doi: 10.17221/254/2015-CJFS

Relationship between Somatic Cell Count and Milk Casein Level Obtained by Two Different Methods

Kristina MUSAYEVA[1], *Antanas SEDEREVIČIUS*[1], *Rasa ŽELVYTĖ*[1], *Ingrida MONKEVIČIENĖ*[1], *Danuta BELIAVSKA-ALEKSIEJŪNĖ*[1] *and Rolandas STANKEVIČIUS*[2]

[1]*Department of Anatomy and Physiology, Faculty of Veterinary Medicine and* [2]*Department of Animal Breeding and Nutrition, Faculty of Animal Husbandry Technology, Lithuanian University of Health Sciences, Kaunas, Lithuania*

Abstract

Musayeva K., Sederevičius A., Želvytė R., Monkevičienė I., Beliavska-Aleksiejūnė D., Stankevičius R. (2016): **Relationship between somatic cell count and milk casein level obtained by two different methods**. Czech J. Food Sci., 34: 47–51.

The purpose of the present work was to investigate the impact of somatic cell count (SCC) on the content of milk casein and to compare the sensitivity of different methods for the determination of the casein level in milk. Two different methods were employed in order to determine the casein level: routine method – infrared spectrophotometry and reference method – Kjeldahl. Data array of 26, 169 milk samples of the livestock control was used to analyse milk casein variation by SCC limits. Mean casein values (investigated by the routine method) varied from 2.47% to 2.8% (2.65 ± 0.09) and casein number (in % on crude protein basis) – CN% varied from 79.5% to 81.1%. The present analysis showed that the relation between SCC and casein content obtained by the routine method correlated significantly (Pearson correlation $r_p = 0.644$, $P < 0.05$, $R^2 = 0.414$, Spearman correlation $r_s = 0.786$, $P < 0.05$). Evaluated results obtained by the least squares method, and linear approximation of variation indicators presented a noticeable decline in casein percentage – 0.27%, when SCC increased by 80×10^3/ml, but only when the studies were done by the reference method.

Keywords: raw milk; proteins; cow

In modern human nutrition milk still gains increasing importance as it is the basic raw material for a great diversity of dairy products. The health of the udder can have a profound effect on the quality and processing characteristics of milk. Many milk processing companies worldwide have incorporated somatic cell count as a key parameter in schemes which reward producers for producing milk of the highest quality. The occurrence of inflammatory process or mastitis generally leads to an increase in SCC in milk, which has been associated with changes in milk components and properties (Barbano *et al.* 1991; Auldist & Hubble 1998; Tripaldi *et al.* 2003; Cunha *et al.* 2008; Barlowska *et al.* 2009).

In Europe, according to the currently valid legislation, Commission Regulation (EC) No. 1662/2006 of 6 November 2006, milk intended for human consumption should not contain more than 400×10^3/ml somatic cells. This level will minimise the effects of mastitis on product quality, although negative effects on product quality have been reported for milk with a SCC as low as 100×10^3/ml. Further, a bulk milk somatic cell count of 400×10^3/ml indicates that around 40% of cows in a herd might be infected (Auldist & Hubble 1998; Barlowska *et al.* 2009). Mastitic milk has a higher proteolytic activity than normal milk, in part due to the increased proteinase plasmin, which hydrolyzes caseins (Le Roux *et al.* 2003). Proteolysis could occur in milk with SCC as low as 250×10^3/ml (Le Roux *et al.* 1995). The variations in milk SCC explain about half of the variations in milk plasmin and casein damage (Le Bars & Gripon 1993; Le Roux *et al.* 1995, 2003; McSweeney & Fox 1995; Barbano 2000). The

Supported by the Ministry of Agriculture of the Republic of Lithuania, Project MT 13/18.

doi: 10.17221/254/2015-CJFS

deterioration of milk continues during storage and may lead to casein proteolysis due to the presence of proteolytic enzymes (Button *et al.* 2011).

