CHANGES IN THE CONTENT OF WHEY PROTEINS DURING LACTATION IN COW'S MILK WITH A DIFFERENT SOMATIC CELLS COUNT

Monika Sobczuk-Szul, Zofia Wielgosz-Groth, Zenon Nogalski, Paulina Pogorzelska-Przybyłek

Department of Cattle Breeding and Milk Quality Evaluation, Faculty of Animal Bioengineering
University of Warmia and Mazury
Oczapowskiego 5, 10-719 Olsztyn

Corresponding author: Monika Sobczuk-Szul
tel.: +48 89 5233816; fax: +48 89 5234413. e-mail: monika.sobczuk@uwm.edu.pl

Abstract. The objective of this study was to determine the effect of stage of lactation and hygienic quality of milk on the content bioactive whey proteins. The experimental material was milk from 56 cows of the Polish Holstein-Friesian breed (phf). In the 1st, 5th and 10th month of lactation, milk samples were collected for analyses from each cow. Each sample of fresh milk was analysed for the proximate chemical composition with a MilkoScan FT 120 apparatus and for somatic cells count (SCC) using a BactoCount apparatus. The contents of α-lactalbumin (α-LA) and β lactoglobulin (β-LG) were assayed electrophoretically following the methodology by Laemmli (1970); the contents of lactoferrin (LF), interleukins (IL-1β, IL-6), and tumor necrosis factor TNF-α were determined by the "sandwich" ELISA method. The achieved results suggest that the stage of lactation affected the content of the analyzed bioactive proteins in cow's milk. The higher content of the analyzed proteins at the last stage of lactation allows concluding that the best raw material for the isolation of functional properties is milk originating from the final stage of lactation. An increasing content of SCC decrease the content α-LA and β-LG. An opposite dependence was observed for proteins that play the role of inflammatory mediators. The concentrations of LF, IL-1β and TNF-α were found to depend on the somatic cells count in milk, what indicates that the analysis of their contents in milk may constitute a complementary indicator of mastitis incidence.
Keywords: lactoferrin, cytokines, mastitis, stage of lactation.

Introduction. Bovine mastitis is still a recurring and severe problem faced by milk producers (Bernatowicz et al., 2004; Petrovski et al., 2006; Sharma et al., 2011). Mastitis causes vast economic losses that are due to decrease of milk yield, deterioration of milk quality and, resultantly, its reduced utility for processing, as well as to additional costs of veterinary treatment. Apart from the main nutrients, milk contains chemical compounds that affect the mammary gland and other organs of cow (Malinowski et al., 2008). Milk contains many peptides and proteins, which exhibit bacteriostatic and bactericidal properties in their intact form (Bagnicka et al. 2010). Especially beneficial nutritive, physiological and functional properties are displayed by whey proteins (Atanasova and Ivanova 2010; Bernatowicz and Reklewska, 2003; Świderski and Waszkiewicz-Robak, 2000). As emphasized by Leman (1995), the whey proteins contain peptides that improve, among other things, the immunomodulatory activity (stimulating defence mechanisms of the body) and antibacterial activity. One of these biologically-active milk constituents is lactoferrin which plays a key role in mastitis prevention at the early stage of infection (Kawai et al., 2003). As a multifunctional protein with antimicrobial properties, lactoferrin is one of the major preventive factors against mastitis in dairy cows (Wojdak-Maksymiec and Mikolajczyk, 2012). Other important inflammatory mediators during mastitis are cytokines and, most of all, interleukins: IL-1β, IL-6 and tumour necrosis factor TNF-α the increased content of which in milk may – according to Hagiwara et al. (2001) – be indicative of inflammatory state incidence. According to

Parameswaran and Patial (2010) tumour necrosis factor (TNF) is one of the main proinflammatory cytokines and, thanks to its pleiotropic properties, activates the entire immune system. However, studies concerning the possible physiological impact of bioactive peptides are continuously developing (Szwajkowska et al., 2011).

Owing to the fact that thus far conducted sparse researches on lactoferrin and cytokines contents of milk have been inconclusive, Cheng et al. (2008) and Hagiwara et al. (2001) point to the need for investigating effects of different factors on the level of these proteins in cow's milk.

