52	0.50	0.59	0.53
Curd yield (g DM/g)	0.14	0.14	0.13	0.11	0.12	0.12
Coagulation time (min)	6.18	6.41	23.63	20.09	13.59	12.68

¹ equivalent mM leucine

In udder quarter, cow composite and separated cow milk samples there was a significant effect of storage duration on the content of casein and fat, which both decreased with increasing storage duration. In addition, there was an effect of storage on total protein, proteolysis and curd yield in udder quarter samples and whey fraction, casein number and coagulation time in cow composite and cow separated milk samples. The curd yield decreased, while the total protein and proteolysis increased during storage in udder quarter milk samples. In cow composite and cow separated milk samples the whey fraction content and coagulation time increased during storage but the casein number decreased.

The total bacteria count increased in the milk during storage. In udder quarter milk casein number, curd yield and content of lactose and fat decreased with increasing bacteria count, while proteolysis increased. In cow composite and cow separated milk samples the content of fat decreased with increasing bacteria count. The mean values of total bacteria count in udder

quarter milk samples for day 0, 2 and 5 was 3.61, 4.19 and 5.77 log cfu/ml, respectively. In cow composite and cow separated milk samples the mean values of total bacteria count was 3.88, 4.67 and 6.06 log cfu/ml for day 0, 2 and 5, respectively.

Total protein, casein, whey fraction, fat and curd yield were significantly affected by the interaction between SCC and duration of storage in udder quarter milk. This meant that these parameters reacted differently during storage depending on the SCC.

6 Discussion

It is a challenge for dairy farmers to deliver milk of high quality to the dairy. Many factors affect milk quality, for example the breed, diet, management and health of the cows. This thesis evaluated milk quality with focus on milk composition at udder quarter level in relation to SCC. The main aim was to increase our knowledge about milk quality alterations at udder quarter level and define the normal composition and its variation in healthy cows. A further aim was to investigate whether milk composition can be improved at cow level by separation of milk at udder quarter level. All this information is needed when assessing the possibility of introducing separation of milk at udder quarter level to increase the quality of milk delivered to the dairy.

When interpreting the results from these studies it is important to note that all cows used in the experiments were delivering milk to the dairy, none of them had clinical signs of mastitis and none was being treated with antibiotics.

6.1 Mastitis may go Unnoticed

Irrespective of milk composition or udder health, cow composite milk samples might hide alterations due to the dilution effect.

6.1.1 Milk Composition Changes in Milk with Relatively Low SCC

Although SCC was low in the experimental animals at cow composite level, individual udder quarters with elevated SCC and altered milk composition could be found in these cows. The affected udder quarters in group 1 (cow SCC < 100 000 cells/ml) of Paper I showed a higher content of total protein, whey protein and SCC, while casein number and lactose content were decreased compared with that in the healthy contralateral udder

quarters. However, the average SCC of the affected udder quarters was only 175 000 cells/ml. In Paper II, the affected udder quarters of cows in group 2, with an average SCC of 285 000 cells/ml, had lower casein number, content of lactose and β -CN, while the content of whey protein, sodium, LDH and α -la increased compared with that in the healthy contralateral udder quarters. The differences in milk composition between healthy and affected udder quarters were higher in group 3 than in group 2 in Paper II, indicating an effect of increasing SCC. These findings show that milk composition starts to change at low levels of SCC increase and continues to change with increasing SCC. The higher content of whey protein combined with the lower content of lactose and sodium might indicate increased permeability of the blood-milk barrier. The lower levels of β -CN in affected quarters of group 2 cows in Paper II could be an indication of increased proteolysis or decreased synthesis.

