# New Markers of Bulk Milk Quality in Relation to Mastitis

## Studies on Polymorphonuclear Leukocytes and α-Lactalbumin

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# New Markers of Bulk Milk Quality in Relation to Mastitis. Studies on Polymorphonuclear Leukocytes and α-Lactalbumin


## Abstract

All dairy processors depend on a continuous supply of high-quality bulk milk from milk producers to be able to distribute liquid milk and dairy products. Mastitis, inflammation of the bovine udder, is the most common disease in dairy cows, and leads to altered milk composition and impaired milk quality. The somatic cell count (SCC) is currently used as a marker for udder health, and indirectly, for bulk milk quality, but because it is somewhat insensitive and unspecific, there has been an interest to find alternative markers.

The main objective of this thesis was to acquire more knowledge about two potential markers for bulk milk quality: polymorphonuclear leukocyte count (PMNC) and α-lactalbumin (α-LA). Another objective was to study if the combination of α-LA, haptoglobin (Hp) and serum amyloid A (SAA) in an acute phase index (API) could be useful as an alternative marker for bulk milk quality.

Bulk milk samples were collected from Swedish dairy farms and analyzed for PMNC, α-LA, SCC, haptoglobin (Hp), serum amyloid A (SAA), fat, lactose, total protein and casein contents, casein number, protein composition, proteolysis and coagulating properties. An API was calculated for each sample by combining results on α-LA, Hp and SAA.

Samples with high PMNC had a lower casein number than samples with low PMNC, while samples with high SCC had lower lactose and casein contents, lower casein number and more proteolysis than low SCC samples. There was no significant difference in the inflammatory markers SCC, PMNC, Hp and SAA between milk samples containing low, medium or high concentrations of α-LA. Differences between α-LA groups were, however, found in some milk quality parameters as high α-LA concentration was related to low concentrations of $\alpha_{s1}$-, $\alpha_{s2}$- and β-casein, and high concentrations of lactose and β-lactoglobulin. High API was related to low lactose content and casein number. In conclusion, PMNC and α-LA were not considered more useful markers of inflammation and milk quality in bulk milk than SCC, which is currently used.



*Keywords:* bulk milk quality, polymorphonuclear leukocyte, alpha-lactalbumin, acute phase protein, haptoglobin, serum amyloid A, somatic cell count, mastitis, protein composition, casein content



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# Dedication

To people who enjoy milk

*Scire tuum nihil est nisi te scire hoc sciat alter?*
Persius

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# List of Publications

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

I Wickström, E., Persson Waller, K., Östensson, K., Lindmark-Månsson, H. and Sternesjö, Å. (2009) Relationships between somatic cell count, polymorphonuclear leukocyte count and milk quality parameters in bulk tank milk. *Journal of Dairy Research,* 76(2), 195-201.

II Wickström, E., Persson Waller, K., Lindmark-Månsson, H. and Sternesjö, Å. (2009) Relationships between α-lactalbumin and milk quality parameters in bulk milk (manuscript).

The contribution of Erik Wickström to the papers included in this thesis was as follows:

I Participated in collection of bulk milk samples and performed parts of the analyses. Refined a method for direct differential cell counting. Main person responsible for statistical analysis and writing the manuscript.

II Planned the experiments together with supervisors and performed parts of the analyses, including protein composition. Main person responsible for statistical analysis and writing the manuscript.

# Abbreviations

| | |
|---|---|
| α-LA | Alpha-lactalbumin |
| API | Acute phase index |
| APP | Acute phase protein |
| β-LG | Beta-lactoglobulin |
| ELISA | Enzyme-linked immunosorbent assay |
| Hp | Haptoglobin |
| HPLC | High performance liquid chromatography |
| LOD | Limit of detection |
| PMN | Polymorphonuclear leukocyte |
| PMNC | Polymorphonuclear leukocyte count |
| SAA | Serum amyloid A |
| SCC | Somatic cell count |

# 1 Introduction

Milk has been an important part of human nutrition for thousands of years, providing energy, proteins with high biological value, vitamins and minerals. In Sweden, about 30% of the total milk production is sold as liquid milk, and the rest is processed into dairy products, such as cheese, butter, fermented milk and milk powder (SCB, 2009).

