Relationship between haptoglobin and serum amyloid A in milk and milk quality

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Abstract

The objective of this study was to evaluate relationships between the presence in milk of the major bovine acute phase proteins, haptoglobin (Hp) and serum amyloid A (SAA), and milk quality parameters. Composite milk samples were collected from 89 clinically healthy dairy cows and analysed for Hp and SAA, total protein, casein, and whey protein levels, casein number, proteolysis, total fat and lactose levels, and somatic cell count (SCC). Milk samples with detectable levels of Hp showed lower total protein and casein levels, whereas milk samples with detectable levels of SAA had lower casein number and lactose level. Samples with detectable levels of acute phase proteins also showed an elevated SCC. The results suggest that the presence of Hp and SAA in milk might indicate unfavourable changes in milk composition, especially in relation to protein quality.

Key words: acute phase proteins, haptoglobin, serum amyloid A, milk quality, casein, milk protein, somatic cell count

1. Introduction

For production of high quality dairy products, the dairy industry is dependent on the composition and processing properties of the raw milk. Since only healthy cows produce milk of normal composition, udder health is a major factor determining milk quality. Mastitis, i.e., inflammation of the udder, is the most common disease in dairy cows, affecting the yield, composition and processing properties of the milk (Munro et al., 1984; Auldist et al., 1995). Also the sub-clinical form of mastitis will induce compositional changes in the milk (Tolle et al., 1971; Hamann, 2002) but, since this condition usually goes unnoticed by the farmer, milk from affected cows enters the bulk tank (Leitner et al., 2007). It is therefore of great importance to identify specific and sensitive biomarkers that can be used for rapid detection of unfavourable changes in milk quality as a consequence of the disease. The somatic cell

count (SCC) is a compulsory parameter in EU raw milk quality programs (EC, 2004). It is considered an indicator of the average udder health in the herd, affecting the milk price to the dairy farmer, and is also the standard method for detection of sub-clinical mastitis. SCC is influenced by several physiological factors, e.g., stage of lactation and lactation number (Harmon, 1994), and is not easily incorporated into todays automated milking systems. SCC has been questioned in relation to its ability to predict milk quality, especially regarding its poor correlation with protein quality (Leitner et al., 2006; Leitner et al., 2007). Whereas much effort has been invested in research to find alternative biomarkers of sub-clinical mastitis (Pyörälä, 2003), relationships between the presence of these biomarkers in milk and unfavourable changes in milk quality have received less attention.

During recent years, there has been an increased interest in the potential of acute phase proteins (APP) in diagnosis of animal disease, including bovine mastitis (Murata et al., 2004; Grönlund et al., 2005; Eckersall et al., 2006). APP constitute a part of the acute phase response, which is a non-specific reaction in animals during tissue damage, infection, inflammation or trauma (Eckersall & Conner, 1988; Alsemgeest et al., 1994; Horadagoda et al., 1999). APPs are species-specific and, in cattle, there are two major APPs: haptoglobin (Hp) and serum amyloid A (SAA) (Eckersall & Conner, 1988; Alsemgeest et al., 1994; Gruys et al., 1994; Eckersall et al., 2001). Whereas elevated serum concentrations of Hp and SAA are generally regarded as non-specific markers of inflammation, their presence in milk may provide specific information regarding the inflammatory status of the udder (Eckersall et al., 2001). If Hp and SAA in milk constitute potential biomarkers for mastitis from the diagnostic perspective, it is important to investigate their value in predicting raw milk quality. The aim of the present study was therefore to investigate relationships between the presence of Hp and SAA and different milk quality traits, i.e., total protein, whey protein and casein levels, casein

number, proteolysis, and levels of total fat and lactose in cow composite milk samples. Since SCC is an important parameter in raw milk quality programmes, indirectly affecting the milk composition, SCC analysis was also included in this study.