In countries where large quantities of milk are processed to make cheese, it is crucial to assess the range of the casein proportion in milk true protein and the factors causing it to vary (Coulon *et al.* 1998). Lack of simple routine analyses to measure casein content in milk is a major factor limiting progress in this direction (Hallén 2008). To calculate milk casein as a percentage of true protein (C% TP) according to Barbano *et al.* (1991), casein and true protein need to be measured. Milk protein can be measured rapidly and cost-effectively by infrared analysis, but the dairy industry does not currently have a practical method for measurement of milk casein content. Development of a rapid, accurate, and economical test for casein would be of great value to the dairy industry for use in milk payment programs (Barbano *et al.* 1991).

The purpose of the present work was to investigate the impact of somatic cell count on the content of milk casein as a percentage of true protein and to compare the sensitivity of two different methods for the determination of the casein level in milk.

MATERIAL AND METHODS

Analysis of database concerning casein and SCC in milk samples from livestock in Lithuania. Milk casein and SCC data array of the livestock control (June 2013) was used for a situation analysis. Total data sample record of the livestock control in Lithuania consisted of 22.6% records (26 169 milk samples). Structured Query Language (SQL) data were processed in the database management system of Linux operating environment. The groupings of milk samples were performed on the basis of SCC with the help of SQL request in the database of the livestock control. The individual animal milk samples were divided into thirteen groups where SCC varied from 10×10^3/ml to 4999×10^3/ml and the average of casein as the mean percentage of true protein in each group was computed.

Investigation of casein and SCC in milk samples from selected herd

Collection of milk samples. A selected herd was participating in the animal productivity control program. Overall 110 cows were randomly selected. All animals enrolled in the study were lactating cows of the holsteinized Black-and-White breed. The milk samples were collected during control evening milking time as total quarter milk from each cow in pairs. After cleaning and disinfection of the teats, 50 ml of milk were aseptically collected in sterile plastic tubes according to LST EN ISO 707:1999 + P:2003 standard. Samples were kept under refrigeration until arrival to laboratory facilities and were tested within 6 h from collection.

Analysis of milk samples. The analysis of raw milk samples was performed in an accredited milk laboratory. As the first step for determination of somatic cell count, 110 collected milk samples were preserved and analysed by the flow cytometric analysis method using a Somascope cell counter according to LST EN ISO 13366-1:2008 + AC:2009 standard for a microscopic method.

As a further step, eighteen milk samples sorted according to SCC results of the first step were investigated for casein content and CN ratio (in %) in milk by reference Kjeldahl method and routine infrared spectrophotometric method. Casein nitrogen content was determined by an indirect reference method. Determination of casein and non-casein nitrogen (NCN) content was done according to ISO 8968-1:2014 Milk and milk products – Determination of nitrogen content – excerpted Part 1: Kjeldahl principle and crude protein calculation. NCN was obtained using precipitation with acetic acid and sodium acetate solutions. The precipitated casein was removed by filtration, so NCN remained in the filtrate. Nitrogen content in the filtrate was determined according to ISO 8968-1:2014 Milk and milk products – Determination of nitrogen content – excerpted Part 1: Kjeldahl principle and crude protein calculation. Casein nitrogen was calculated from the total nitrogen minus the obtained NCN content of milk. Casein number (in % on crude protein basis) and casein mean values were analysed by the routine infrared spectrophotometric method using a LactoScope FTIR analyser according to LST ISO 9622:2000 standard.

Statistical analysis. SQL data store and GraphPad Prism Version 4.0 statistical package were used for biometric data analysis. Arithmetic means ($\bar{x}$), medium standard deviations (SD) and standard error (SE), coefficients of variation (*CV*), minimum and maximum values were calculated for cow's milk casein parameters and somatic cell count. Pearson and Spearman correlation coefficients and linear regression were used to investigate the relationship between

doi: 10.17221/254/2015-CJFS

Table 1. Variation indicators of casein distribution by SCC limits

No of groups	SCC groups (× 10^3/ml)	Number of cows (*n*)	$\bar{x}$	CN (%)	SD	*CV*
1	10–99	8737	2.47	79.5	0.31	12.52
2	100–199	6970	2.53	79.8	0.35	13.79
3	200–299	3790	2.59	80.0	0.38	14.48
4	300–399	2230	2.65	80.2	0.40	15.14
5	400–499	1421	2.64	80.1	0.40	15.13
6	500–599	1099	2.66	80.3	0.42	15.69
7	600–699	793	2.68	80.6	0.44	16.35
8	700–799	471	2.71	80.7	0.43	15.79
9	800–999	338	2.70	80.5	0.44	16.25
10	1000–1499	187	2.60	80.1	0.35	13.48
11	1500–1999	90	2.74	80.7	0.52	19.08
12	2000–2999	32	2.66	80.3	0.30	11.22
13	3000–4999	11	2.80	81.1	0.30	10.61