For all dairy products, especially those with a long shelf life, the milk composition of the raw bulk milk is of great importance. In dairy cows with mastitis, inflammation of the udder, the synthesis of milk components decreases and components from blood leak into the milk. This will have adverse effects on the quality of the raw milk (Auldist & Hubble, 1998).
Mastitis is associated with an increased somatic cell count (SCC) in milk. For several decades, SCC has been used as a marker for udder health in quarter and cow composite milk, and indirectly, for bulk milk quality. The producers' milk price will depend on the composition and the SCC of the bulk milk. However, the SCC is also influenced by factors apart from the udder health, and therefore, there is an interest in finding new markers for milk quality (Pyörälä, 2003). Among others, the polymorhonuclear leukocyte count (PMNC) and the alpha-lactalbumin (α-LA) content have been suggested as alternative markers (Caffin et al., 1985; Kelly et al., 2000).

## 1.1 Milk Composition

The major components of milk are given in Figure 1. The composition may vary depending on breed, season and feeding regime. In bulk milk, the contents of the major nutrients fat, protein and lactose are approximately 4.3%, 3.4% and 4.8%, respectively (Swedish Dairy Association, 2009; Walstra et al., 2006).

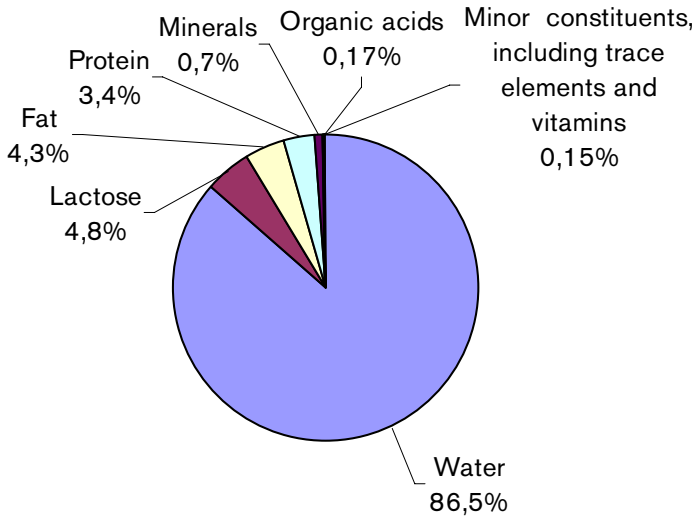


*Figure 1.* Approximate composition of milk (Swedish Dairy Association, 2009; Walstra et al., 2006).

Most (98%) of the milk fat consists of triglycerides, and the rest is mainly phospholipids, cholesterol and carotenoids. Milk fat is unique in its high content of short chain fatty acids with 4-10 carbon atoms (Jensen, 2002). As a consequence of feeding regime, the fatty acid composition varies depending on season. This holds true especially for the essential fatty acids linoleic and linolenic acid, which tend to increase during the summer (Lock & Bauman, 2004).

Milk proteins are made up of caseins (~80%) and whey proteins (~20%). As the caseins form the cheese curd, they constitute the most important milk proteins for the dairy industry. The caseins, mainly composed of $\alpha_{s1}$-, $\alpha_{s2}$-, β- and κ-casein in an approximate ratio of 4:1:4:1, precipitate after rennet addition or in an acidic solution (Davies & Law, 1980; Farrell et al., 2004; Walstra & Van Vliet, 1986). In response to rennet addition, the negatively charged tail of the κ-casein, the glucomacropeptide, is cleaved off. This will prevent the casein micelles to repel each other and instead they aggregate and form a strong coagulum, i.e. the cheese curd. The coagulum formed by lowering the pH of the solution is not as strong as the coagulum produced after addition of rennet. Instead of aggregation of the casein micelles, the individual caseins are liberated into the serum phase of the milk where they aggregate (Fox et al., 2004).

The whey proteins include β-lactoglobulin (β-LG; 50%), α-LA (20%), immunoglobulins (11%), bovine serum albumin (6%) and others (13%) (Farrell et al., 2004). For a long time, the whey proteins have been considered to be a less significant ingredient in dairy production, but in recent years they have attracted interest because of the high biological value of their amino acid composition (Smithers, 2008).

