Haptoglobin and serum amyloid A in bulk tank milk in relation to raw milk quality

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Summary

The aim of the present study was to evaluate relationships between the presence of the two major bovine acute phase proteins haptoglobin (Hp) and serum amyloid A (SAA) and raw milk quality parameters in bulk tank milk samples. Hp and SAA have been suggested as specific markers of mastitis but recently also as markers for raw milk quality. Since mastitis has detrimental effects on milk quality, it is important to investigate if the presence of Hp or SAA indicates such changes in the composition and properties of the milk. Bulk tank milk samples (n=91) were analysed for Hp, SAA, total protein, casein, whey protein, proteolysis, fat, lactose, somatic cell count and coagulating properties. Samples with detectable levels of Hp had lower casein content, casein number and lactose content, but higher proteolysis than samples without Hp. Samples with detectable levels of SAA had lower casein number and lactose content, but higher whey protein content than samples without SAA. The presence of APP in bulk tank milk is suggested as an indicator for unfavourable changes in the milk composition, e.g. protein quality, due to udder health disturbances, with economical implications for the dairy industry.

The bulk tank milk composition has a decisive influence on the production of safe, high quality dairy products. One major factor responsible for the deterioration of the quality of the raw milk is mastitis, and its negative effect on the milk composition is well established (Kitchen, 1981; Munro *et al*. 1984). It is the sub-clinical form of mastitis that constitutes problems to the dairies, since these cases often go undetected and the milk is delivered to the dairy (Leitner *et al*. 2008). Studies show that during mastitis the casein content, valuable for the cheese making industry, will decrease while the whey protein content will increase (Barbano *et al*. 1991; Auldist *et al*. 1996; Urech *et al.* 1999). In addition, increased proteolysis is often observed in milk from cows with mastitis (Schaar, 1985; Auldist *et al*. 1996). Proteolysis in milk is one of the major product deteriorating factors with negative impact on the quality and stability of milk and dairy products (Mara *et al*. 1998; Kelly *et al*. 2006). Saeman *et al*. (1988) found that after an udder infection the proteolytic activity may sustain even though the SCC has returned to normal levels. Larsen *et al.* (2004) established that casein degradation not only occurred in the infected quarter but also in the neighbouring quarters, even though there was no effect on the SCC. In the bulk tank, milk from healthy udder quarters will be commingled with milk from infected quarters, and thus, the entire bulk tank may be affected by protein degradation. This is a problem, especially for the cheese making industry, since curd formation properties will be impaired and yield reduced (Mara *et al.* 1998, Leitner *et al*. 2008). Likewise, proteolytic activity in UHT-milk may cause off flavours and gelation, and consequently reduced shelf-life of the products (Ma *et al*. 2000; Santos *et al*. 2003; Barbano *et al*. 2006; De Noni *et al*. 2007).

Milk somatic cell count (SCC) has been used extensively since the 1960s in the diagnosis of mastitis, and the BTMSCC (bulk tank milk somatic cell count) is widely used in the assessment of raw milk quality. In many EU countries milk payment systems favours a low BTMSCC. There is, however, no clear scientific data defining the level of BTMSCC that is associated with additional benefits in terms of milk quality. Several authors have reported that SCC is not a suitable indicator of proteolysis in quarter milk samples (Le Roux *et al*. 1995; Urech *et al*. 1999) and recent studies have also shown that the BTMSCC gives a poor prediction of raw milk quality for cheese production (Leitner *et al*. 2008). Research to find new sensitive and specific markers for disadvantageous changes in raw milk composition due to udder health disturbances is therefore warranted.