Objective. In view of the above, it was found advisable to analyze changes in the content of whey proteins, including lactoferrin and cytokine, in cow's milk as affected by the health status of udder expressed by somatic cells count and by lactation stage.

Material and methods

Material. The experimental material was milk of 56 cows of the Polish Holstein-Friesian breed (phf) selected from six loose barns with an average herd size of ca. 150 cows, located in the north-eastern Poland. These were mainly cows in the 1st and 2nd lactation and the average number of lactations of the analyzed cows was 1.81±0.86. The annual milk yield in the barns the cows originated from amounted to ca. 7,000 kg milk, whereas the average daily yield of cows to 23.56±8.89 kg. The feeding of cows was alike in all barns. Throughout the year, the cows were kept in cowsheds and fed preserved feed mixtures in the Total Mixed Ration (TMR) or Partly Mixed Ration (PMR) system. In the 1st, 5th and 10th month of lactation, during trial milking, milk samples were

collected for analyses from each cow. Having been collected, the milk samples were transported in chilled conditions to a laboratory of the Department of Cattle Breeding and Milk Evaluation.

Methods. Samples of fresh milk were determined for the proximate chemical composition (protein, fat, lactose, and dry matter) by the spectrophotometric method in infrared with a MilkoScan FT 120 apparatus (FossElectric) and for somatic cells count (SCC) by the flow cytometry method using a BactoCount apparatus (Bentley). The data regarding cows yield originated from the SYMLEK system and were provided by the Polish Federation of Cattle Breeders and Milk Producers (PFHBiPM). The content of α-lactalbumin (α-LA) and β lactoglobulin (β-LG) was assayed electrophoretically. Proteins were separated in a 14.5% polyacrylamide gel in the presence of SDS, in a Mini-PROTEAN 3–cell apparatus following the methodology by Laemmli (1970), modified in terms of the applied voltage according to methodological guidelines of the Bio-Rad company. The content of lactoferrin, interleukins (IL-1β, IL-6), and tumor necrosis factor TNF-α was determined with the "sandwich" ELISA method, in which the protein to be detected is bound between two "layers" of antibodies. Determinations were carried out in whey obtained by centrifugation of 10 ml of fresh milk for 45 minutes at a temperature of 4°C and at the speed of 3,000 rpm (Singh et al., 2007). The resultant whey was stored frozen at a temperature of -20°C until analyzed. The concentration of lactoferrin in milk was assayed using a ready kit for bovine lactoferrin determination (Bovine Lactoferrin Elisa Quantitation Kit, Bethyl Laboratories Inc), following the procedure recommended by the manufacturer. For analyses, the milk samples were diluted at the ratio of 1:1,000. In determinations of interleukins (IL–1β, IL-6) and tumour necrosis factor TNF-alfa, use

was also made of commercial kits for bovine cytokines assays (respectively: Bovine IL-1β Screening Set and Bovine IL-6 Screening Set, ThermoScientific; Bovine TNF-α DuoSet, R&D). Analyses were carried out according to protocols provided by producers, and buffers necessary for these analyses were purchased in Sigma Aldrich and prepared following producer's instructions. For analyses, milk samples were diluted at the ratio of 1:2. In all analyses, a standard curve was plotted for each plate separately.

Measurements were carried out with an ELx808 reader (Biokom Systems), measuring absorbance at a wavelength of 450 nm. Absorbance results were expressed as lactoferrin concentration in milk using KCJunior software (Biokom Systems).

Statistical analysis. For statistical analysis, the numerical material was divided into four groups, based on somatic cells count in milk (Tab. 1): group I – up to 100,000 SCC/ml milk (n=56); group II – from 101,000 to 400,000 SCC/ml milk (n=66); group III – from 400,000 to 1 mln SCC/ml milk (n=21); and group IV – over 1 mln SCC/ml milk (n=18). According to the Polish Standard (PN–A–86002:1999), the milk from groups I and II was classified to Extra class (i.e. containing less than 400,000 SCC/ml). As reported by Hamann (2002), Lindmark-Mansson et al. (2000) and Cheng et al. (2008), milk containing less than 100,000 somatic cells per 1 ml originates from a healthy udder, hence the first class was distinguished that represented milk with the highest hygienic quality. The second analyzed factor was the stage of lactation. Three groups were distinguished in this respect: early stage (1st month of lactation; n=56), middle stage (5th month of lactation; n=49), and late stage (10th month of lactation; n=56).