Lactose is a disaccharide consisting of glucose and lactose and it is the major carbohydrate in milk. Compared to other mammals, bovine milk has a relatively high concentration of lactose, but the content is still 30% lower than in human milk (Sjaastad et al., 2003). Being the main osmotic component, lactose regulates the volume of the secreted milk. As one of two components of the lactose synthase complex, α-LA is required for the production of lactose (Caffin et al., 1985).

Milk also contains high amounts of many important vitamins and minerals, such as vitamin A and D, calcium and phosphorous (Miller et al., 2000).

## 1.2 Mastitis in Dairy Production

Mastitis is the most common disease in dairy cows, accounting for almost half of all veterinary-treated diseases in Swedish dairy cows (Swedish Dairy Association, 2007). Mastitis can be caused by mechanical stress, but the most common way to acquire the disease is by bacterial infection through the teat canal. This initiates an inflammatory reaction, which leads to decreased synthesis of milk components and an increased leakage in the tight junctions at the blood-milk barrier. This implies that less e.g. lactose and caseins are synthesized, and that levels of whey proteins and salts will increase in milk (Pyörälä, 2003; Sandholm et al., 1995; Table 1). During mastitis, there is also a large increase of proteolytic and lipolytic enzymes and somatic cells in the milk. The cells may both release enzymes and stimulate enzymes present (Paape et al., 2003), such as the major proteolytic enzyme in milk, plasmin (Bastian & Brown, 1996).

Mastitis can appear in clinical or subclinical form. Clinical mastitis is characterized by visual abnormalities of the milk and the udder. Clots, and even blood, can be found in the milk and the udder may be swollen, hard and painful. Clinical mastitis is relatively easy to detect and the milk from affected cows must not be included in the bulk milk (Sandholm et al., 1995). In cows with subclinical mastitis, no visible signs can be observed, and it is therefore common that this milk is delivered to the dairy plant.

Both types of mastitis will result in changes in the milk composition, but to a varying degree depending on the severity of the disease (Pyörälä, 2003).

Economically, mastitis is a nemesis for farmers in many aspects: medication costs, discarded milk during and shortly after treatment, loss of milking days, reduced milk production, reduced milk price, increased labor and increased recruitment costs due to culling. Mastitis is also costly for the dairy processors as it will affect the quality of dairy products, leading to e.g. poor-coagulating milk during cheese production and reduced shelf life of dairy products (Barbano et al., 1991; Fernandes et al., 2008). Consequently, it is of great importance to have sensitive markers to distinguish milk with an altered composition due to mastitis.

Table 1. *Main changes in the production and composition of milk caused by mastitis (from Pyörälä, 2003). Number of pluses and minuses indicates the degree of decrease/increase for a specific component*

| Decrease | | Increase | |
|---|---|---|---|
| Quarter milk yield | -(--) | Somatic cell count | +++ |
| Dry matter | - | Whey proteins | +++ |
| Lactose | - | Bovine serum albumin | + |
| Fat | - | Immunoglobulins | +++ |
| Long-chained fatty acids | - | κ-casein | +(+) |
| Total casein | -- | Proteose peptones | ++ |
| $\alpha_{s1}$-casein | -- | Free fatty acids | ++ |
| β-casein | --- | Short-chained fatty acids | + |
| α-lactalbumin | - | Sodium | ++ |
| β-lactoglobulin | --- | Chloride | ++ |
| Calcium | --- | Lactate | +++ |
| Magnesium | --- | *Enzyme activity* | |
| Phosphorous | --- | Lipase | ++ |
| Zinc | - | Lysozyme | +++ |
| Potassium | - | N-acetyl-β-D-glucosaminidase | +++ |
| | | β-glucuronidase | +++ |
| | | Plasmin | +++ |