The acute phase proteins (APP) have become important diagnostic markers of disease in human medicine and are also being evaluated in veterinary diagnostics (Eckersall, 2004). The major bovine APP are haptoglobin (Hp) and serum amyloid A (SAA), which both increase dramatically upon infection, inflammation or trauma. Hp and SAA are mainly produced by the liver but are also produced locally in the mammary gland (McDonald *et al.* 2001; Hiss *et al*. 2004). Some studies have found that Hp and SAA have antibacterial effects (Eaton *et al*. 1982; Hari-Dass *et al*. 2005; Larson *et al*. 2005) and considering that they are locally produced, their role in the inflammatory defence is interesting. In several studies Hp and SAA in milk have been evaluated as markers for mastitis (Horadagoda *et al*. 1999; Eckersall *et al*. 2001; Grönlund *et al*. 2003; Nielsen *et al*. 2004; Grönlund *et al*. 2005; Eckersall *et al*. 2006; Hiss *et al*. 2007), but so far little attention has been paid regarding their potential in predicting changes in milk composition and technological properties of the raw milk. In a

previous study we reported that detectable levels of Hp and SAA could be found in bulk tank milk samples (Åkerstedt *et al*. 2007). In a more recent study we also investigated APP in relation to raw milk quality parameters in cow composite milk samples (Åkerstedt *et al.* 2008). To our knowledge, these papers are the only studies examining APP in bovine bulk tank milk or applying APP research in the field of product quality. However, no studies have been reported on Hp and SAA in relation to the quality of the raw bulk tank milk. Quality programs for milk payment and advisory measures to improve the raw milk quality are mostly based on analysis of bulk tank milk samples. For APP to be a potential candidate as indicator for unfavorable changes in milk composition due to udder health disturbances in the herd, it is important that levels of APP in the bulk tank milk are related to important quality traits of the raw milk.

The aim of this study was to investigate relationships between the presence of Hp and SAA, and different raw milk quality parameters, i.e. total protein, casein, whey protein, proteolysis, fat, lactose and SCC in bulk tank milk samples. In addition, APP in relation to the coagulating properties of the bulk tank milk samples were evaluated.

Materials and methods

Bulk tank milk samples

The study included 91 bulk tank milk samples collected from different dairy farms in cooperation with the Milko dairy cooperative (Grådö, Hedemora, Sweden). One representative sample from each farm was taken by the tanker driver just before emptying the bulk tank, in connection with the ordinary milk collection, which occurred every second day. At the sampling occasion the farms delivered 90-13,025

kg milk (average 1,610 kg), indicating that the herd size of the participating farms varied markedly. The average BTMSCC for the samples was 195,000 cells/ml, ranging from 33,000 to 1,365,000 cells/ml (median 146,000 cells/ml). The bulk tank milk samples were collected at the dairy plant for further transportation to the university laboratory the same day. Sample aliquots for the analyses of Hp, SAA and proteolysis were frozen and stored at -70^{o}C until analysis, whereas the other parameters were analysed using fresh milk samples.

Assay of haptoglobin and serum amyloid A

Hp was analysed by an earlier described optical biosensor assay (Åkerstedt *et al.* 2006; Åkerstedt *et al.* 2008) with some additional modifications. In this study, the Hp surface was prepared by using a solution of 20 mg/l instead of 500 mg/l Hp in 0.01 M acetate buffer, and the activation of the surface during immobilisation was reduced from 7 minutes to 3 minutes. For regeneration of the sensor surface the concentration of sodium dodecyl sulphate (SDS) was increased from 2 mM to 3 mM. An extra reconditioning step was added, in which 50 mM glycine pH 9.5 was injected over the sensor surface for 30 seconds, after the ordinary regeneration step. Bovine Hp (Life diagnostics, Clarkston, GA, USA) was used for immobilization and standards, and the limit of detection (LOD) of the modified assay was 0.3 mg/l.

SAA was determined using a commercial ELISA with a LOD of 0.3 mg/l (Phase^{TM} Serum Amyloid A Assay, Tridelta Development Ltd, Wicklow, Ireland).