Table 1. **Division into SCC groups based on somatic cells count in milk**

SCC Group	SCC (10^3/ml)	Milk class *according to the Polish Standard PN–A–86002:1999*	Number of samples
I	≤100	Extra class	56
II	101–400		66
III	401–1000	Unclassified milk	21
IV	≥1000		18
Total			161

The numerical data achieved were elaborated statistically in a STATISTICA data analysis software system Ver. 9.0 (StatSoft, Inc.), using a one- and two-way analysis of variance with interaction. To normalize distribution of lactoferrin content, the real results were subjected to logarithmic transformation. The significance of differences between mean values of the evaluated groups was determined with the Fisher's LSD test. The analysis of variance was conducted based on the following linear model:

$$Y_{ij} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk,}$$

where: Yij - dependent variable, μ – effect of total mean, a_i – effect of somatic cell counts (SCC) (i = I, II, III, IV), b_j – effect of stage of lactation (j = early, middle,

late), $(ab)_{ij}$ – effect of interaction SCC group x stage of lactation, e_{ijk} – random error

Results. The analysis of the proximate composition demonstrated that the lowest content of total protein and fat, and the highest content of lactose were typical of milk characterized by the highest hygienic quality (Tab. 2). A decrease was noted in lactose content along with an increase of SCC in milk, and differences between groups 1 and 4 turned out to be statistically significant (P≤0.05). Higher milk yields of the cows analyzed were reported in classes 1 and 4, compared to classes 2 and 3, and the lowest daily yield was noted for cows whose milk contained from 400,000 to 1 mln somatic cells per 1 ml.

Table 2. **Basic chemical composition of milk according to the somatic cell count** (SCC)

Characteristic		Average	SCC group				SEM
			I n=56	II n=66	III n=21	IV n=18	
Daily yield (kg)	$\bar{x}$	23.56	23.86	23.70	21.83	24.16	1.51
Protein (%)		3.50	3.36	3.63	3.52	3.46	0.06
Fat (%)		4.34	3.93^B	4.76^A	4.22	4.29	0.13
Lactose (%)		4.70	4.78^a	4.69	4.64	4.61^b	0.03
Dry matter (%)		13.50	13.16^b	14.04^a	13.15^b	13.19	0.15
Non-fat dry matter (%)		9.25	9.22	9.42	9.27	8.76	0.14

Mean values denoted by different letters in rows are significantly different: a, b – P < 0.05; A, B – P < 0.01

The somatic cell count was found to exert a statistically significant (P≤0.05) effect on the content of α-lactalbumin, the mean content of which in milk reached 1.03 g/l (Tab. 3). An increase in the content of this protein in milk was determined at SCC of up to 1 mln/ ml and a decrease in α-LA in milk from group IV. The highest content of this protein was noted in milk from group III (1.16 g/l), and the differences compared to group I were statistically significant (P≤0.05). A similar tendency was reported for the main whey protein, i.e. β-LG. Yet, in its case, the increase occurred only between groups I and II, whilst its lowest content was assayed in milk from group IV (3.40 g/l) (P≤0.05). An opposite dependence was noted for proteins that fulfil the function of inflammatory mediators. The average content of lactoferrin in the analyzed milk samples reached 198.84±188.12 µg/ml. Along with an increasing SCC in milk, an increase was also observed in lactoferrin content. The differences between the highest content of this protein in the samples with the highest SCC in groups III and IV (respectively:

233.20 and 246.77 µg/ml), when compared to group I, were statistically significant (P≤0.01). Likewise, milk from group IV was characterized by the highest concentration of IL-1β (0.21 µg/ml) and TNF-α (1.26 µγ/ml), and the concentration of the cytokines analyzed was observed to increase along with an increasing SCC in milk. Only in the case of IL-6, was its content the highest in milk from group II (0.11 µg/ml) and diminished in milk samples with the highest somatic cells count.