## 1.3 Markers for Bulk Milk Quality in Relation to Mastitis

The SCC is currently used world wide as a direct marker of udder health and an indirect marker of milk quality in the dairy industry, and it is considered the "golden standard" and reference method. The SCC can, however, be affected by other factors than mastitis, e.g. number of

lactations, stage of lactation, season and milking frequency. Thus, it is not the ultimate marker of milk quality, which has resulted in studies searching for more sensitive and specific markers (Pyörälä, 2003). Some markers, e.g. electrical conductivity and lactate dehydrogenase, are used today to detect mastitis on cow composite level in commercial farms, and other markers, e.g. N-acetyl-β-D-glucosaminidase (NAGase), are mainly used in research studies. Recent studies on the acute phase proteins (APP) haptoglobin (Hp) and serum amyloid A (SAA) have indicated that presence of Hp and SAA is associated with impaired milk quality, e.g. lower casein content and increased proteolysis, both at cow composite (Åkerstedt et al., 2008) and bulk milk level (Åkerstedt et al., 2009). Other parameters that have been suggested as potential markers for bulk milk quality are PMNC (Kelly et al., 2000; Pillai et al., 2001; Vangroenweghe et al., 2002; Le Roux, 2003; O'Brien et al., 2003) and α-LA content (Bortree et al., 1962; Caffin et al., 1985).

### 1.3.1 Existing Standard – the Somatic Cell Count

During mastitis, the SCC increases significantly due to an influx of somatic cells from the blood into the milk, indicating that the milk composition is adversely affected (Shukken et al., 2003). According to European Union regulations, the bulk milk SCC must not exceed 400 000 cells/mL (EC, 2004), but in Sweden, many dairy cooperatives reward farmers that deliver milk with less than 200 000 cells/mL. Numerous studies have shown that high SCC is associated with changes in milk composition in quarter, cow composite and bulk milk (for a review, see Auldist & Hubble, 1998), such as decreased contents of lactose (Kitchen, 1981), caseins (Barbano et al., 1991), and an increase of sodium (Rogers et al., 1989). Deterioration of dairy product quality has also been associated with elevated SCC, e.g. increased rennet clotting time and reduced curd yield (Leitner et al., 2006; Politis & Ng-Kwai-Hang, 1988).

### 1.3.2 Markers Evaluated in this Study – Polymorphonuclear Leukocytes and α-Lactalbumin

There are four types of somatic cells in milk: macrophages, lymphocytes, polymorphonuclear leukocytes (PMN) and epithelial cells, the macrophages being the dominating cell type in healthy udder quarters (Schröder & Hamann, 2005). At the onset of an infection, PMN migrate across the blood-milk barrier and serve as the first line of cellular defence by phagocytosing foreign particles like bacteria. Consequently, PMN is the cell

type that accounts for most of the large increase in SCC during mastitis, and in severe cases >90% of all somatic cells are PMN (Saad & Östensson 1990; Kehrli & Shuster, 1994: Kelly et al., 2000).

PMN may cause direct adverse effects on milk quality, such as increased proteolysis (Ballou et al., 1995; Politis, 1996; Haddadi et al., 2006). This is mediated by up-regulation of cytokines due to PMN enzymes (e.g. elastase and collagenase) and stimulation of plasmin activity resulting in increased protein degradation, potentially shortening shelf life of liquid milk and reducing cheese curd yield (Barbano et al., 2006; Hurley et al., 2000; Le Roux, 2003; Somers et al., 2003; Mehrzad et al., 2005; Haddadi et al., 2006; Kelly et al., 2006; Paape et al., 2003). Because of the negative effects on milk components associated with PMN, the PMNC has been suggested as an alternative marker for milk quality (Kelly et al., 2000; Le Roux, 2003; O'Brien et al., 2003; Pillai et al., 2001; Vangroenweghe et al., 2002), but such studies have so far not been performed.

In the course of mastitis, the synthesis of many milk components decreases in varying degrees. The whey protein α-LA has been shown to decrease concurrently with an increase in SCC as a response to mastitis (Auldist & Hubble, 1998; Bortree et al., 1962; Harmon et al., 1976). α-LA is part of the lactose synthase complex, which plays an important part in regulating the lactose content, and since lactose is the main osmoregulator in milk, α-LA indirectly influences milk yield (Caffin et al., 1985). Hogarth et al. (2004) used a proteomic approach to identify new diagnostic markers for bovine mastitis. In agreement with previous studies, a reduction in the α-LA concentration in mastitic whey was observed. α-LA was suggested to be a negative APP in bovine milk since the concentration decreases, potentially as a consequence of the onset of the acute phase response. In humans, several negative APP have been identified, such as albumin, transferrin and transthyretin (Ritchie et al., 1999), but none has so far been described to have the same function in bovine milk. α-LA might play a role as a negative APP in milk and could potentially be used as an indicator for milk quality, but this has never been investigated in bulk milk.