Measurement of the somatic cell count, total protein, whey protein and casein content, casein number, fat and lactose content

149 SCC in the bulk tank milk samples was measured by an electronic fluorescence based
150 cell counting technique (Fossomatic 5000, Foss, Hillerød, Denmark). Total protein,
151 fat and lactose contents were measured on fresh milk using mid infrared spectroscopy
152 (Fourier Transform Instrument, FT 120, Foss). The casein content was determined by
153 an indirect method which was described earlier (Åkerstedt *et al*. 2008).
154
155 *Measurement of proteolysis*
156 The extent of proteolysis in the milk sample was measured according to a
157 fluorescamine method as previously described (Wiking *et al.* 2002).
158
159 *Measurement of coagulating properties*
160 The coagulating properties of the milk was measured with a Bohlin VOR Rheometer
161 (Malvern Instruments Nordic AB, Uppsala, Sweden) according to Hallén *et al.* (2007)
162 with one minor modification, i.e. Chymax Plus, strength 200 IMCU per gram
163 (Christian Hansen A/S, DK-2970, Hørsholm, Denmark), was used instead of pure
164 chymosin. The coagulation time was measured, i.e. the time (s) elapsed from
165 chymosin addition until a weak coagulum corresponding to 5 Pa was formed. In
166 addition, curd firmness (Pa) was measured 25 min after chymosin addition.
167
168 *Statistical analyses*
169 Parametric t-test using SAS (Version 9.1, SAS Institute Inc., Cary, NC, USA) was
170 used to evaluate the relationships between APP and the different raw milk quality
171 parameters analysed. The bulk tank milk samples were categorised into two groups;
172 detectable or non-detectable levels of Hp or SAA, based on the detection limits of the
173 assays used to determine the proteins in milk. SCC and curd firmness were

174 logarithmically transformed before statistical analyses to obtain normally distributed
175 data. Differences between groups were considered significant if $p \leq 0.05$.
176
177 **Results**
178 Descriptive statistics for the milk quality parameters analysed in the study are
179 presented in Table 1. Detectable levels of Hp were found in 19 (21%) of the 91 bulk
180 tank milk samples. The average Hp concentration in these 19 samples was 1.02±0.99
181 mg/l and the average SCC in these samples was 387,000±266,000 cells/ml. Detectable
182 levels of SAA were found in 68 (75%) of the 91 bulk tank milk samples. The average
183 SAA concentration in these 68 samples was 1.12±1.16 mg/l and the average SCC in
184 these samples was 218,000±179,000 cells/ml. Samples not containing detectable
185 levels of Hp had the average SCC of 144,000±78,000 cells/ml while samples not
186 containing detectable levels of SAA had the average SCC of 127,000±116,000
187 cells/ml.
188
189 Table 1 near here.
190
191 Table 2 and 3 present differences in milk composition between samples with and
192 without detectable levels of Hp and SAA, respectively. Bulk tank milk samples in
193 which Hp could be detected had a lower casein number, contained less casein, had
194 increased proteolysis, a lower lactose content and higher SCC compared to samples
195 without detectable levels of Hp. Bulk tank milk samples in which SAA could be
196 detected had a lower casein number, increased whey protein, lower lactose content
197 and higher SCC compared to samples not containing detectable levels of SAA.
198

199 Table 2, 3 and 4 near here.

200

201 In Table 4, results obtained for differences in milk composition between samples
202 containing high BTMSCC (>195,000 cells/ml) and low BTMSCC (<195,000 cells/ml)
203 are presented. Samples with higher BTMSCC had lower casein number than samples
204 with lower BTMSCC. In the high BTMSCC group, 16 of the 29 samples (55%)
205 contained Hp, while 26 of the 29 (90%) samples contained SAA. In the low BTMSCC
206 group, 3 of the 62 samples (5%) contained Hp, while 42 of the 62 (68%) contained
207 SAA.

208

209 **Discussion**

210 The present study is, to our knowledge, the first investigating relationships between
211 the quality of raw bulk tank milk and the presence of APP as indicator of mastitis.
212 When studying markers for udder health disturbances and effects on milk composition
213 it is important to have in mind that the type of milk sample, i.e. quarter, cow
214 composite or bulk tank milk, may affect the results. Due to the dilution effect from
215 quarter milk to composite and bulk tank milk, significant relationships found at
216 quarter level may not be present at cow or bulk tank level and vice versa. However,
217 the main findings of the present study, i.e. unfavourable changes in protein
218 composition in bulk tank milk samples with detectable levels of APP, are largely in
219 agreement with the results of our earlier study in cow composite milk (Åkerstedt *et al*.
220 2008).