Some previous studies have reported alterations in milk composition and proteolysis at low SCC levels, but most research is carried out in cows with more severe mastitis. Le Roux *et al.* (1995) found that milk proteins were degraded at very low levels of SCC in udder quarter milk and that proteolysis began to occur at udder quarter SCC of only 250 000 cells/ml. Urech *et al.* (1999) noted changes in the proportion of casein and whey protein in milk from cows with subclinical mastitis and udder quarter SCC of approximately 300 000 cells/ml. In addition, a greater potential for proteolysis was found in these subclinical udder quarters. Tolle *et al.* (1971) found that milk composition starts to change already at SCC of 50 000 cells/ml in individual udder quarters. It can be concluded that milk composition can undergo changes at udder quarter level despite the level of SCC in cow composite milk being low, *i.e.* 100 000 cells/ml. These changes in milk composition might not be very severe but in the case of increased enzyme activity and higher proteolysis, even small changes can contribute to lower overall milk quality, especially during storage as seen in Paper IV.

6.1.2 Level of SCC for Healthy Cows

Many researchers have examined the level of SCC at which milk should be declared abnormal. Hamann (2003; 2002) suggests that milk at udder quarter level should not have SCC higher than 100 000 cells/ml to be defined as physiologically normal. Hillerton (1999) believes that udder quarter milk with SCC below 100 000 cells/ml should be defined as healthy. Deggweiler and Hess (1983) reported the SCC of healthy first parity cows to be approximately 20 000 cells/ml at udder quarter level. However, these are

mainly udder quarter levels and the normal procedure is to evaluate milk at cow level. When interpreting values of SCC it is extremely important to bear in mind the type of sample mentioned. SCC at udder quarter level cannot be interpreted in the same way as SCC at cow composite or bulk tank level. In addition, levels of SCC differ between foremilk samples and strip milk samples (Östensson, 1993; Paape & Tucker, 1966).

A relative high number of cows with SCC < 100 000 cells/ml have udder quarters with elevated SCC, *i.e.* > 100 000 cells/ml. For example, in Paper I we found that 29% of the cows studied were affected in this way. According to Berglund *et al.* (2004), 10% of cows with low SCC at cow level had individual udder quarters with increased SCC and 50% of these were infected with bacteria. In Paper II the group of cows with four healthy udder quarters (group 1) had an average cow composite SCC of 20 000 cells/ml, which is in agreement with the findings by Doggweiler and Hess (1983). However group 2, which had one quarter with elevated SCC, had an average cow composite SCC of 100 000 cells/ml. According to this, a SCC in cow composite milk of 100 000 cells/ml will not guarantee that the cow has four healthy udder quarters. The number of cows that were presumed to be healthy when evaluated at cow composite level but turned out not to be when studied at quarter level can be rather high. In addition, it has been observed in both cows and goats that declining milk production in one mammary gland is compensated for by increased milk secretion in the other gland, which could further enhance the dilution effect (Hamann & Reichmuth, 1990; Henderson & Peaker, 1983). Whether this phenomenon happens during mastitis in udder quarters of cows has not been fully evaluated.

Consequently, cows with deteriorated milk composition, elevated SCC and infected with bacteria can often go unnoticed when milk is evaluated at cow level, due to the dilution effect. This results in infected cows remaining undetected and continuing to be contagious to other cows, and also milk of lower quality entering the bulk tank without the farmer's knowledge. This ultimately leads to milk products of lower quality that are not attractive to consumers. Therefore evaluation of milk quality and udder health ought to be carried out at udder quarter level.

6.2 Detecting Milk with Deteriorated Milk Composition

Today, milk quality is often equated with SCC. However, the major use of SCC is for indicating udder health and therefore this parameter could be misinterpreted when evaluating milk quality. While SCC is often related to

changes in milk composition there may be alternative parameters that are better for this purpose.