The contents of all analyzed whey proteins in milk were also affected by lactation stage (Tab.3). With time elapsing since parturition, an increase was noted in their content, however it was found statistically significant only in the cases of β-LG, lactoferrin and IL-1β. The highest concentrations of these proteins were assayed in milk from the last stage of lactation (4.10 g/l, 261.5 µg/ml, and 0.19 ng/ml, respectively), and these values were significantly higher than the respective values determined in milk from the early and middle stages of lactation.

Table 3. **Average protein content in milk with different somatic cell count and from different stage of lactation**

Characteristic		Average	SEM	SCC group				SEM	Stage of lactation			SEM	Effect		
				I n=56	II n=66	III n=21	IV n=18		Early n=56	Middle n=49	Late n=56		SCC	Stage of lactation	Interaction SCC x Stage of lactation
α-LA (g/l)	$\bar{x}$	1.03	0.03	0.93^b	1.07	1.16^a	0.96	0.02	0.98	0.99	1.09	0.02	*	ns	ns
β-LG (g/l)		3.72	0.07	3.64	3.87^a	3.68	3.40^b	0.04	3.41^B	3.52^B	4.10^A	0.04	ns	**	ns
Lactoferrin (µg/ml)		198.84	16.76	149.16	212.69	233.20	246.77	16.76	167.30^b	140.14^B	261.45^{Aa}	16.76	ns	*	ns
Lactoferrin (log)		2.13	0.04	1.94^B	2.19^A	2.30^A	2.29^A	0.04	1.97^B	2.09^B	2.32^A	0.04	**	**	ns
IL-1β (ng/ml)		0.13	0.02	0.08^b	0.15	0.19	0.21^a	0.02	0.10^b	0.09^b	0.19^a	0.02	*	*	*
IL-6 (ng/ml)		0.08	0.01	0.06	0.11	0.08	0.04	0.01	0.05	0.10	0.10	0.01	ns	ns	ns
TNF-α (ng/ml)		0.87	0.08	0.81^b	0.63^B	0.90	1.26^{Aa}	0.08	0.99	0.70	0.89	0.08	*	ns	ns

Mean values denoted by different letters in rows within trait are significantly different at: a,b - p≤0.05; A,B - p≤0.01
Mean values are significantly different at: * p≤0.05; ** p≤0.01; ns – non significant

The coupled effect of both experimental factors was observed in the case of IL-1β content (Fig. 1). At all lactation stages, the highest contencentrations of this protein were noted in milk from class IV. Simultaneously, the milk from the 10th month was characterized by the highest content of IL-1β, irrespective of hygienic quality class.
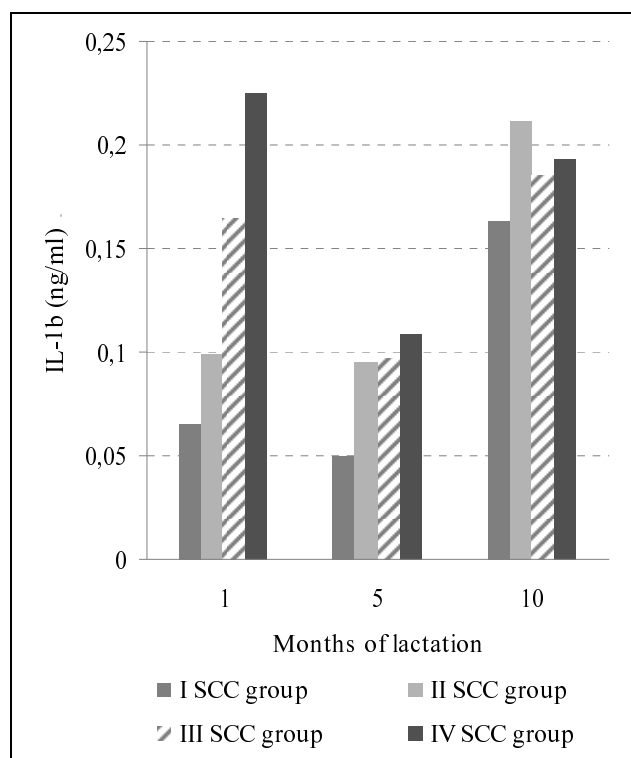