# 2 Aims of Thesis

The main objective of this thesis was to acquire more knowledge about two potential markers for bulk milk quality: PMNC and α-LA. This was achieved by the following specific aims:

1. To study relationships between PMNC, SCC and milk quality parameters in bulk milk (**Paper I**).

2. To study relationships between α-LA, inflammatory markers and milk quality parameters in bulk milk (**Paper II**).

3. To evaluate an index combining α-LA, Hp and SAA as an alternative marker for bulk milk quality (**Paper II**).

# 3 Materials and Methods

This section is a summary of the analyses used in the studies. More detailed descriptions are found in **Papers I & II**.

## 3.1 Sample Collection

In **Papers I & II**, bulk milk samples from 91 Swedish farms were collected in collaboration with the Milko Dairy Cooperative. Each sample was made up of commingled milk from two morning milkings and two afternoon milkings. Samples for analyses of PMNC, α-LA, SCC, Hp, SAA, contents of total protein, casein, fat and lactose, casein number, individual protein composition, proteolysis and rheological properties were transported to the laboratory the day of collection. All analyses were carried out on fresh milk except for Hp, SAA, proteolysis and individual protein composition, where samples were frozen and stored at -70$^{o}$C until analysis. In **Paper I**, 228 additional bulk milk samples were collected from the Swedish milk grading laboratory (Eurofins Steins, Jönköping). These were only analyzed for SCC and PMNC.

## 3.2 Markers of Inflammation

Bulk milk SCC (**Papers I & II**) was analyzed using direct fluorescence-based cell counting (Fossomatic 5000, Foss, Hillerød, Denmark) and PMNC (**Papers I & II**) was determined using cell staining and direct microscopy. Hp (**Paper II**) was analyzed using an optical biosensor assay based on the strong interaction between Hp and hemoglobin. Depending on the level of Hp in the sample, different amounts of added, non-inhibited hemoglobin will bind to covalently bound Hp on the sensor surface, and give rise to a response signal inversely proportional to the change in mass on the sensor

surface. SAA (**Paper II**) was analyzed with a commercial ELISA (PhaseTM Serum Amyloid A Assay, Tridelta Development Ltd, Wicklow, Ireland). An acute phase index (API) was calculated as Hp [mg/L] x SAA [mg/L] / α-LA [g/L] and all values of Hp and SAA lower than 0.3 mg/L was set to limit of detection (0.3 mg/L).

## 3.3 Milk Quality Parameters

Total protein, fat and lactose contents (**Papers I & II**) were determined by mid-infrared spectroscopy (Fourier Transform Instrument, FT 120, Foss). Casein content (**Papers I & II**) was determined indirectly according to Åkerstedt et al. (2008). In brief, whey proteins were determined after coagulation of caseins by rennet addition. Casein number was obtained by dividing casein content by total protein content and multiplying by 100.

Reversed-phase HPLC was used to determine milk protein composition (**Paper I**) according to Hallén et al. (2009). $\alpha_{s1}$-, $\alpha_{s2}$-, β- and κ-casein, and the whey proteins α-LA and β-LG were separated using a BioBasic-4 $C_4$-column (Thermo Electron Corporation, Runcorn, UK). A gradient program was used at room temperature with Eluent A (10% acetonitrile in ultrapure water and 0.1% trifluoroacetic acid) and B (10% ultrapure water in acetonitrile and 0.1% trifluoroacetic acid) as mobile phase. Separations were carried out with a flow rate of 0.3 mL/min.

Proteolysis (**Papers I & II**) was analyzed using a fluorescamine method (Wiking et al., 2002), where the fluorescence resulting after coupling fluorescamine to free amino terminals was measured by a LS-50B Luminescence Spectrophotometer (Perkin Elmer Corporation, Norwalk, CT, USA).

Clotting time and curd strength (**Papers I & II**) were measured with a Bohlin VOR Rheometer (Malvern Instruments Nordic AB, Uppsala, Sweden) according to Åkerstedt et al. (2008). Rennet was added to milk and clotting time (s) was determined as the time from rennet addition to the the coagulum attained a strength of 5 Pa. Curd strength (Pa) was measured 25 min after rennet addition.