6.2.1 Markers for Milk Composition

The use of SCC as a milk quality parameter, especially for processing properties, has been discussed. Barbano *et al.* (1991) found that SCC in bulk tanks is not the best parameter to measure the processing quality of milk. Leitner *et al.* (2008) indicated that SCC was a limited marker when milk was evaluated with respect to curd yield, especially after storage. In another study, those authors found that SCC in comparison to measurements of proteolysis of casein provided the poorest prediction of milk quality when cheese-making abilities were evaluated, and suggested that direct measurements of milk clotting time and curd firmness might provide the best prediction of milk quality for cheese-making (Leitner *et al.*, 2006). Bulk tank SCC is influenced by the amount of milk in the tank and thus the herd size (Emanuelson & Funke, 1991). Consequently two bulk tanks with a certain SCC can differ in milk quality. In light of these facts, a better method for evaluating overall milk quality and the processing properties of milk is needed. A rapid and accurate method for evaluating the content of casein in milk would be of great value. Since there may be better markers for milk quality than SCC, one disadvantage with our studies was that SCC was used for finding udder quarters with deteriorated milk composition. In the future, an alternative could be to use a more direct marker, such as casein or whey protein content to find milk with deteriorated milk composition, irrespective of udder health status.

Many efforts have been made to find alternative methods for evaluating milk quality. Åkerstedt *et al.* (2008) suggested that the major bovine acute phase proteins, haptoglobin and serum amyloid A, could predict the protein quality of milk. In this thesis (Papers I, II and IV), the content of lactose was shown to decline at udder quarter level when the content of whey protein increased. Lactose is normally a stable component of milk, with low day-to-day variation. These findings indicate that at udder quarter level, lactose content can be used as a marker for changes in milk composition. When LDH was analysed in udder quarter milk samples in Paper II, compositional changes were found when elevated values of LDH were detected. LDH was the only parameter (except SCC) at cow composite level that was significantly different between groups where the casein number differed. This suggests that LDH might indicate the compositional milk quality, in addition to acting as a marker for mastitis.

Irrespective of the constituent used for detection of deteriorated milk composition, if technical systems are used for deciding on automatic separation of milk, the sensitivity and specificity of the analytical method need to be high (Brandt *et al.*, 2010). Automatic separation of milk requires knowledge about the levels at which for the specific components analysed should be separated and also how this should be done.

6.2.2 Within-Udder Comparisons

As milk SCC is affected by various factors, such as lactation stage, lactation number, stress and season it can sometimes be difficult to decide from the SCC whether the milk is normal and the cow healthy (Harmon, 1994). However, Paper III found that the day-to-day variation in milk composition and SCC of healthy cows is small and equal within the four udder quarters. In another study, performed on the same material as Paper III, Åkerstedt *et al.* (2010) found generally higher day-to-day variations in the biomarkers LDH, NAGase, serum amyloid A and alkaline phosphatase compared with the compositional parameters used in Paper III. However, this study also found that within-udder comparisons are useful in detecting abnormalities. Similarly to Åkerstedt *et al.* (2010) and Paper III, Linzell & Peaker (1972) found that the day-to-day variation in milk components should be equal within the four udder quarters of healthy cows. However, the level of milk components differs naturally between individual cows, lactation number, lactation stage *etc.*. For example in Paper III a cow with quite a high lactation number (7) had slightly higher SCC than the other cows, but the day-to-day variation in SCC and the other milk components of the four udder quarters was low and no bacteria were found in the udder quarters, indicating that this cow was healthy. This further suggests that using a cut-off value can be misleading, since each cow has its own level. In general, the mean composition and variation in the four udder quarters of healthy cows are similar. This suggests that analysing milk at udder quarter level, especially repeated measurements, can be a sensitive method for detecting udder health and milk composition disturbances. It can also be an economical method if the milk components tested in repeated measurements at udder quarter are cheap to analyse.

6.3 Day-to-Day Variation in Milk Composition at Udder Quarter Level

As the automation of dairy production continues to increase, AM systems will become used to a greater extent on farms. AM systems involve udder quarter milking *i.e.* each udder quarter is milked separately, thus enabling milk components to be recorded at udder quarter level. A fundamental consideration in analysing milk components is to know the normal composition and variation in healthy animals, in order to recognise the alterations that can occur during disturbances. Previous studies have determined the day-to-day variation in milk composition and milk yield in cow composite milk samples (Millogo *et al.*, 2009; Quist *et al.*, 2008; Čandek-Potokar *et al.*, 2006; Rook *et al.*, 1992; Sjaunja, 1986; Syrstad, 1977; Gilbert *et al.*, 1973). However, with recording possible at udder quarter level, day-to-day variation in milk composition and milk yield was determined in Paper III.