Figure 1. **Simultaneous effect of stage of lactation and the somatic cell count (SCC) on the interleukin 1β content**

SCC – somatic cell count
I SCC group – up to 100.000 SCC/ml milk
II SCC group – from 101.000 to 400.000 SCC/ml milk
III SCC group – from 400.000 to 1 mln SCC/ml milk
IV SCC group – over 1 mln SCC/ml milk
IL-1β – interleukin 1β

Discussion
Microbial infections change milk composition and render milk less suitable for consumption and processing (Shariff and Muhammad, 2008). The decreasing lactose concentration in blood along with an increasing SCC is consistent with results described by other authors (Philpot and Nickerson, 1991; Bernatowicz et al., 2004). In addition, Bernatowicz et al. (2004), Brzozowski et al. (1999) and Jakiel et al. (2011) reported, likewise in this study, an insignificant decrease in protein and fat contents of milk containing over 1 mln SCC/ml. Litwińczuk et al. (2011) reported also, as in our study, that daily yield of milk declined progressively with the increase of SCC.
Own results achieved for contents of α-LA and β-LG in milk from various lactation stages are in agreement with the findings of Ostersen et al. (1997), who noted an increase in β-lactoglobulin content of milk in the 24th and 46th week of lactation compared to the second and the ninth week. Contrary to our results, Ostersen et al. (1997) reported a decrease in α-LA along with time elapsing since parturition, with the highest content of this protein (1.28 g/l) noted in the 2nd week of lactation. A significant effect of lactation stage on the content of whey proteins in milk was also demonstrated by Maas (2002), however the highest contents of α-LA and β-LG were noted by this author in samples from the first and the last stage of lactation, which is in part consistent with results of our study. In addition, our study confirmed the results achieved by Bernatowicz et al. (2004), who determined a negative effect of a high SCC on α-LA and β-LG levels in cow's milk and those obtained by Reklewska et al. (2004) who observed increasing concentrations of α-LA and β-LG along with decreasing TBC and SCC in goat milk. Also Litwińczuk et al. (2011) showed that elevation of somatic cell count (SCC) produced a decrease in alpha-LA and beta-LG and a significant rise in immunoactive proteins (lactoferrin and lysozyme).
Likewise in the reported study, the highest mean concentration of lactoferrin in milk originating from the final stage of lactation (over 200 days after parturition) was also obtained by Cheng et al. (2008). In their research, milk from the initial stage of lactation was characterized by the lowest content of lactoferrin, and the content of this protein in all analyzed samples ranged from 100 to 300 µg/ml. Cheng et al. (2008) also reported a positive correlation (r = 0.0557) between lactoferrin concentration and lactation stage, what indicates that along with time elapsing since parturition the concentration of this protein in milk was increasing. The increase in lactoferrin concentration in the successive months of lactation was also obtained by Harmon et al. (1975). In contrast, Hagiwara et al. (2003) did not observe any correlation between lactoferrin content in milk and lactation stage. As reported by Arnould et al. (2009), determinations of LF concentration in cow's milk have been inexplicit and results achieved so far point only to a high variability of its concentration depending on somatic cells count, yield and breed. Both the average content of lactoferrin in milk determined in our study as well as the correlation between LF concentration and both lactation stage and SCC confirm earlier literature data (Fox, 1992; Lindmark-Mansson et al., 2000; Steijns & van Hooijdonk, 2000; Vorland, 1999). Lactoferrin is a constituent of immunoactive proteins that prevent mastitis (Bernatowicz et al., 2004), hence its concentration in milk is increasing along with an increasing somatic cells count. Furthermore, amongst factors affecting the content of this specific protein in cow's milk, Cheng et al. (2008) enumerate also a higher daily yield, contents of protein and lactose, as well as the number of milk samples subjected to analyses.
The immunoactive proteins that are co-acting with host's defence factors and exhibit antibacterial properties include also cytokines. As claimed by Malinowski et al. (2008), their increase is proportional to the SCC in milk, which may be indicative of mastitis occurrence. It was