221

222 In this study, bulk tank milk samples with detectable levels of Hp had lower casein
223 content as well as lower casein number. This was most likely due to increased

224 proteolytic activity, since these samples also had significantly higher proteolysis than
225 samples without detectable levels of Hp. Bulk tank milk samples containing
226 detectable levels of SAA also had a lower casein number compared to samples
227 without detectable levels of SAA. In this case, however, the effect was likely due to
228 the observed increased whey protein content in the samples. It is thus likely that
229 decreased synthesis, proteolysis and influx of components from the blood will occur
230 simultaneously but to evaluate to what extent was outside the scope of this paper.
231 In this study, detectable levels of Hp in bulk tank milk was related to increased
232 proteolytic activity, which is in contrast to the results in our previous study on cow
233 composite milk samples (Åkerstedt *et al.* 2008). One possible explanation for not
234 observing proteolysis in cow composite milk but in bulk tank milk may be related to
235 the fact that bulk tank milk consists of commingled milk from different milkings, as
236 well as milk from non-infected and sub-clinically infected glands (Leitner *et al.*
237 2008). Moreover, the storage time before collection and freezing was longer for bulk
238 tank, than for cow composite milk samples, allowing proteolysis to proceed for a
239 longer time. The bulk tank milk samples were collected at the dairy plant and were
240 kept at +4°C during the entire chain from farm to laboratory. Since the milk is
241 collected every second day, the oldest batch of milk in the tank was stored for
242 approximately 2.5 days before it was frozen. Refrigerated storage of raw milk is
243 known to favor the growth of psychrotropic bacteria, which may produce heat-
244 resistant extra-cellular proteases and lipases. Proteases are mainly secreted at the end
245 of the log phase of the bacterial growth, at numbers in the order of 10^7 cfu/ml,
246 indicating that very high numbers of bacteria are required to result in proteolysis
247 (Sørhaug & Stepaniak, 1997). In a study by Haryani *et al*. (2003), such high numbers
248 were reached first after 7 days at 4°C, and proteolysis, as measured by the

fluorescamine method, was observed on day 6. Considering the very high numbers of bacteria needed for proteolysis to become a problem, it is unlikely that microbial contamination of milk samples would explain the observed relationships between the presence of Hp and increased proteolysis. An influence of microbial proteases cannot, however, be excluded at this stage.

Bulk tank milk samples with detectable levels of SAA contained less lactose in agreement with earlier studies on udder quarter and cow composite milk samples (Lindmark-Månsson *et al*. 2006; Åkerstedt *et al*. 2008). In the present study, bulk tank milk samples with detectable levels of Hp also contained reduced levels of lactose. The most common explanation to decreased lactose content is reduced synthesis due to damaged epithelial cells. Another explanation, suggested by Silanikove *et al.* (2000), might be that proteolysis of β-casein will result in release of peptides with a regulatory effect on lactose secretion.

Bulk tank milk samples with detectable levels of Hp and SAA had significantly higher SCC compared to samples without Hp and SAA, respectively. In our previous study, bulk tank milk samples with detectable levels of SAA, but not samples with detectable levels of Hp, had higher SCC (Åkerstedt *et al*. 2007). The discrepancy between the studies might be explained by the use of different statistical methods, differences in the categorisation of the samples and the selection of milk samples. To assess the potential of APP as markers for milk quality in comparison with the commonly applied SCC, we also investigated relationships between SCC and the different quality traits in the same samples. In this study, bulk tank milk samples with