2. Materials and methods

2.1. Animals and milk sampling

The study included 89 cows from two University dairy farms in Uppsala. The cows were of the two main Swedish dairy breeds, Swedish Red, and Swedish Holstein, and were in lactation number 1-9 (median 1), lactation week 5-62 (median 36), and produced 12-51 kg milk per day (median 24). The cows were clinically healthy, i.e., without systemic symptoms and clinical signs of disease or abnormalities in the udder or in the milk, when observed by visual examination and palpation of the udder. The total milk volume from each cow was collected in a separate vessel for the whole milking and, after milking was completed, a representative composite milk sample was taken. All analyses were performed using fresh milk samples, except the analyses of Hp, SAA and proteolysis, where sample aliquots were frozen and stored at -70^{o}C until analysis.

2.2. Assay of haptoglobin and serum amyloid A

Analysis of Hp was performed according to a recently described optical biosensor assay (Åkerstedt et al., 2006), with some minor modifications. In short, the method is based on the strong interaction between Hp and haemoglobin. Bovine haemoglobin is added to the milk before injection over a sensor surface with covalently bound Hp. When there is no or small amounts of Hp present in the sample, haemoglobin will bind to immobilized Hp on the sensor surface. This results in an increased response signal due to increased mass on the sensor surface. In contrast, when Hp is present in the sample, it will form a complex with added

haemoglobin, inhibiting binding of haemoglobin to the surface. Thus, the biosensor response is inversely proportional to the amount of Hp in the sample. In the modified assay, the concentration of added haemoglobin was 1.5 mg L^{-1} and the contact time 75 sec, instead of 2.5 mg L^{-1} and 60 sec. Analysis was performed on whole milk samples and bovine Hp (Life diagnostics, Clarkston, GA, USA) was used for immobilization and standards. The limit of detection (LOD) of the assay was 1 mg L^{-1}. A commercial ELISA (Phase™ Serum Amyloid A Assay, Tridelta Development Ltd, Wicklow, Ireland) was used to determine the SAA concentration in milk. Optical densities were read on an automatic plate reader (Model ELx 800; Bio-tek Inc, Winooski, VT, USA) at 450 nm with a reference at 630 nm. The LOD of the ELISA was 0.3 mg L^{-1} , according to the manufacturer.

2.3. Measurement of the somatic cell count, total protein, whey protein and casein levels, casein number, and total fat and lactose levels

SCC was measured on fresh milk by electronic fluorescence based cell counting (Fossomatic 5000, Foss). Total protein, total fat and lactose were measured directly on fresh milk using mid infrared spectroscopy (Fourier Transform Instrument, FT 120, Foss, Hillerød, Denmark). The casein level was determined by an indirect method, whereby protein in the whey fraction (whey protein) was determined by mid infrared spectroscopy after rennet coagulation of the caseins. In short, 60 µl calcium chloride (48%) was added to the milk (40 ml) and the sample was incubated in a water bath at 40°C. Upon reaching this temperature, rennet (200 µl; 180±10 international milk clotting units) was added, the sample was mixed and incubated for approximately 15-20 min to allow coagulation of the milk. The resulting curd was then cut in smaller pieces and passed through a filter (42 µm) to remove the caseins. The casein level in the sample was then calculated by subtracting whey protein, as determined by mid infrared

spectroscopy from total protein in the milk, and casein number was calculated as casein divided by total protein.

2.4. Measurement of proteolysis

To evaluate the extent of proteolysis in the milk sample, free amino termini were measured in skimmed milk samples according to a fluorescamine method (Wiking et al., 2002). In short, intact milk proteins are precipitated with trichloroacetic acid and, after centrifugation, free amino acids and peptides produced as a result of proteolytic activity in the milk sample, will be found in the supernatant. These are then coupled to the reagent fluorescamine which, after reaction with amino terminals, will fluoresce. The fluorescence (excitation 390 nm, emission 480 nm) was measured by a Luminescence spectrometer (Perkin-Elmer, LS 50 B, Norwalk, CT, USA) and the extent of proteolysis was expressed as leucine equivalent (mM), using a standard curve constructed by analysis of leucine diluted in HCl.

2.5. Statistical analyses

Relationships between APP and the milk quality parameters were evaluated by parametric t-test using SAS (Version 9.1, SAS Institute Inc., Cary, NC, USA). The observations were categorized into two groups; detectable or non-detectable levels of APP, based on the detection limits of the methods used for determination of Hp and SAA respectively.