The different levels of day-to-day variation found in the milk components in Paper III indicate that some milk components need more frequent recording than others to obtain reliable results. Fat content and milk yield showed high day-to-day variation, which is in agreement with earlier studies performed at cow level, and hence repeated sampling is needed (Quist *et al.*, 2008; Čandek-Potokar *et al.*, 2006; Rook *et al.*, 1992; Syrstad, 1977; Gilbert *et al.*, 1973). However, lactose and protein content showed less day-to-day variation, allowing less frequent sampling of these parameters. The day-to-day variation in lactose content was found to have the overall lowest variation, confirming findings by Čandek-Potokar *et al.* (2006), Sjaunja (1986) and Millogo *et al.* (2009) at cow level.

The common test routine today in the official milk recording is to analyse milk for composition and SCC at one or two milkings once a month. In healthy cows this can give reliable result, due to the low day-to-day variation of milk components within cow. However, as the day-to-day variation of milk components seemed to be higher in udder quarters with elevated SCC, sampling only at one or two milkings every month when cows are examined for udder health might give misleading results.

6.4 Interdependence between Udder Quarters?

Some studies have raised the question of whether the udder quarters within cows are interdependent, *i.e.* whether there are any reactions in the healthy udder quarters of cows with quarters affected by mastitis. This issue has to be considered when separation of milk at udder quarter level is being

discussed. If the neighbouring quarters to a quarter with high SCC are affected, the effect of separation of milk from the affected udder quarter will be impaired. Larsen *et al.* (2004) suggest that separation of individual udder quarter with high SCC cannot be recommended, since they found that the milk quality of the contralateral glands can be affected. Fragments of casein degradation were found not only in the infected quarters but also in the uninfected udder quarters of the same cow. Merle *et al.* (2007) found higher SCC and percentage of neutrophils in neighbouring quarters to infected quarters compared with quarters from cows with four healthy quarters. Further studies by Merle *et al.* (2008) confirmed this change in percentage of neutrophils and NAGase in healthy quarters of cows with an infected quarter compared with healthy quarters from healthy animals. Bansal *et al.* (2005) reported that electrical conductivity, SCC, NAGase and lactose differed significantly when healthy udder quarters of cows with at least one mastitic udder quarter were compared with healthy quarters of cows with four healthy quarters, indicating some interdependence between udder quarters.

This indicates that an infected udder quarter might influence the cell activity of neighbouring udder quarters. These studies suggest that the udder quarters of the same cow are dependent on each other and that there is a reaction in the healthy neighbouring quarters to the infected quarter. However, the anatomy of the cow's udder prevents milk moving from one udder quarter to another. Consequently, milk in one udder quarter with high SCC and deteriorated milk composition cannot pass to another quarter. This is supported by the results in this thesis, since significant differences in milk composition between healthy and affected udder quarters were frequently observed.

However, there is a common blood supply to the four quarters and blood circulates from one udder quarter to another (Akers, 2002). This suggests that there is communication between udder quarters through blood, rather than milk moving from one udder quarter to another. With our present level of knowledge, we cannot assume that the neighbouring udder quarters to an infected udder quarter are highly affected in milk composition, although we know that the udder quarters are not completely independent of each other. Hence, separation at udder quarter level will most likely improve milk composition, regardless of the degree of interdependence of udder quarters.