elevated SCC (>195,000 cells/ml), had a lower casein number than samples with a lower SCC (<195,000 cells/ml). In our previous study (Åkerstedt *et al*. 2008) with cow composite milk, samples with elevated SCC (>83,000 cells/ml) had reduced lactose and increased whey protein content compared to samples with a lower SCC (<83,000 cells/ml). The threshold values used in those studies (195 000 cells/ml and 83,000 cells/ml) are median and mean values, respectively. These threshold values are relevant since several studies demonstrate that the composition is deteriorated on quarter and cow level between 50,000-100,000 cells/ml, while the Swedish milk payment system give additional bonus payment when the bulk tank contains less than 175,000-200,000 cells/ml. Our studies suggest that the presence of APP in milk is related to disadvantageous changes in several milk quality parameters and that these relationships are valid in both cow composite and bulk tank milk. SCC, on the other hand, is not related to milk quality traits to the same extent as APP and the type of relationships observed differs between cow composite and bulk tank milk.

No significant relationships between APPs and coagulation properties, i.e. coagulation time and curd firmness, were found in this study. Since there are no other studies published investigating APP in relation to the coagulating properties of milk, it is difficult to evaluate the results obtained. In earlier studies, significant correlations between mastitis and impaired coagulating properties were observed (for review see Munro *et al.* 1984). In contrast, Leitner *et al.* (2008) found no correlation between coagulation time and SCC at bulk tank or silo level. In general, most published research on coagulating properties is based on studies with milk from a small number of animals (Barbano *et al.* 1991; Mazal *et al.* 2007). It is also common that batches of milk with a specific SCC are constructed by pooling milk with very high SCC, often

originating from cows with clinical mastitis, and milk with a low SCC. Such a procedure is not ideal, as the composition of milk from cases of clinical mastitis is very deviant from milk originating from cows without clinical signs. Consequently, this type of constructed milk samples are not representative for real bulk tank milk samples, representing commingled milk from a large number of clinically healthy cows.

The casein content is an important quality parameter in cheese production and decreased casein content implies large losses for the dairy industry. At present, the total protein content is used as a major quality parameter, largely affecting the milk price to the producer. The total protein content, however, also includes the whey proteins, of which those originating from blood, with no interest for the dairies, will increase during mastitis. The extent of proteolysis is another important factor influencing milk quality, although not presently assessed. There are thus no techniques in place allowing reliable, large-scale analyses of the protein quality of the milk, although research and development in this field is ongoing. In a future perspective, the dairies might have the possibility to differentiate raw milk based on quality to be used for different purposes. SCC is a good marker for udder health disturbances at udder quarter level but several studies have demonstrated that SCC is not a strong candidate for predicting the processing quality of the bulk tank milk (Le Roux *et al*. 1995; Urech *et al*. 1999; Leitner *et al*. 2006; Leitner *et al*. 2008). Since many dairy products like cheese and fermented products require high protein quality it should be of great importance to have a sensitive and specific marker for disadvantageous changes in milk composition, e.g. those associated to poor udder health. Changes in levels of such a marker should preferably be associated to the

protein composition of the raw milk. In this study, Hp and SAA have shown to be potential candidates for predicting the raw bulk tank milk quality, specifically in relation to protein quality. This study and our previous studies therefore suggest that APP may be used as indicators for changes in milk composition as consequence of udder health disturbances, in quarter or cow composite milk samples at the farm, as well as in bulk tank milk at the dairy plant or milk grading laboratory.

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References

Åkerstedt M, Björck L, Persson Waller K & Sternesjö Å 2006 Biosensor assay for determination of haptoglobin in bovine milk. *Journal of Dairy Research* **73** 299-305

Åkerstedt M, Persson Waller K & Sternesjö Å 2007 Haptoglobin and serum amyloid A in relation to the somatic cell count at quarter, cow composite and bulk tank milk samples. *Journal of Dairy Research* **74** 198-203

Åkerstedt M, Persson Waller K, Bach Larsen L, Forsbäck L & Sternesjö Å 2008 Relationship between haptoglobin and serum amyloid A in relation to milk quality. *International Dairy Journal* **18** 669-674