$\bar{x}$ – mean casein values; CN – casein number values (in % on crude protein basis); SD – standard deviations; *CV* – coefficients of variation

SCC levels and milk casein. Results were statistically significant when R^2 was not lower than 0.25.

RESULTS

Mean casein values by somatic cell count limits of database analysis from the livestock of Lithuania ranged from 2.47 to 2.8 (2.648 ± 0.09; SE 0.02); these data are reported in Table 1 and Figure 1. The present analysis shows only a slightly notable change in casein of controlled cow's milk by somatic cells count limits. When milk samples were estimated by the infrared spectrophotometric method, a high level of SCC had a statistically positive relation to the casein content ($P < 0.05$).

CN (in %) estimated by the reference method was 2–5% higher than the casein percentage tested by the infrared spectrophotometric method when SCC was low and ranged from 33 × 10^3/ml to 293 × 10^3/ml. Though, when SCC was high and ranged from 2203 × 10^3/ml to 5395 × 10^3/ml, the casein percentage decreased to 5%, in comparison with the casein level estimated by the infrared spectrophotometric method. The data obtained by reference (Kjeldahl) and routine (infrared spectrophotometry) methods correlate significantly (Pearson correlation $r_p = -0.634$, $P < 0.05$, $R^2 = 0.4023$). These data are reported in Figure 2.

Evaluated results obtained by the least squares method, and linear approximation of variation indicators are presented in Figure 3. When the studies were done by the reference Kjeldahl method with

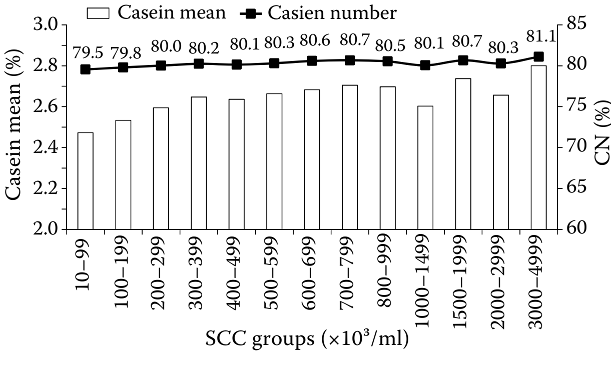

Figure 1. Changes in casein percentage (CN%) by somatic cells count (SCC) limits tested by infrared spectrophotometric method

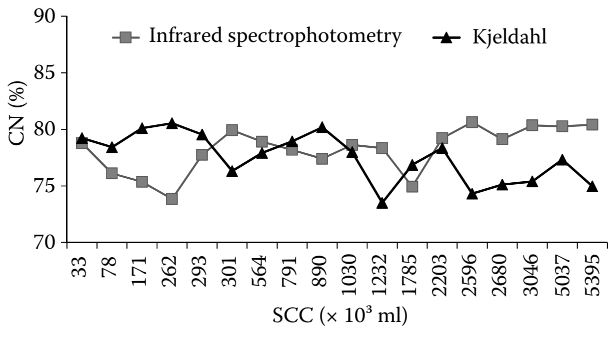

Figure 2. Changes in casein percentage (CN%) by somatic cell count (SCC) limits tested by reference Kjeldahl and routine infrared methods

doi: 10.17221/254/2015-CJFS

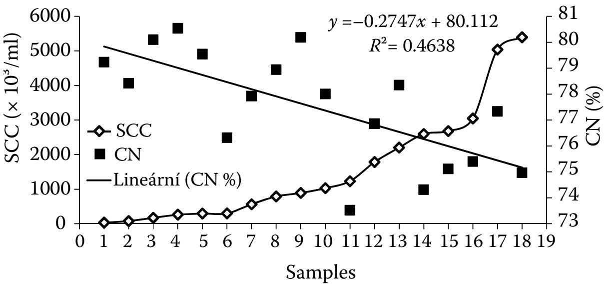

Figure 3. Linear regression model of the effect of somatic cell (SCC) count on milk casein number (CN in % on crude protein basis)

eighteen milk samples, SCC increased from about 30×10^3/ml to 5000×10^3/ml, and casein number decreased from 81% to 74%. Results were statistically significant ($R^2 = 0.4638$).