6.5 Effect of Storage on Milk Quality

The common procedure in dairy production is to send milk to the processing dairy every second day. Therefore it is highly important that the milk produced can be stored for some days before it is processed, without undergoing major spoilage due to storage. Paper IV, evaluated the storage characteristics of milk in relation to SCC. It has been shown in earlier studies that milk composition and processing properties of milk are affected by storage and SCC (Barbano *et al.*, 2006; Santos *et al.*, 2003; O'Brien *et al.*, 2001; Barbano *et al.*, 1991). In Paper IV, there were indications of milk with elevated SCC undergoing enhanced deterioration of some milk composition parameters in udder quarter milk samples due to storage. Total protein, casein, whey fraction, fat and curd yield were affected by both SCC and duration of storage, indicating an interactive effect of these spoiling factors during storage. However, the effect on curd yield is rather uncertain, since it seemed to be higher in udder quarter milk samples with elevated SCC.

During cold storage of milk, proteinases and lipases originating from psychrotrophic bacteria contribute to the degradation of protein and fat (Haryani *et al.*, 2003; Sørhaug & Stepaniak, 1997). It has been suggested that the enzymes from psychrotrophic bacteria might even make a higher contribution to the degradation during storage than the indigenous enzymes (Kelly *et al.*, 2006). In Paper IV, storage duration affected casein, whey fraction, casein number, fat, proteolysis, curd yield and coagulation time. This indicated a major impact of milk quality due to storage. The increasing total bacteria count due to storage duration further confirmed that this spoilage of milk was related to the increasing amount of bacteria, presumably psychrotrophic bacteria. However, five days, as tested in our study, is a long time of storage for raw milk. In practice, the milk is probably pasteurised within 24–36 h after arriving at the dairy and then further processed within 10 h of that¹. The duration of storage before processing, including storage on the farm and at the dairy, is probably enough for the milk to start to degrade, although it will probably not be as long as five days.

One way to improve the overall quality of milk supplied to the dairy would be to deliver milk more often, *i.e.* every day. In economic terms this might be worthwhile for milk used for processing, *i.e.* cheese and fermented milk products, since milk quality is particularly important in producing large quantities of these products at high quality. However, delivering milk to the

¹ Personal communication, T. Lund, 2009, Milko, Bollnäs.

dairy every day would increase the transportation cost and also contribute to negative effects on the environment, which make this practice questionable.

As milk quality is altered by both SCC and storage, it is important to produce milk from healthy cows and to reduce the duration of storage.

6.6 Yields of Milk Components in Relation to SCC

When evaluating compositional changes in relation to SCC, the common practice is to record the content of the milk parameters. However, it is ultimately the mass of milk components that determines the outcome of dairy products. Since the milk yield is decreased due to high SCC, this determines how the total yield of milk components is affected (Fox *et al.*, 1985; Miller *et al.*, 1983; Tolle *et al.*, 1971). The reduction in milk yield is often more significant than the changes in contents of milk components. It was observed in Paper I that the affected udder quarters always produced lower masses of the milk components analysed, although the differences were not always significant. The decrease in milk production related to high SCC is the major determinant of the yield of a milk component, especially when the percentage changes are small.

6.7 Economics of Separating Milk with High SCC

The question of whether milk should be separated to obtain higher milk quality is mainly an economic consideration. If the farmer can make a profit due to separating out milk with impaired composition, this could be one possibility to improve the finances of the business. The outcome of delivering milk with a higher quality to the dairy processor will mainly be decided by the payment system of the dairy. Payment for milk is generally made according to amount of milk delivered, fat content, protein content and SCC. Exclusion of milk with impaired composition would result in a lower amount but higher quality of milk delivered. Therefore the payment system needs to encourage this, since otherwise milk separation will not be profitable and will not be introduced on dairy farms. However, all excluded milk does not necessarily need to be discarded. It might be worthwhile having a quality separation system where milk with high quality is used for processing and consumption milk is delivered separately.

Nielsen (2009) simulated discarding milk with high SCC and found it never to be profitable, with none of the simulated scenarios giving any beneficial effect on the net return per cow-year. The problem is that the higher milk quality achieved does not pay enough to compensate for the

lower amount of milk supplied. The quality of milk supplied has a lower impact on income than the amount supplied milk. However, the simulation by Nielsen (2009) represented sorting of milk at cow level and this may have involved milk of good quality being discarded, since we know that it is very seldom that all four udder quarters of a cow are infected. Separation of milk at cow level would contribute to higher amounts of milk being discarded than separation of milk at udder quarter level.