## 3.4 Data Analyses

Pearson correlation and linear regression was used to assess relationships between SCC and PMNC (SAS Institute Inc., version 9.1, Cary NC, USA; Minitab Inc., version 15, State College, PA, USA). To investigate relationships between SCC, PMNC, α-LA, API and milk quality

parameters, bulk milk samples were categorized as below the 25th percentile (low), the 25th to the 75th percentile (medium) and above the 75th percentile (high) for SCC, PMNC, α-LA and API, respectively. Differences in milk quality parameters between groups of SCC, PMNC, α-LA and API were analyzed using the Student's unpaired *t* test and the chi-square test. SCC, PMNC, API and proteolysis were log-transformed to obtain normal distribution. A level of $P < 0.05$ was considered significant.

# 4 Results and Discussion

## 4.1 Polymorphonuclear Leukocyte Count as a Marker of Bulk Milk Quality in Relation to Mastitis

Our results (**Paper I**) showed that samples with high PMNC had lower casein number than samples with low PMNC (Table 2), but no other milk quality parameter differed significantly in relation to PMNC. The SCC, however, was related to several milk quality parameters (Table 2) as the contents of lactose and casein, and the casein number were lower in the high SCC group compared with the low SCC group, while it was the opposite for proteolysis.

Table 2. *Differences in quality parameters between bulk milk samples with low, medium and high polymorphonuclear leukocyte count (PMNC) and somatic cell count (SCC), respectively. Means±SD within a row with different superscripts were significantly different (P<0.05). Milk quality parameters where no significant differences were observed are not shown*

| Parameter | Low PMNC[1] | Medium PMNC[2] | High PMNC[3] |
|---|---|---|---|
| N | 23 | 45 | 23 |
| PMNC (× 1000/mL) | 15.45±6.73[a] | 43.66±13.07[b] | 137.82±81.16[c] |
| SCC (× 1000/ml) | 80.61±29.96[a] | 157.89±47.68[b] | 381.83±243.30[c] |
| Lactose (g/100 ml) | 4.63±0.13 | 4.62±0.11 | 4.58±0.07 |
| Casein content (g/100 ml) | 2.61±0.16 | 2.57±0.14 | 2.56±0.08 |
| Casein number | 73.22±0.70[a] | 72.98±0.67[a] | 72.61±0.52[b] |
| Proteolysis (mM) | 1.05±0.07 | 1.07±0.06 | 1.07±0.07 |

| Parameter | Low SCC[1] | Medium SCC[2] | High SCC[3] |
|---|---|---|---|
| N | 23 | 45 | 23 |
| SCC (× 1000/ml) | 74.70±22.40[a] | 155.60±31.25[b] | 392.22±237.10[c] |
| PMNC (× 1000ml) | 16.66±8 270[a] | 45.33±17 850[b] | 133.35±84.80[c] |
| Lactose (g/100 ml) | 4.65±0.13[a] | 4.61±0.10[ab] | 4.57±0.08[b] |
| Casein content (g/100 ml) | 2.63±0.16[a] | 2.58±0.13[ab] | 2.54±0.11[b] |
| Casein number | 73.17±0.69[a] | 73.06±0.56[b] | 72.51±0.70[b] |
| Proteolysis (mM) | 1.03±0.06[a] | 1.07±0.06[ab] | 1.08±0.07[b] |

[1] <25th percentile of the sample values
[2] 25th–75th percentile of the sample values
[3] >75th percentile of the sample values

Thus, our data could not support that PMNC is a better marker for bulk milk quality than SCC, as previously proposed (Kelly et al., 2000; Pillai et al., 2001; Vangroenweghe et al., 2002; Le Roux, 2003; O'Brien et al., 2003). PMN account to a large degree for the increase in SCC during mastitis, especially in the acute phase, and the PMNC and SCC were highly correlated (r=0.849; $P < 0.001$; data not shown). However, most milk that is delivered to the dairy processor comes from healthy udder quarters. Although milk from subclinically affected quarters will enter the bulk milk, this milk contains less SCC and a lower proportion of PMN than milk from clinical cases (Saad & Östensson 1990; Kehrli & Shuster, 1994: Kelly et al., 2000).

