LACTOFERRIN AND IMMUNOGLOBULIN G CONTENT IN COW MILK IN RELATION TO SOMATIC CELL COUNT AND NUMBER OF LACTATIONS

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Abstract. Dairy cattle vary considerably in their susceptibility to mastitis, perhaps due to innate levels of milk antimicrobial proteins. **The aim of the study** was to evaluate the amount of chosen antimicrobial proteins, i.e. lactoferrin (LTF) and immunoglobulin G (IgG) in cow milk in relation to somatic cell count (SCC), and number of lactations. Milk samples were collected from individual udder quarters from thirty selected normal lactating cows. Milk samples were obtained from quarters of 1st (n = 32), 2nd (n = 44) and 3rd (n = 44) lactations. The quarters health status was set on the base of SCC in milk. The affected udder quarters showed increased concentration of LTF and IgG by 17 % and 15 % respectively, in compare to healthy quarters ($P < 0.05$). Evaluation of the influence of the lactations number on the distribution of antimicrobial proteins in the milk showed that the highest concentrations of LTF (0.08 mg/mL) occur in first-calf heifers and IgG (0.3 mg/mL) in second lactation cows. Analysis of the relations between the health status of udder quarters established by SCC in milk and antimicrobial proteins showed a strong positive correlation between LTF and IgG in quarters affected by inflammation ($r = 0.817$; $P < 0.05$).

Keywords: mastitis, lactoferrin, immunoglobulins, cow

Introduction. Lactoferrin (LTF) is a glycoprotein naturally present in milk with known favorable associations with the immune system (Soyeurt et al., 2012). LTF is a multifunctional, iron-binding glycoprotein naturally present in milk and secreted mainly by the mammary epithelial cells (Shimazaki and Kawai, 2017). LTF can also be released by polymorphonuclear neutrophils during inflammation (Kutila et al., 2004). Bovine milk contains between 0.02 and 0.35 mg/ml of LTF, depending on the period of lactation (Madureira et al., 2007). In the colostrum, lactoferrin concentration is very high and then gradually decreases (Shimazaki and Kawai, 2017). High LTF concentration in milk (2.3 g/l of milk) may indicate clinical or subclinical mastitis (Kutila et al., 2004). Clinical mastitis presents physiological abnormalities in the udder such as swelling, heat, redness, hardness, or pain. Bacterial infection without any visible signs or clinical appearance is called subclinical mastitis. A high infection ratio has been observed when lactoferrin content is low in the milk of nonlactating cows (Farke et al. 2008). Therefore, the selection and breeding of cows with lactoferrin rich milk has been attempted (Arnould et al. 2009).

Immunoglobulins (Igs) link various parts of the cellular and humoral immune system. They are able to prevent the adhesion of microbes, inhibit bacterial metabolism, agglutinate bacteria, augment phagocytosis of bacteria, kill bacteria through activation of complement-mediated bacteriolytic reactions, and neutralize toxins and viruses.

Somatic cell count is often used as an indicator of subclinical mastitis in dairy cows, but knowledge on the milk LTF and IgG content could aid in mastitis detection (Schukken et al., 2003; Malinowski et al., 2008).

Summarizing theoretical achievements reported in literary sources the objective of the present study was to evaluate the concentration of natural antimicrobial proteins in cow milk in relation to somatic cell count (SCC), and number of lactations.

Materials and methods

Sampling. In total, 120 milk samples were collected individually from thirty normal lactating dairy cows of 1st (n = 7), 2nd (n = 12) and 3rd (n = 11) lactation. Milk samples from individual quarters were collected. For estimation of the udder health status of all cows included in the investigation, we evaluated the udder and teats visually and palpated before milking. All cows were clinically healthy with no signs of udder infection (by checking the presence of redness, swelling, hardness, and pain in the udder, or the presence of clots in milk) at sampling time.

Milk samples were collected for laboratory examination aseptically in accordance with the method recommended by the standard ISO 707:2008 (Milk and milk products - Guidance on sampling). In the middle of the milking samples of milk were taken for somatic cell count and antimicrobial proteins investigation (100 mL).

Estimation of somatic cell count. For determination of SCC, 40 ml of total milk samples were taken and preserved with bronopol (2-bromo-2-nitropropane-1,3-diol and 2-bromo-2-nitropropanol) in microtabs and analysed with the flow cytometric analysis method using a Somascope MK2 cell counter (Foss, 3400 Hillerod, Denmark) according to standard EN ISO 13366-1:2008/Cor.1:2009 (Milk - Enumeration of somatic cells - Part 2: Guidance on the operation of fluoro-opto-electronic counters). The direct measurement reading is SCC in thousands per one milliliter of milk (thou/mL).

Estimation of LTF and IgG concentration. The LTF and IgG concentration in whole milk samples was measured at least in duplicate with a commercial ELISA kit (Bovine Lactoferrin ELISA Quantification Kit, Bethyl Laboratories Inc., Montgomery, TX, USA). The procedure was carried out according to the manufacturer's instructions. For LTF analyses, the milk samples were diluted at the ratio of 1:1,000 and for IgG 1:2,000. In both analyses, a standard curve was plotted for each plate separately.