6.8 Whether to Sort Milk at Udder Quarter Level

The calculations on separating milk from individual udder quarters with high SCC in Paper I showed that the bulk tank SCC could be decreased to a low level by only excluding 1.1% of the milk. However, the milk composition showed no changes. The small amount of milk excluded and the lower bulk tank SCC would probably lead to some increase in the payment for that milk. This was only a calculation, and this issue needs to be studied in practice to fully evaluate the effects. As the milk is stored for some days before processing, the effect of storage on milk also needs to be studied. However, if the outcome is as indicated in Paper I, that only the SCC is decreased and there are no effects on the milk composition, separation will not give any positive effect on actual milk quality. The milk payment system today is mainly based on the amount of milk and fat and protein content, which does not reward the production of milk with higher quality, for example a higher content of casein.

In a study in which milk from bacteria-free individual udder quarters was compared with milk from bulk tanks, it was found that the milk from the bulk tank deteriorated much faster than milk from individual udder quarters (Leitner *et al.*, 2008). This suggests that milk from subclinically infected cows in the bulk tank accounts for the difference in storage behaviour compared with milk from individual cows. A low number of cows producing milk with elevated SCC and deteriorated milk composition that enters the bulk tank will probably not affect the composition to a high extent. However, if many subclinically infected cows go unnoticed the milk from these infected udder quarters may contribute to lower milk quality in the bulk tank.

In Paper I, it was observed that 91% of cows in group 2 (cow SCC 100 000 - 300 000 cells/ml) and 72% of those in group 3 (cow SCC > 300 000 cells/ml) had at least one udder quarter with SCC < 100 000 cells/ml. These results suggest that cows with elevated SCC in cow composite milk samples probably have udder quarters with low SCC and

normal composition. This indicates that it is a waste of resources to discard all milk from these cows, whereas sorting milk at udder quarter level could be worthwhile. However, the European Union Directive 853/2004 states that milk from any cow presenting organoleptic or physico-chemical abnormalities is not permitted for use for human consumption (EC, 2004). This can be interpreted as meaning that all milk from cows presenting abnormalities in milk should be discarded, which will further prevent farmers separating milk from individual udder quarters.

In Paper IV the effect of sorting milk from individual udder quarters with elevated SCC was studied at cow level. It was observed that the total protein and whey fraction contents were lower and the content of lactose was higher in the milk when udder quarter milk with elevated SCC was excluded. Although the number of cows in this study was only 11, the results give an indication that there are some improvements in milk quality, at least at cow level, when milk from individual udder quarters with elevated SCC is excluded.

This thesis presents indications that milk quality could be improved by separating out milk from individual udder quarters with deteriorated milk composition. However, in order to deciding whether milk should be separated at udder quarter level, the effect of milk quality at bulk tank level needs to be evaluated. The total milk quality delivered from the dairy farm needs to be enhanced to justify separation at udder quarter level.

7 Main Findings and Conclusions

The results presented in this thesis show how milk composition is related to SCC at udder quarter level. The main conclusions from the studies are:

- In 29% of cows with SCC below 100 000 cells/ml at cow level, milk from individual udder quarters had elevated SCC and deteriorated milk composition.
- The majority of cow milk with SCC > 100 000 cells/ml contained individual udder quarter milk with low SCC. This milk could be utilised if milk were to be sorted at udder quarter level.
- Calculated bulk tank values after exclusion of individual udder quarter milk with high SCC indicated a reduction in SCC but no changes in milk composition.
- Milk yield losses should be considered when the contents of milk components are evaluated, since there will not be a major effect on the total yield of a milk component unless the milk yield is also affected.
- Deteriorated milk composition, with signs of proteolysis and decreased blood-milk barrier, can be found in individual udder quarters of cows producing milk with a low to moderate increase in SCC
- The lowest day-to-day variation in milk components at udder quarter and cow composite level was found in lactose and the highest in fat content.