Auldist MJ, Coats S, Sutherland BJ, Mayes JJ, McDowell GH & Rogers GL 1996 Effects of somatic cell count and stage of lactation on raw milk composition and the yield and quality of cheddar cheese. *Journal of Dairy Research* **63** 269-280

Barbano DM, Rasmussen RR & Lynch JM 1991 Influence of milk somatic cell count and milk age on cheese yield. *Journal of Dairy Science* **74** 369-388

Barbano DM, Ma Y & Santos MV 2006 Influence of raw milk quality on fluid milk shelf life. *Journal of Dairy Science* **89** (E.Suppl.):E15-E19

De Noni I, Pellegrino L, Cattaneo S & Resmini P 2007 HPLC of proteose peptones for evaluating ageing of packaged pasteurized milk. *International Dairy Journal* **17** 12-19

Eaton JW, Brandt P, Mahoney JR & Lee JT 1982 Haptoglobin: A natural bacteriostat. *Science* **215** 691-693

Eckersall PD, Young FJ, McComb C, Hogarth CJ, Safi S, Weber A, McDonald T, Nolan AM & Fitzpatrick JL 2001 Acute phase proteins in serum and milk from dairy cows with clinical mastitis. *Veterinary Record* **148** 35-41.

Eckersall PD 2004 The time is right for acute phase proteins assay. *The Veterinary Journal* **168** 3-5

Eckersall PD, Young FJ, Nolan AM, Knight CH, McComb C, Waterston MM, Hogarth CJ, Scott EM & Fitzpatrick JL 2006 Acute phase proteins in bovine milk in an experimental model of Staphylococcus aureus subclinical mastitis. *Journal of Dairy Science* **89** 1488-1501

Grönlund U, Hulten C, Eckersall PD, Hogarth C & Persson Waller K 2003 Haptoglobin and serum amyloid A in milk and serum during acute and chronic experimentally induced Staphylococcus aureus mastitis. *Journal of Dairy Research* **70** 379-386

Grönlund U, Hallén Sandgren C & Persson Waller K 2005 Haptoglobin and serum amyloid A in milk from dairy cows with chronic sub-clinical mastitis. *Veterinary Research* **36** 191-198

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

Haryani S, Datta N, Elliott AJ & Deeth HC 2003 Production of proteinases by psychrotropic bacteria in raw milk stored at low temperature. *Australian Journal of Dairy Technology* **58** 15-20

Hari-Dass R, Shah C, Meyer DJ & Raynes JG 2005 Serum amyloid A protein binds to outer membrane protein A of gram-negative bacteria. *Journal of Biological Chemistry* **280** 18562-18567

Hiss S, Mielenz M, Bruckmaier R M & Sauerwein H 2004 Haptoglobin concentrations in blood and milk after endotoxin challenge and quantification of mammary Hp mRNA expression. *Journal of Dairy Science* **87** 3778-3784

Hiss S, Mueller U, Neu-Zahren A & Sauerwein H 2007 Haptoglobin and lactate dehydrogenase measurements in milk for the identification of subclinically infected quarters. *Veterinarni Medicina* **52** 245-252

Horadagoda NU, Knox KMG, Gibbs HA, Reid SWJ, Horadagoda A, Edwards SER & Eckersall PD 1999 Acute phase proteins in cattle: discrimination between acute and chronic inflammation. *Veterinary Record* **144** 437-441

Kelly AL, O´Flaherty FO & Fox PF 2006 Indigenous proteolytic enzymes in milk: A brief overview of the present state of knowledge. *International Dairy Journal* **16** 563-572

Kitchen BJ 1981 Review of the progress of dairy science: Bovine mastitis: milk compositional changes and related diagnostic tests. *Journal of Dairy Research* **48** 167-188

Larsen LB, Rasmussen MD, Bjerring M & Nielsen JH 2004 Proteases and protein degradation in milk from cows infected with Streptococcus uberis. *International Dairy Journal* **14** 899-907