3. Results and Discussion

Table 1 presents descriptive statistics for the parameters measured in the study. In 35 (39%) of 89 cow composite milk samples concentrations of both Hp and SAA were below LOD. The average composite SCC for these 35 cows was 69,300±100,800 cells mL^{-1}. Hp was detected in 28 (31%) of 89 milk samples, and the average Hp concentration in these 28 samples was

3.4±6.1 mg L^{-1}. SAA was detected in 46 (52%) of the 89 milk samples and the average SAA concentration in these samples was 8.3±12.1 mg L^{-1}. In 20 of 89 milk samples (22%) both Hp and SAA were detected. The average SCC in milk samples with detectable Hp levels was 709,000±1,319,000 cells mL^{-1}, in samples with detectable levels of SAA 533,000±1,051,000 cells mL^{-1} and in samples with both Hp and SAA 974,000±1,487,000 cells mL^{-1}.

In Table 2, differences in milk composition between samples with and without detectable levels of Hp are presented. Milk with detectable levels of Hp showed lower total protein and casein levels and higher SCC. Differences between samples with and without detectable levels of SAA and the different milk quality traits are presented in Table 3. Milk samples with detectable levels of SAA showed lower casein number, lower lactose level and higher SCC. The total protein level in the milk is presently one of the most important milk quality parameters, highly affecting the milk price to the producer. For the dairy industry, however, protein composition, and for the cheese industry casein content in particular, is a more valuable parameter, since cheese yield increases with higher casein level (Auldist et al., 1996). Moreover, casein is a more specific quality parameter since total protein may include less valuable serum proteins that are known to increase during mastitis. Total protein may, thus, be unchanged or even increase during mastitis (Munro et al., 1984; Urech et al., 1999), with the change in protein quality remaining unnoticed. The casein content but also the degree of casein degradation affects the processing properties of the milk and the yield and quality of the dairy products. Some properties that will be affected is the heat stability, sensory properties and texture, e.g., in the production of yoghurt and cheese (Lynch et al., 1995; Barbano et al., 2006; Kelly et al., 2006; De Noni et al., 2007). For these reasons, it would be more relevant to measure casein instead of total protein level in the milk. Today, the experience from routine analysis of casein in raw milk is limited, although instrumentation

based on mid-infrared spectroscopy is available. The relationship between APP and casein content/casein number is interesting, suggesting that the presence of APP in milk may indicate an unfavourable protein composition.

No relationship between APP and proteolysis was observed in this study. One reason for this could be that the fluorescamine method is not sensitive enough to detect small differences in proteolysis (Harayani et al., 2003). There were, however, a decreased casein level and lower casein number in samples with detectable levels of APP and proteolysis could be one possible explanation for this. Plasmin, which is the most important proteolytic enzyme in milk, binds to casein and hydrolyses the proteins, resulting in soluble peptides (Bastian & Brown, 1996). The enzyme is heat stable and the degradation of caseins will continue during storage of milk and dairy products. Other indigenous enzymes may, however, also participate in casein degradation (Kelly et al., 2006) and many of these, e.g., the cathepsins, originate in leakage from milk leukocytes (Hurley et al., 2000). In addition, exogenous, heat stable proteases originating in psychrotrophs and pathogenic bacteria associated to the infected gland, will also contribute to the degradation of the caseins (Harayani et al., 2003; Haddadi et al., 2006).

Presence in milk of APPs correlated with an elevated SCC (Table 2 and Table 3), which is in agreement with previous findings related to milk SAA (Lindmark-Månsson et al., 2006; O´Mahony et al., 2006; Åkerstedt et al., 2007) and Hp, respectively (Nielsen et al., 2004; Åkerstedt et al., 2007). SAA in milk, but not Hp, showed significant relationship with lower lactose content. Lactose has for many years been evaluated as an indirect marker for mastitis with varying degree of success; it seems to be useful only if applied on quarter level with healthy udder quarters as controls (Berglund et al., 2007). It has been suggested that during casein degradation by plasmin, the release of specific peptides may serve as regulators of

mammary gland function, reducing lactose secretion (Leitner et al., 2006). Since plasmin activity increases during mastitis, this seems a possible explanation for the commonly found correlations between lactose and biomarkers for mastitis. It does, however, not explain why samples with detectable levels of SAA but not Hp contained less lactose. In bulk tank milk, lactose is not sensitive or specific enough to be used as indicator of udder health and it is has never been considered a milk quality parameter.