DISCUSSION AND CONCLUSIONS

Our objective in this investigation was to assess the influence of somatic cell count on the casein level in cow's milk. It has been suggested that a somatic cell count in milk higher than 400×10^3/ml probably indicates that the cow suffers from udder inflammation (Akers 2002). High SCC influences milk quality, as it is associated with the increased proteolytic degradation of caseins (Barbano *et al.* 1991; Bastian & Brown 1996).

It was hypothesized that the relationship between increasing milk SCC and decreasing casein would be linear. The data collected in this study demonstrate a statistically positive relation ($P < 0.05$) between SCC and the content of casein when milk samples were tested by the routine infrared spectrophotometric method.

At the beginning of this study, data array of the livestock control was taken for a situation analysis to estimate the casein variation by SCC limits in milk. The average percentage of casein by somatic cells count limits ranged from 79.5% to 81.1%. The present analysis showed that an increase of somatic cell counts in milk was associated with increased casein content. The casein content was related with somatic cells by a positive correlation (Pearson correlation r_p = 0.644, $P < 0.05$, $R^2 = 0.414$, Spearman correlation r_s = 0.786, $P < 0.05$). According to other authors, to obtain better values of these parameters SCC 200×10^3/ml should not be exceeded (Barbano *et al.* 1991; Tripaldi *et al.* 2003). This may be linked to increased endogenous proteolysis due the elevation of plasmin or other proteases derived from somatic cells, leading to the breakdown of casein and the influx of blood proteins (immunoglobulins – IgG, and bovine serum albumin) into milk due to increased permeability of the mammary epithelium, which results in an elevated non-casein nitrogen content (Le Roux *et al.* 1995; Coulon *et al.* 2002). The results obtained in this study are in contradiction with the results reported by some authors. According to Tripaldi *et al.* (2003) the content of casein decreased when somatic cells increased (from 3.90% to 2.69%). Similar results were reported by Ballou *et al.* (1995) and Litwinczuk *et al.* (2011).

The investigation of casein by different methods was analysed in a scientific study performed by Barbano and Dellavalle (1987). These authors determined that protein, casein, or non-casein protein in 36 different individual herd milk samples subjected to analysis of variance indicated no statistically significant differences between the results from Kjeldahl and infrared analyses. Casein as a percent on the total nitrogen basis varied from 76.3% to 78.8% (Barbano & Dellavalle 1987). Hanuš *et al.* (2010) reported that the casein number varied from 79.4% to 80.56% (two breeds, Holstein and Czech Fleckvieh, six herds) in bulk milk samples in three years. The variability was from 1.4% to 1.5% relatively, which means low. It was shown on relatively reliable casein analyses by indirect methods of infrared spectroscopy (MIR – Michelson interferometer and MIR-FT – Fourier transform) (Hanuš *et al.* 2010). We found out that high SCC showed a negative correlation with casein when milk samples were tested by the routine infrared spectrophotometric method. When the studies were done by the reference Kjeldahl method and SCC was increased, linear approximation of variation indicators showed a noticeable decline in casein number from 81% to 74 %.

Based on the results, it can be concluded that from the data array of the livestock control the relationship between increasing milk SCC and increasing casein content demonstrates a statistically positive relation ($P < 0.05$). There was a slight change in CN (in %) with milk SCC increasing from 10×10^3/ml to 2999×10^3/ml when milk samples were tested by the infrared spectrophotometric method. Determination of casein by different methods indicated that CN decreased from 78% to 75% with milk SCC increasing from 2203×10^3/ml to 5395×10^3/ml when milk samples were tested by the reference Kjeldahl method.

doi: 10.17221/254/2015-CJFS

Further studies are needed to clarify the relationships between casein content and SCC in cow's milk. To develop a method for the determination of milk casein content more practical studies and analyses are required.