SCC had a significant influence on bulk milk quality in our study, which could not be referred to the PMNC. There is an array of proteolytic enzymes in PMN, such as cathepsin C and G, collagenase and elastase (Kelly

et al., 2006). However, in bulk milk, the proportion of PMN is low, and therefore, it might be of interest to investigate the importance of other cell types present in milk. In low SCC milk, the dominating proteinases are the major indigenous proteinase plasmin and cathepsin D (Barry & Donnelly, 1981), and the latter is mainly regarded as a macrophage proteinase (Cohn, 1975). It has also been reported that macrophages have much higher proteinase activity than PMN (Verdi & Barbano, 1991). For that reason, and because macrophages are much more abundant than PMN in low SCC bulk milk, they are an interesting candidate for further research.

## 4.2 α-Lactalbumin as a Marker of Bulk Milk Quality in Relation to Mastitis

When bulk milk samples were divided into groups according to α-LA, no significant differences between groups were seen in relation to the inflammatory markers PMNC, SCC, Hp and SAA (**Paper II**). However, increasing α-LA was related to numerically lower numbers of cells and concentrations of acute phase proteins. Previous studies have shown that α-LA is negatively correlated to SCC (Auldist & Hubble, 1998; Bortree et al., 1962; Harmon et al., 1976). These studies were performed on quarter and cow composite milk samples, and consequently, the dilution effect was less pronounced compared to the situation for bulk milk.

Åkerstedt et al. (2009) suggested that Hp and SAA may serve as markers for bulk milk quality. In their study, presence of Hp was related to increased proteolysis and lower contents of casein and lactose and presence of SAA was related to increased whey protein content and lower lactose content.

Significant differences in several milk quality parameters (**Paper II**) were found between α-LA groups (Table 3). Milk with low α-LA concentration contained less lactose and β-LG compared to milk with high α-LA concentration. In contrast, the contents of $\alpha_{s1}$-, $\alpha_{s2}$- and β-casein were highest in the low α-LA group. α-LA is one part of the enzyme lactose synthase, which is of major importance for lactose synthesis. Several studies (Nielsen et al., 2005; Bleck et al., 2009; Sarikaya et al., 2006) have shown a strong association between α-LA and lactose in bovine milk, but none have to our knowledge, like ours, been conducted on bulk milk. α-LA was related to several individual caseins and whey proteins. However, relationships between α-LA concentration and total protein and casein contents, and casein number supporting the data on the individual proteins were not found. Therefore, these results must be interpreted cautiously.

Table 3. *Milk quality parameters (mean±SD) in bulk milk samples with low, medium and high α-lactalbumin (α-LA) concentrations. Means within a row with different superscripts were significantly different (P < 0.05)*

| Bulk milk quality parameters | Low α-LA[1] (n=23) | Medium α-LA[2] (n=45) | High α-LA[3] (n=23) |
|---|---|---|---|
| Lactose (g/100 mL) | 4.57±0.10[a] | 4.62±0.10[ab] | 4.63±0.09[b] |
| Casein content (g/100 mL) | 2.56±0.09 | 2.60±0.15 | 2.57±0.11 |
| Casein number | 72.83±0.50 | 72.93±0.69 | 73.11±0.63 |
| $\alpha_{s1}$-casein (g/L) | 9.42±1.24[a] | 9.45±1.48[a] | 9.16±0.80[b] |
| $\alpha_{s2}$-casein (g/L) | 3.62±0.71[a] | 3.63±0.72[ab] | 3.55±0.93[b] |
| α-lactalbumin (g/L) | 1.04±0.06[a] | 1.21±0.03[b] | 1.30±0.03[c] |
| β-casein (g/L) | 16.14±1.87[a] | 16.09±2.93[ab] | 15.73±1.85[b] |
| κ-casein (g/L) | 4.09±1.24 | 4.55±1.16 | 4.46±0.93 |
| β-lactoglobulin (g/L) | 4.24±0.25[a] | 4.29±0.43[b] | 4.29±0.23[b] |
| Proteolysis (mM) | 1.14±0.15 | 1.17±0.18 | 1.13±0.17 |

[1] <25th percentile of the sample values
[2] 25th-75th percentile of the sample values
[3] >75th percentile of the sample values

## 4.3 Acute Phase Index as a Marker of Bulk Milk Quality in Relation to Mastitis

When bulk milk samples were divided into groups based on their API (**Paper II**), differences were only observed for lactose content and casein number (Table 4).