Relationships between the presence of APPs in milk and milk quality traits were also evaluated using groups of samples with detectable levels of both Hp and SAA versus samples not meeting this criterion (Table 4). Milk samples with detectable levels of both APPs in milk had lower casein, total protein and whey protein contents and higher SCC. As expected, differences were more significant than when studying differences in milk quality from samples with and without detectable levels of one of the APPs and the milk quality parameters. An unexpected result was that the whey protein content was lower when both APPs were present in the sample. Since the total protein and casein contents also decreased, the most possible explanation is that there was a reduced synthesis of the milk proteins, including the whey proteins.

Relationships between SCC and the different milk quality parameters were analysed to verify that previously established relationships were present also in this set of samples. The relationships with total protein and casein levels/casein number observed for Hp and SAA, were not found for SCC. Elevated SCC coincided with decreased lactose level and increased whey protein level (results not shown), a relationship commonly observed in this type of studies (Auldist et al., 1995; Urech et al., 1999).

To our knowledge, there are no reports on the effect of physiological factors, e.g., oestrus, lactation number and stage of lactation, on the presence of APP in milk. It is important to point out, that such studies are needed for full assessment of the value of new biomarkers for milk quality, and for evaluation of advantages and disadvantages of APP in comparison to the existing standard marker, the SCC. These aspects were, however, outside the scope of the present study.

5. Conclusions

The results of this study suggest that the presence of the major bovine APPs, Hp and SAA, may predict the protein quality of composite milk. The casein content and the casein number are very important traits in assessment of raw milk quality although not measured routinely at present. The observed relationships between APP and milk protein quality parameters should be verified in further studies.

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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.

Alsemgeest, S.P.M., Kalsbeek, H.C., Wensing, T., Koeman, J.P., Vanederen, A.M., & Gruys, E. (1994). Concentrations of serum amyloid-A (SAA) and haptoglobin (Hp) as parameters of inflammatory diseases in cattle. *Veterinary Quarterly, 16*, 21-23.

Auldist, M.J., Coats, S., Rogers, G.L., & McDowell, G.H. (1995). Changes in the composition of milk from healthy and mastitic dairy cows during the lactation cycle. *Australian Journal of Dairy Technology*, *35*, 427-436.

Auldist, M.J., Coats, S., Sutherland, B.J., Mayes, J.J., McDowell, G.H., & Rogers, G.L. (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, D.M., Ma, Y., & Santos, M.V. (2006). Influence of raw milk quality on fluid milk shelf life. *Journal of Dairy Science, 89 E,* Supplement E15-E19.

Bastian, E.D., & Brown, R.J. (1996). Plasmin in milk and dairy products: an update. *International Dairy Journal, 6,* 435-357.

Berglund, I., Pettersson, G., Östensson, K., & Svennersten-Sjaunja, K. (2007). Quarter Milking for Improved Detection of Increased SCC. *Reproduction in Domestic Animals 42*:427-432. Online. Available: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1439-0531.2006.00803.x

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.

EC (2004). Regulation No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. *Official Journal of the European Union L226*, 22-82

Eckersall, P.D., & Conner, J.G. (1988). Bovine and canine acute phase proteins. *Veterinary Research Communications, 12,* 169-178.

Eckersall, P.D., Young, F.J., McComb, C., Hogarth, C. J., Safi, S., Weber, A., McDonald, T., Nolan, A.M., & Fitzpatrick, J.L. (2001). Acute phase proteins in serum and milk from dairy cows with clinical mastitis. *Veterinary Record, 148,* 35-41.