Larson MA, Weber A, Weber AT & McDonald TL 2005 Differential expression and secretion of bovine serum amyloid A (SAA3) by mammary epithelial cells stimulated with prolactin or lipopolysaccahride. *Veterinary Immunology and Immunopathology* **107** 255-264

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

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

Le Roux Y, Colin O & Laurent F 1995 Proteolysis in samples of quarter milk with varying somatic cell counts: 1. Comparison of some indicators of endogenous proteolysis in milk. *Journal of Dairy Science* **78** 1289–1297

Lindmark-Månsson H, Bränning C, Aldén G & Paulsson M 2006 Relationship between somatic cell count, individual leukocyte populations and milk components in bovine udder quarter milk. *International Dairy Journal* **16** 717-727

Ma Y, Ryan C, Barbano DM, Galton DM, Rudan MA & Boor KJ 2000 Effects of somatic cell count on quality and shelf-life of pasteurized fluid milk. *Journal of Dairy Science* **83** 264-274

Mara O, Roupie C, Duffy A & Kelly AL 1998 The curd-forming properties of milk as affected by the action of plasmin. *International Dairy Journal* **8** 807-812

Mazal G, Vianna PCB, Santos MV & Gigante ML 2007 Effect of somatic cell count on prato cheese composition. *Journal of Dairy Science* **90** 630-636

McDonald TL, Larson MA, Mack DR & Weber A 2001 Elevated extrahepatic expression and secretion of mammary-associated serum amyloid A 3 (M-SAA3) into colostrum. *Veterinary Immunology and Immunopathology* **83** 203-211

Munro GL, Grieve PA & Kitchen BJ 1984 Effects of mastitis on milk yield, milk composition, processing properties and yield and quality of milk products. *Australian Journal of Dairy Technology* **39** 7-16

Nielsen BH, Jacobsen S, Andersen PH, Niewold TA & Heegaard PMH 2004 Acute phase protein concentrations in serum and milk from healthy cows, cows with clinical mastitis and cows with extramammary inflammatory conditions. *Veterinary Record* **154** 361-365

Saeman AI, Verdi RJ, Galton DM & Barbano DM 1988 Effect of mastitis on proteolytic activity in bovine milk. *Journal of Dairy Science* **71** 505-512

Santos MV, Ma Y & Barbano DM 2003 Effect of somatic cell count on proteolysis and lipolysis in pasteurized fluid milk during shelf-life storage. *Journal of Dairy Science* **86** 2491-2503

Schaar J 1985 Plasmin activity and proteose-peptone content of individual milks. *Journal of Dairy Research* **52** 369-378

Silanikove N, Shamay A, Shinder D & Moran A 2000 Stress down regulates milk yield in cows by plasmin induced β-casein product that blocks K+ channels on the apical membranes. *Life Sciences* **67** 2201-2212

Sørhaug T & Stepaniak L 1997 Psychrotrophs and their enzymes in milk and dairy products: Quality aspects. Trends in Food Science & Technology **18** 35-41

Urech E, Puhan Z & Schallibaum M 1999 Changes in milk fraction as affected by subclinical mastitis. *Journal of Dairy Science* **82** 2402-2411

Wiking L, Frost MB, Larsen LB & Qvist KB 2002 Effects of storage conditions on lipolysis, proteolysis and sensory attributes in high quality raw milk. *Milchwissenschaft* **57** 190-194

Table 1. Milk composition, including contents of haptoglobin (Hp) and serum amyloid A (SAA), and coagulating properties of 91 bulk tank milk samples.