Statistical analysis. The data obtained were statistically processed by using Microsoft Excel 2010® and the SPSS program 20.0 for Windows (Inc., Chicago, IL) using a one-way ANOVA procedure. The results are presented as mean and standard error of mean (mean ± SEM). The significance of differences between mean values of the evaluated groups was determined with the *post-hoc* Fisher's *LSD* test. A value of $P < 0.05$ was considered significant. The inner correlation of the parameters was evaluated according to Pearson correlation coefficients (r) and their statistical reliability (P). The results are considered to be statistically reliable when $P < 0.01$, $P < 0.05$.

The numerical material was divided into groups according to the health status of the udder quarters, milk SCC and lactation.

Based on the milk SCC, the data were allocated into four groups:

- group I – up to 100 thou/mL (n = 76);
- group II – from 101 to 200 thou/mL (n = 15);
- group III – from 201 to 400 thou/mL (n = 9);
- group IV – over 401 thou/mL (n = 20).

Udder quarters from group I and group II corresponded to the healthy quarters (n = 91) and quarters from group III and group IV – to the diseased quarters (n = 29).

To test the reliance of LTF and IgG on subsequent lactation the data were classified into three age classes: 1st (n = 32), 2nd (n = 44) and 3rd (n = 44) lactation.

Results

Distribution of antimicrobial protein concentrations according to SCC in the milk. By grouping the data according to SCC in the milk and evaluation of the health status of udder quarters, 76 % of healthy and 24 % of affected quarters were determined. The obtained results showed that LTF and IgG concentrations (0.07±0.01 and 0.27±0.05 mg/mL respectively) in the affected udder quarters were higher than in the healthy quarters (0.05±0.01 and 0.20±0.02 mg/mL respectively) by 17 % and 15 % respectively. The difference between the healthy and affected quarters was statistically significant ($P < 0.05$).

As is shown in Fig. 1, the distribution of the average values of LTF in the groups distinguished by SCC is uneven. A statistically significant difference ($P < 0.05$) was determined between the lowest (first group) and highest (second group) LTF concentrations. The distribution of IgG average values between the groups followed a similar pattern as that of LTF yet it was not statistically significant.

By correlation analysis of antimicrobial proteins statistically significant relations was observed between LTF and IgG ($r = 0.731$, $P < 0.01$). Analysis of the relations between the health status of udder quarters established by SCC in milk and antimicrobial proteins showed a strong positive correlation between LTF and IgG in healthy quarters (SCC < 200 thou/mL) and quarters affected by inflammation (SCC > 201 thou/mL) ($r = 0.698$ and $r = 0.817$ respectively).

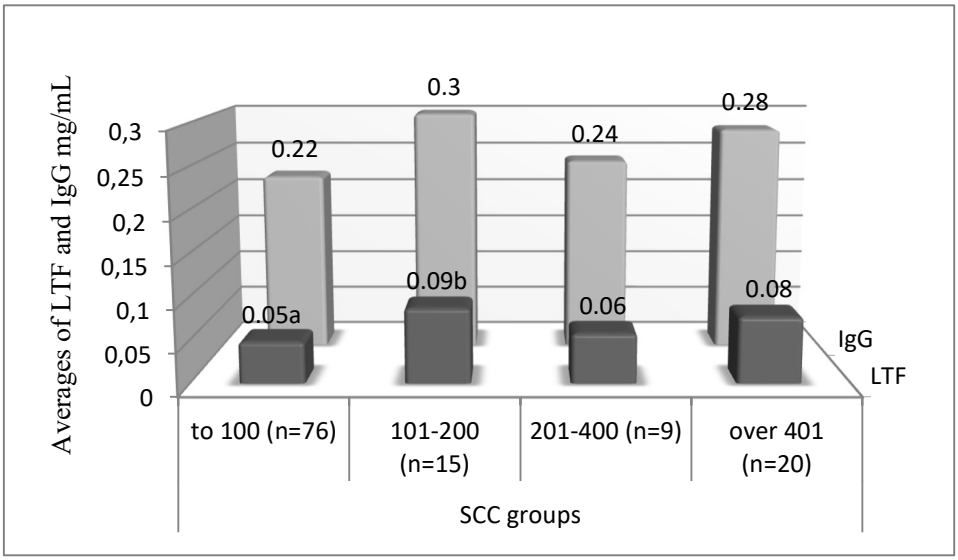

Fig. 1. *Distribution of the average values (mean ± SEM) of LTF and IgG in SCC groups*
Note: Mean values denoted by letters a and b are significantly different at P < 0.05

Results of investigation of the influence of lactation number on the concentrations of antimicrobial proteins in the milk. Assessment of the influence of cow age, i.e. lactation number, on the distribution of proteins in milk (Fig. 2) showed that during the second lactation the concentration of IgG was higher than during the first or

third lactations. The milk of the first-calf heifers contained the highest concentration of LTF. The average value of the second and third lactation groups differed IgG by 25% and the differences were statistically significant (P < 0.05)