Table 4. *Milk quality parameters (mean±SD) in bulk milk samples with low, medium and high acute phase index (API). Means within a row with different superscripts were significantly different (P < 0.05)*

| Bulk milk quality parameters | Low API[1] (n=23) | Medium API[2] (n=45) | High API[3] (n=23) |
|---|---|---|---|
| API[4] | 0.07±0.01[a] | 0.17±0.06[b] | 2.86±8.56[c] |
| Lactose (g/100 ml) | 4.70±0.07[a] | 4.59±0.10[b] | 4.56±0.10[b] |
| Casein content (g/100ml) | 2.56±0.16 | 2.59±0.13 | 2.57±0.12 |
| Casein number | 73.32±0.68[a] | 72.96±0.65[b] | 72.56±0.51[c] |
| $\alpha_{s1}$-casein (g/L) | 9.10±1.33 | 9.50±1.43 | 9.37±1.09 |
| $\alpha_{s2}$-casein (g/L) | 3.41±0.65 | 3.68±0.79 | 3.66±0.72 |
| β-casein (g/L) | 15.66±2.86 | 16.28±2.70 | 15.83±2.00 |
| κ-casein (g/L) | 4.43±0.90 | 4.49±1.30 | 4.26±1.06 |
| β-lactoglobulin (g/L) | 4.39±0.37 | 4.25±0.37 | 4.21±0.26 |
| Proteolysis (mM) | 1.16±0.15 | 1.21±0.18 | 1.04±0.09 |

[1] < 25th percentile of the sample values.
[2] 25th-75th percentile of the sample values
[3] >75th percentile of the sample values
[4] All values of Hp and SAA lower than limit of detection (LOD) was set to 0.3 mg/L, i.e. the detection limit of the assays. The proportions of samples above LOD (0.3 mg/L) were 0%, 9% and 74% for Hp, and 26%, 89% and 100% for SAA, in the low, medium and high API group, respectively.

The inclusion of α-LA in combination with Hp and SAA in an index did not result in a more sensitive or specific marker for bulk milk quality than using Hp or SAA alone, or Hp and SAA in combination (data not shown). Probably, the decrease in α-LA during mastitis is of a much lower magnitude than the increase in Hp and SAA. The variation in α-LA is also much lower than the variation in Hp and SAA.

# 5 Main Findings and Conclusions

- PMNC and SCC were strongly correlated in bulk milk
- High PMNC was related to low casein number, but to no other bulk milk quality parameters measured.
- High SCC was related to lower contents of lactose and casein, lower casein number and higher proteolysis in bulk milk.
- High α-LA concentration was related to low contents of $\alpha_{s1}$-, $\alpha_{s2}$- and β-casein, and high contents of β-LG and lactose, but not related to SCC, PMNC, Hp and SAA.
- An API based on α-LA, Hp and SAA did not give any additional information about bulk milk quality compared to Hp and SAA alone.

The studies in this thesis show that neither PMNC, α-LA nor an API appear to be better markers for bulk milk quality than SCC, which is currently the standard marker of raw bulk milk quality in relation to mastitis.

# 6 Future Research

- There are other cell types apart from PMN that need to be evaluated with respect to their effect on milk components and as markers for bulk milk quality. Macrophages are the most abundant cell type in low SCC milk and they contain many proteases that may adversely affect the quality of bulk milk.

- α-LA needs to further investigated and the mechanisms behind its variation in milk are still not elucidated. α-LA might decline during infection only due to decreased synthesis in the mammary gland and increased leakage in the blood-milk barrier. Another explanation for the reduction of α-LA in mastitis might be that α-LA has a regulating role in milk synthesis, and so, during mastitis α-LA would be down-regulated.

- It could be of interest to test another approach by evaluating direct measurement of important quality faults, such as proteolysis and lipolysis, in relation to various dairy products. The degree of proteolysis was used in this study, but methods need to be refined to become more sensitive and specific.

- It is plausible that optimum raw milk quality traits differ between dairy products such as cheese and butter, but this has still not been investigated.

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