References

Akers R.M. (2002): Lactation and the Mammary Gland. Iowa, Iowa State Press: 100–200.

Auldist M.J., Hubble I.B. (1998): Effects of mastitis on raw milk and dairy products. Australian Journal of Dairy Technology, 53: 28–36.

Ballou L.U., Pasquini M., Bremel R.D., Everson T., Dean Somme R.D. (1995): Factors affecting herd milk composition and milk plasmin at four levels of somatic cell counts. Journal of Dairy Science, 78: 2186–2195.

Barbano D.M. (2000): Practical Guide for Control of Cheese Yield. International Dairy Federation, Brussels: 19–27.

Barbano D.M., Dellavalle M.E. (1987): Rapid method for determination of milk casein content by infrared analysis. Journal of Dairy Science, 70: 1524–1528.

Barbano D.M., Rasmussen R.R., Lynch J.M. (1991): Influence of milk somatic cell count and milk age on cheese yield. Journal of Dairy Science, 74: 369–388.

Barlowska J., Litwinczuk Z., Wolanciuk A., Brodziak A. (2009): Relationship of somatic cell count to daily yield and technological usefulness of milk from different breeds of cows. Polish Journal of Veterinary Science, 12: 75–79.

Bastian E.D., Brown R.J. (1996): Plasmin in milk and dairy products: an update. International Dairy Journal, 6: 435–457.

Button P.D., Roginski H., Deeth H.C., Craven H.M. (2011): Improved shelf life estimation of UHT milk by prediction of proteolysis. Journal of Food Quality, 34: 229–235.

Coulon J.B., Hurtaud C., Remond B., Verite R. (1998): Factors contributing to variation in the proportion of casein to cows' milk true protein: a review of recent INRA experiments. Journal of Dairy Research, 65: 375–387.

Coulon J.B., Gasqui P., Barnouin J., Ollier A., Pradel P., Pomiès D. (2002): Effect of mastitis and related-germ on milk yield and composition during naturally-occurring udder infections in dairy cows. Animal Research, 51: 383–393.

Cunha R.P.L., Molina L.R., Carvalho A.U., Facury Filho E.J., Ferreira P.M., Gentilini M.B. (2008): Subclinical mastitis and relationship between somatic cell count with number of lactations, production and chemical composition of milk. Brazilian Journal of Veterinary and Animal Science, 60: 19–24.

Hallén E. (2008): Coagulation Properties of Milk Association with Milk Protein Composition and Genetic Polymorphism. [PhD Thesis.]. Uppsala, Swedish University of Agricultural Sciences.

Hanuš O., Yong T., Kučera J., Genčurová V., Hanušová K., Kopec T., Kopecký J., Jedelská R. (2010): Analýza výsledků kalibrací pro stanovení kaseinu nepřímou metodou infračevené spektroskopie. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 58: 123–136.

Le Bars D., Gripon J.C. (1993): Hydrolysis of α-s1-casein by bovine plasmin. Lait, 73: 337–344.

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.

Le Roux Y., Laurent F., Moussaou F. (2003): Polymorphonuclear proteolytic activity and milk composition change. Veterinary Research, 34: 629–645.

Litwińczuk Z., Król J., Brodziak A., Barłowska J. (2011): Changes of protein content and its fractions in bovine milk from different breeds subject to somatic cell count. Journal of Dairy Science, 94: 684–691.

McSweeney P.L.H., Fox P.F. (1995): Proteolysis of bovine caseins by cathepsin D: preliminary observations and comparison with chymosin. International Dairy Journal, 5: 321–336.

Tripaldi C., Terramoccia S., Bartocci S., Angelucci M., Danese V. (2003): The effects of the somatic cell count on yield, composition and coagulating properties of mediterranean buffalo milk. Asian-Australasian Journal of Animal Sciences, 16: 738–742.

Received: 2015–05–18
Accepted after corrections: 2015–12–29

Corresponding author:

Kristina Musayeva, PhD., Lithuanian University of Health Sciences; Faculty of Veterinary Medicine, Department of Anatomy and Physiology, Tilžės st. 18, LT-47181 Kaunas, Lithuania; E-mail: kristina.musayeva@lsmuni.lt