- Within healthy cows, the day-to-day variation in milk components in the four udder quarters was similar, which suggests that within-udder comparisons can be useful in detecting alterations.
- Milk composition and processing properties are affected by both SCC and storage duration. Therefore milk should be obtained from healthy cows and the storage time of milk should be minimised.
- Excluding milk from udder quarters with elevated SCC and deteriorated milk composition gave a effect on content of total protein, whey protein and lactose at cow level.

8 Future Research

- This thesis focused on milk composition at udder quarter and cow composite level. However, for the farmer the composition, quality and quantity of bulk tank milk are essential for the financial outcome of milk production. Further studies are needed to evaluate the effect of excluding udder quarter milk with altered milk composition and elevated SCC at bulk tank level. The next step would be to evaluate the effect on milk composition at the dairy silo level, which is the milk that will actually be further processed.
- In order to detect milk with deteriorated milk composition, a reliable marker that is suitable for in-line analysis and can rapidly detect changes in milk quality is needed. The next step would be to investigate the critical levels at which milk should be excluded.

9 Populärvetenskaplig Sammanfattning

Dagens mjölkproduktion ställer höga krav på lantbrukaren att leverera mjölk av god kvalitet och hög kvantitet. Detta för att mejeriet ska kunna producera produkter av högsta kvalitet. För att producera stora volymer mjölk av god kvalitet krävs friska och välmående kor. Ett stort problem bland dagens mjölkkor världen över är mastit (juverinflammation), vilket orsakar både försämrade djurvälstånd och mjölk kvalitet. Mastit finns i två olika former, klinisk och subklinisk. Gemensamt för båda är att mjölkens sammansättning och kvalitet försämras, samt att det s.k. cellalet höjs. Den lägre kvaliteten på mjölken leder till sämre förutsättningar att tillverka processade mjölkprodukter, t.ex. ost. Den kliniska formen uppvisar tydliga symptom hos kon och även mjölken kan vara påverkad med klumpar och blodinblandning. Den subkliniska formen däremot visar inga synliga symptom i mjölken eller hos kon, utan måste detekteras med hjälp av analyser av mjölken. Detta gör att den subkliniska formen av mastit är svår att upptäcka för lantbrukaren, varvid mjölk med försämrade kvalitet ofta hamnar i mjölk tanken utan att lantbrukaren vet om detta. Följaktligen kommer denna mjölk att påverka tankmjölkens sammansättning och kvalitet.

Den tilltagande mekaniseringen av dagens mjölkproduktion har lett till en ökad användning av robotmjölkning s.k. automatiska mjölkningssystem (AMS). Mjölkningen är automatiserad i dessa system, vilket innebär att roboten själv identifierar kon, tvättar spenarna, utför mjölkningen och sedan desinficerar spenarna efter avslutad mjölkning. Mjölkningen i AMS sker på juverfjärdedelsnivå, vilket innebär att varje spene mjölkas var för sig.

Då kor som är drabbade av subklinisk mastit upptäcks väljer ofta lantbrukaren att separera mjölken från dessa kor så den inte hamnar i mjölk tanken. Idag görs detta på konivå, d.v.s. all mjölk från kon kasseras. Då mastit oftast bara drabbar en juverdel åt gången skulle dagens

robotmjölkning kunna möjliggöra frånskiljning av mjölk på juverfjärdedelnivå. Vilket skulle kunna bidra till en minskad mängd kasserad mjölk då enbart den juverdelen med mastit och försämrad mjölkkvalitet separeras.

Syftet med denna avhandling är att öka kunskapen om mjölkens sammansättning och kvalitet i relation till celltalet på juverfjärdedelnivå. Detta för att kunna komma närmre svaret på frågan om det är värt att frånskilja mjölk på juverfjärdedelnivå.