Eckersall, P.D., Young, F. J., Nolan, A.M., Knight, C.H., McComb, C., Waterston, M.M., Hogarth, C.J., Scott, E.M., & Fitzpatrick, J.L. (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., 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.

Gruys, E., Obwolo, M. J., & Toussaint, M.J. M. (1994). Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry: a review. *Veterinary Bulletin*, *64*, 1009-1018.

Haddadi, K., Prin-Mathieu, C., Moussaoui, F., Faure, G.G., Vangroenweghe, F., Burvenich, C., & Le Roux, Y. (2006). Polymorphonuclear neutrophils and *Escherichia coli* proteases involved in proteolysis of casein during experimental *E.coli* mastitis. *International Dairy Journal*, *16*, 639-647.

Hamann, J. (2002). Relationships between somatic cell count and milk composition. *Bulletin of the International Dairy Federation*, *372*, 56-59.

Harmon, R.J. (1994). Symposium: Mastitis and genetic evaluation for somatic cell count-Physiology of mastitis and factors affecting somatic cell counts. *Journal of Dairy Science, 77*, 2103-2112.

Harayani, S., Datta, N., Elliott, A.J., & Deeth, H.C. (2003). Production of proteinases by psychrotropic bacteria in raw milk stored at low temperature. *Australian Journal of Dairy Technology, 58*, 15-20.

Horadagoda, N.U., Knox, K.M.G., Gibbs, H.A., Reid, S.W.J., Horadagoda, A., Edwards S.E.R., & Eckersall, P.D. (1999). Acute phase proteins in cattle: discrimination between acute and chronic inflammation. *Veterinary Record, 144*, 437-441.

Hurley, M.J., Larsen, L.B., Kelly, A.L., & McSweeney, P.L.H. (2000). The milk acid proteinase cathepsin D; a review. *International Dairy Journal 10*, 673-681.

Kelly, A.L., O´Flaherty, F.O., & Fox, P.F. (2006). Indigenous proteolytic enzymes in milk: A brief overview of the present state of knowledge. *International Dairy Journal, 16*, 563-572.

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. (2007). The influence of storage on the farm and in dairy silos on milk quality for cheese production. *International Dairy Journal*, doi:10.1016/j.idairyj.2007.09.001

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.

Lynch, J.M., Barbano, D.M., & Fleming, J.R. (1995). Indirect and direct determination of the casein content of milk by Kjeldahl nitrogen analysis: Collaborative study. *Journal of AOAC International 81*, 763-774.

Munro, G. L., Grieve, P. A., & Kitchen, B. J. (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.

Murata, H., Shimada, N., & Yoshioka, M. (2004). Current research on acute phase proteins in veterinary diagnosis: an overview. *Veterinary Journal, 168*, 28-40.

O'Mahony, M.C., Healy, A.M., Harte, D., Walshe, K.G., Torgerson P.R., & Doherty, M.L. (2006). Milk amyloid A: Correlation with cellular indices of mammary inflammation in cows with normal and raised serum amyloid A. *Research in Veterinary Science, 80,* 155-161.

Nielsen, B. H., Jacobsen, S., Andersen P. H., Niewold, T. A., & Heegaard P.M.H. (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.

Pyörälä, S. (2003). Indicators of inflammation in the diagnosis of mastitis. *Veterinary Research, 34,* 565-578.

Tolle, A., Heeschen, W., Reichmoth, J., & Zeidler, H. (1971). Counting of somatic cells in milk and possibilities for automation. *Dairy Science Abstracts, 33*, 875-879.

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, M.B., Larsen, L.B., & Qvist, K.B. (2002). Effects of storage conditions on lipolysis, proteolysis and sensory attributes in high quality raw milk. *Milchwissenschaft, 57,* 190-194.

Table 1. Contents in milk of haptoglobin (Hp) and serum amyloid A (SAA), and various milk quality parameters for cow composite milk samples.