Parameter	Unit	Mean (SD)	Minimum	Maximum
Hp	mg/l	ND[†]	<0.3	4.70
SAA	mg/l	ND	<0.3	8.79
Total protein	%	3.54 (0.18)	3.12	4.21
Casein	%	2.58 (0.14)	2.20	3.07
Casein number		0.73 (0.01)	0.71	0.75
Whey protein	%	0.91 (0.05)	0.82	1.09
Proteolysis	eq leu[‡]	1.11 (0.11)	0.89	1.71
Fat	%	4.48 (0.43)	3.54	6.25
Lactose	%	4.61 (0.11)	4.30	4.91
Somatic cell count	cells/ml	195,000 (169,600)	33,000	1,365,000
Coagulation time	s	120 (42)	32	278
Curd firmness	Pa	350 (118)	162	798

[†]ND= not determined. Since many of the samples did not contain detectable levels of Hp or SAA, i.e levels were below 0.3 mg/l, it was not considered relevant to calculate a mean value.

[‡]eq leu = equivalent mM leucine.

Table 2. Differences in milk composition between bulk tank milk samples with (Hp+) and without (Hp-) detectable levels of Hp. Differences between Hp+ and Hp- samples were evaluated by parametric t-test and were considered significant if $p \leq 0.05$.

	Hp+ (n=19)	SE [†]	Hp- (n=72)	SE	p-value
Total protein (%)	3.493	0.025	3.549	0.023	NS [‡]
Casein (%)	2.532	0.018	2.593	0.017	0.016
Casein number	0.725	0.001	0.731	0.001	<0.001
Whey protein (%)	0.913	0.008	0.908	0.006	NS
Proteolysis (eq leu)[§]	1.159	0.019	1.100	0.014	0.038
Fat (%)	4.455	0.049	4.491	0.055	NS
Lactose (%)	4.579	0.014	4.618	0.014	0.05
Log SCC (cells/ml)	5.528	0.049	5.104	0.027	<0.001
Coagulation time (s)	110	9.862	123	4.963	NS
Log curd firmness (Pa)	2.547	0.039	2.517	0.015	NS

[†] SE = Standard Error

[‡] NS = not significant

[§] eq leu = equivalent mM leucine.

Table 3. Differences in milk composition between bulk tank milk samples with (SAA+) and without (SAA-) detectable levels of SAA. Differences between SAA+ and SAA- samples were evaluated by parametric t-test and were considered significant if $p \leq 0.05$.

	SAA+ (n=68)	SE [†]	SAA- (n=23)	SE	p-value
Total protein (%)	3.553	0.021	3.488	0.042	NS [‡]
Casein (%)	2.588	0.016	2.559	0.032	NS
Casein number	0.728	0.001	0.734	0.001	<0.001
Whey protein (%)	0.918	0.006	0.883	0.011	0.004
Proteolysis (eq leu) [§]	1.122	0.014	1.082	0.023	NS
Fat (%)	4.477	0.049	4.504	0.102	NS
Lactose (%)	4.583	0.012	4.692	0.018	<0.001
Log SCC (cells/ml)	5.261	0.029	4.989	0.063	<0.001
Coagulation time (s)	122	5.055	113	9.276	NS
Log curd firmness (Pa)	2.521	0.016	2.533	0.030	NS

[†] SE = Standard Error

[‡] NS = not significant

[§] eq leu = equivalent mM leucine.

Table 4. Differences in milk composition between bulk tank milk samples with high SCC (>195,000 cells/ml) and low SCC (<195,000 cells/ml). Differences between high SCC and low SCC samples were evaluated by parametric t-test and were considered significant if $p \leq 0.05$.

	high SCC (n=29)	SE [†]	low SCC (n=62)	SE	p-value
Total protein (%)	3.498	0.027	3.555	0.025	NS
Casein (%)	2.541	0.021	2.599	0.018	NS
Casein number	0.726	0.001	0.731	0.001	0.002
Whey protein (%)	0.909	0.008	0.909	0.007	NS
Proteolysis (eq leu)[§]	1.126	0.020	1.105	0.014	NS
Fat (%)	4.453	0.059	4.498	0.060	NS
Lactose (%)	4.585	0.014	4.622	0.015	NS
Coagulation time (s)	118	8.053	121	5.359	NS
Log curd firmness (Pa)	2.507	0.030	2.531	0.015	NS

[†] SE = Standard Error

[‡] NS = not significant

[§] eq leu = equivalent mM leucine.