I den första av fyra delstudier studerades mjölken från varje enskild juverdel samt den sammanslagna mjölken från varje ko hos 90 kor vid ett mjölkningstillfälle. Detta för att undersöka hur mjölken sammansättning ser ut på juverfjärdedelnivå samt se förekomsten av juverdelar med förhöjda celltal och förändrad mjölksammansättning. Resultatet från denna studie visade att så många som 29% av korna, som hade ett celltal som låg under det som anses normalt på konivå, hade en eller flera juverdelar med förhöjt celltal. Dessutom var mjölkens sammansättning i dessa juverdelar påverkad. Hos kor som hade förhöjda celltal på heljuvernivå kunde det konstateras att en mycket stor andel av dessa kor hade enskilda juverdelar med låga celltal.

I den andra studien undersöktes mjölkens sammansättning mer detaljerat hos kor med förhöjda celltal i en juverdel. I denna studie kunde förändringar i mjölkens sammansättning påvisas i enskilda juverdelar redan vid förhållandevis låga celltal på heljuvernivå. Förändringen i mjölkens sammansättning såg ut att öka med ökat celltal.

I den tredje studien undersöktes mjölksammansättningens naturliga variation över tre veckors tid hos 9 friska kor på juverfjärdedels- och konivå. Denna studie visade att den lägsta och högsta variationen från dag till dag fanns i koncentrationen av mjölkkomponenterna laktos respektive fett. Dessutom visade resultaten att hos friska kor är variationen från dag till dag lika i de fyra juverdelarna, vilket tyder på att jämförelser på individnivå mellan de fyra juverdelarna skulle vara ett värdefullt redskap för att hitta förändringar i mjölkens sammansättning och kvalitet.

I den fjärde och sista studien studerades mjölkens sammansättning, proteinnedbrytning och processgenskaper på juverdels- och konivå hos kor med en eller två juverdelar med förhöjt celltal. Denna mjölk analyserades dels färsk, direkt efter mjölkning, och dels efter kylskåpslagring i två respektive 5 dagar. Detta för att simulera den lagring som idag sker i verkligheten på gården och mejeriet. Dessutom studerades effekten av att skilja ifrån mjölken från den eller de juverdelar som hade förhöjt celltal på konivå. Resultaten visade att såväl celltalet som lagringen påverkar mjölkens sammansättning och kvalitet. Celltalet påverkade lagringsförändringarna hos

koncentrationen av totalproteinet, kasein, vassleproteiner, fett samt ostutbyte. Genom att skilja ifrån juverdelar med förhöjt celltal och slå samman mjölk till ett nytt koprov påverkades halten av totalproteinet, vassleproteiner och laktos.

Sammanfattningsvis, det finns juverdelar med förhöjt celltal och förändrad sammansättning i mjölk från kor med relativt lågt celltal på konivå. Förändringarna i mjölken från dessa juverfjärdedelar är svåra att påvisa såvida mjölken inte analyseras på juverfjärdedelsnivå. Provtagning och registrering av parametrar på juverfjärdedelsnivå är ett mycket bra verktyg för att hitta förändringar i mjölkens sammansättning. Detta eftersom mjölkens sammansättning och dess variation är lika hos de fyra juverdelarna hos en frisk ko. Innehållet av totalproteinet, vassleproteiner och laktos hos mjölken från enskilda kor går att påverka genom att separera juverfjärdedelar med förhöjt celltal och förändrad sammansättning. För att motivera frånskiljning av mjölk på juverfjärdedelsnivå behöver det kunna påvisas en förbättring av mjölk kvaliteten på tanknivå, då det är denna mjölk som levereras till mejeriet och den som lantbrukaren får betalt för. Det behövs därför fler studier för att visa om separeringen av mjölk på juverfjärdedelsnivå kan påverka den totala kvaliteten av mjölken på tanknivå. Om det skulle visa sig att separering av mjölk på juverfjärdedelsnivå skulle kunna förbättra tankmjölken är det en framtidsvision att kvalitetssortera mjölken redan på gården. Detta för att kunna leverera mjölk av mycket hög kvalitet till t.ex. osttillverkning samt övrig mjölk till konsumtionsmjölk.

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