Parameter	Unit	Mean (SD) n=89	Minimum	Maximum
Hp	mg L^{-1}	ND[a]	<1.0	33.00
SAA	mg L^{-1}	ND[a]	<0.3	48.66
Total protein	%	3.65 (0.39)	2.76	4.40
Casein	%	2.68 (0.30)	1.97	3.22
Whey protein	%	0.972 (0.14)	0.62	1.32
Casein number		0.73 (0.02)	0.67	0.80
Proteolysis	eq Leu[b]	1.13 (0.27)	0.64	2.29
Fat	%	4.94 (0.98)	2.88	7.53
Lactose	%	4.49 (0.20)	3.83	4.97
Somatic cell count	cells mL^{-1}	306,800 (789,600)	5,000	5,083,000

[a]ND= not determined. Since many of the samples did not contain detectable levels of Hp or SAA, i.e levels were below 1.0 mg L^{-1} and 0.3 mg L^{-1}, respectively, it was not considered relevant to calculate a mean value.

[b]eq Leu = equivalent mM Leucine.

Table 2. Differences in milk composition between cow composite samples with (Hp+) and without (Hp-) detectable levels of Hp. Differences for the different parameters were evaluated by parametric t-test and were considered significant if $p<0.05$.

	Hp+ (n=28)	S.E. [a] Hp+	Hp- (n=61)	S.E. Hp-	p-value
Total protein (%)	3.506	0.071	3.714	0.050	0.020
Casein (%)	2.575	0.054	2.724	0.038	0.030
Whey protein (%)	0.931	0.022	0.991	0.018	NS [b]
Casein number	0.734	0.003	0.733	0.003	NS
Proteolysis (eq Leu)[c]	1.154	0.044	1.119	0.038	NS
Fat (%)	4.655	0.175	5.064	0.125	NS
Lactose (%)	4.505	0.034	4.487	0.026	NS
LogSCC (mL^{-1})	5.274	0.131	4.788	0.070	0.0005

[a] S.E. = Standard Error

[b] NS = not significant

[c] eq Leu = equivalent mM Leucine.

Table 3. Differences in milk composition between cow composite samples with (SAA+) and without (SAA-) detectable levels of SAA. Differences for the different parameters were evaluated by parametric t-test and were considered significant if $p<0.05$.

	SAA+ (n=46)	S.E.[a] SAA+	SAA- (n=43)	S.E. SAA-	p-value
Total protein (%)	3.632	0.063	3.667	0.056	NS [b]
Casein (%)	2.646	0.048	2.710	0.041	NS
Whey protein (%)	0.986	0.021	0.957	0.020	NS
Casein number	0.728	0.004	0.739	0.003	0.026
Proteolysis (eq Leu) [c]	1.131	0.044	1.128	0.038	NS
Fat (%)	4.940	0.150	4.930	0.144	NS
Lactose (%)	4.441	0.030	4.547	0.027	0.010
LogSCC (mL^{-1})	5.288	0.086	4.569	0.069	<0.0001

[a] S.E. = Standard Error

[b] NS = not significant

[c] eq Leu = equivalent mM Leucine.

Table 4. Differences in milk composition between cow composite samples with detectable levels of both haptoglobin (Hp) and serum amyloid A (SAA) and samples not meeting these criteria. Differences were evaluated by parametric t-test and were considered significant if $p<0.05$.

	Hp+/SAA+ n=(20)	S.E. [a] Hp+/SAA+	Not Hp+/SAA+ (n=69)	S.E. Not Hp+/SAA+	p-value
Total protein (%)	3.435	0.083	3.711	0.046	0.005
Casein (%)	2.519	0.062	2.723	0.035	0.007
Whey protein (%)	0.915	0.026	0.988	0.017	0.033
Casein number	0.734	0.004	0.734	0.003	NS [b]
Proteolysis (eq Leu) [c]	1.179	0.055	1.115	0.034	NS
Fat (%)	4.719	0.224	4.998	0.116	NS
Lactose (%)	4.473	0.038	4.498	0.025	NS
LogSCC (mL^{-1})	5.537	0.142	4.768	0.063	<0.0001

[a] S.E. = Standard Error

[b] NS = not significant

[c] eq Leu = equivalent mM Leucine