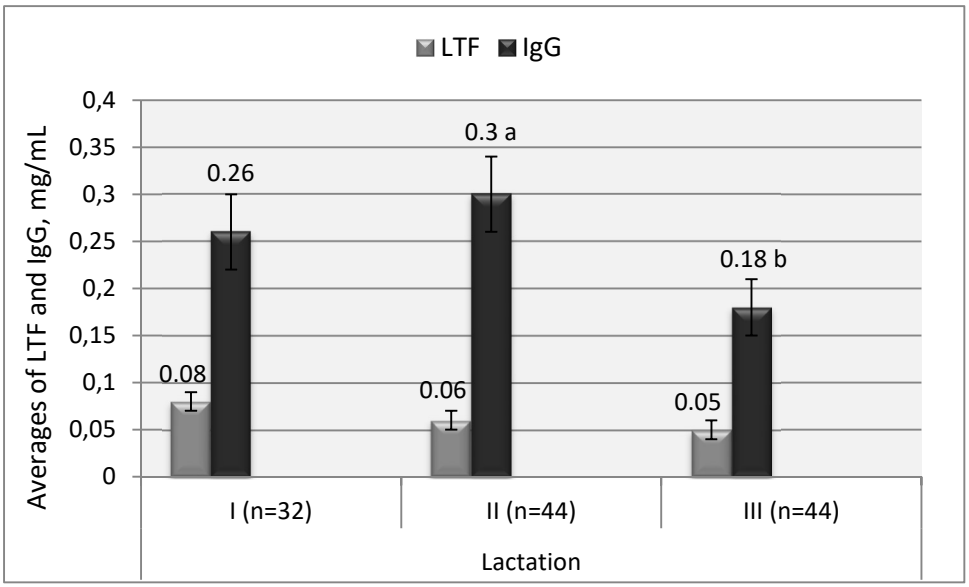

Fig. 2. ***Variations of antimicrobial proteins in milk during different lactations (mg/mL)***
Mean values denoted by letters a and b are significantly different at P < 0.05

Analysis of the links between the factors revealed LTF and IgG strong, positive correlations during all stages of first (r = 0.922), second (r = 0.684) and third (r = 0.619) lactations (P<0.01).

Discussion. Somatic cell count is recognized as the main indicator of milk quality and health status of cows (Schukken et al., 2003; Green et al., 2004; Piccinini et al., 2006). Smith (2002) and Pyorala (2003), reported that milk from udder quarter in which SCC exceeds 200 thou/mL is indicative of inflammatory reaction (subclinical mastitis). The data obtained by milk SCC usually are grouped and the groups serve as a basis for identification of healthy cows and cows with mastitis. Researchers conducted in Poland showed that increasing SCC contributed to decreasing values of the main albumins but the concentrations of immunologically active proteins (lactoferrin and lysozyme) and bovine serum albumin increased (P < 0.001) (Litwinczuk et al., 2011). Krol and co-authors (2012) supplemented Litwinczuk's et al. (2001) data. They determined that the increasing SCC statistically significantly influenced the increasing concentrations of LTF, IgG and lysozyme in the milk. In the mentioned researches, the influence of SCC on the content of LTF and IgG was proved by high correlation coefficients, which are in agreement with our calculations. The positive correlation coefficients between SCC and LTF also were confirmed by Hutton at al. (1990), Lindmark-Mansoon et al. (2000; 2006), and Cheng et al. (2008) and between SCC and IgG by Liu et al. (2009) and Krol et al. (2012; 2013). Our results and results obtained by other researchers allow assuming that identification of subclinical mastitis should be based on assessment of LTF and IgG indices and SCC in milk.

Contrasting results were obtained on the influence of the number of lactations on the content of antimicrobial proteib in the milk. Our results are comparable with the results obtained by Hagiwara et al. (2003) showing that LTF concentration in the milk of older cows is lower than in the milk of first-calf heifers (P < 0.05). However, other studies disproved the theory that LTF and Ig concentrations decrease in the milk of older cows (Soyeurt et al., 2007; Krol et al., 2010; Krol et al., 2012). Krol et al. (2010) maintain that Ig and lysozyme concentrations in the milk increase with cow ageing and reach their peak values during the fourth lactation. Besides, the highest LF concentrations in the cow milk occur during the second and fourth lactations (Krol et al., 2010). Subsequent investigations conducted by Krol et al. (2012) backed up the previous results. They showed that LTF and IgG concentrations in the milk of first-calf heifers was significantly lower than in the milk of cows of the second–third lactations (P ≤ 0.05) and even older cows (P ≤ 0.01).

Conclusion. It suggests the usefulness of these indicators associated to SCC to detect the presence of mastitis. Moreover, the knowledge of milk LTF and IgG could also improve the milk nutritional quality.

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