confirmed in our study, where contents of the analyzed cytokines were observed to increase along with an increasing somatic cells count in milk. Owing to the fact that sparse investigations have so far been devoted to cytokines content of milk of healthy cows, Alluwaimi (2004) emphasizes the necessity of determining their content in milk at various stages of lactation. Our study demonstrated an increase in concentrations of IL-1β and IL-6 along with time elapsing since parturition and a negligible decrease of TNF-α at the middle stage of lactation. Likewise in our research, Hagiwara et al. (2001) demonstrated the highest concentration of interleukin 6 in milk samples from the middle stage of lactation and the lowest one in the samples from the initial lactation stage. The values obtained by these authors were higher compared to those reported in our study and accounted for 1.7 and 3.5 ng/ml at, respectively, the initial and final stage of lactation. These differences were, however, not confirmed statistically either. In another study, Hagiwara et al. (2000) determined the concentration of five cytokines in colostrum and milk. They demonstrated significant differences in cytokine concentrations in both colostrum and milk and in successive lactation stages. The tendency for higher concentrations of TNF-α and IL-1β at the final stage of lactation observed in our study was similar to results achieved by the above-cited authors. In contrast, Alluwaimi and Cullor (2002) observed a significant increase in TNF-α activity in mammary glands at the final stage of lactation, compared to the middle one, and were explaining this increase by the role played by this cytokine, as it is an indispensable component regulating immune functions of cells and a factor engaged in physiological changes proceeding in mammary glands during lactation. The contents of cytokines analyzed in our study were, however, almost twofold as low as those reported by Hagiwara et al. (2000) The differences between the results achieved in our study and those obtained by the said authors, manifested mainly in the contents of particular cytokines in milk, are likely to be due to the number of analyzed samples and impact of other environmental factors.

Conclusions

Despite the discrepancies between own findings and literary data, the results achieved suggest that the stage of lactation affect contents of the analyzed bioactive proteins in cow's milk. The higher content of the analyzed proteins at the last stage of lactation allows concluding that the best raw material for the isolation of the functional properties is milk originating from the final stage of lactation.

Concentrations of LF, IL-1β and TNF-α were found to depend on the somatic cells count in milk, which indicates that the analysis of their contents in milk may constitute a complementary indicator of mastitis incidence.

References

1. Alluwaimi A.M. The cytokines of bovine mammary gland: prospects for diagnosis and therapy. Research in Veterinary Science. 2004. 77. P.211–222.

2. Alluwaimi A.M., Cullor J.S. Cytokines gene expression patterns of bovine milk during mid and late stages of lactation. Journal of Veterinary Medicine B. 2002. 49. P.105–110.

3. Arnould V. M. R., Soyeurt H., Gengler N., Colinet F. G., Georges M. V., Bertozzi C., Portetelle D., Renaville R. Genetic analysis of lactoferrin content in bovine milk, Journal of Dairy Science. 2009. 92. P. 2151–2158.

4. Atanasova J., Ivanova I. Antibacterial peptides from goat and sheep milk proteins. Biotechnology & Biotechnological Equipment. 2010. 24 (2). P. 1799–1803.

5. Bagnicka E., Strzałkowska N., Jóźwik A., Krzyżewski J., Horbańczuk J.O., Zwierzchowski L. Defensins in farm animals, their expression and polymorphism. Acta Biochimica Polonica 2034. 2010. 57(4). P. 487–497.

6. Bernatowicz E., Reklewska B. Bioaktywne składniki białkowej frakcji mleka. Przegląd Hodowlany. 2003. 3. P. 1-10 (in Polish).

7. Bernatowicz E., Reklewska B., Zdziarski K., Karaszewska A. Poziom bioaktywnych składników w mleku krów zależnie od zdrowia gruczołu mlekowego. Zeszyty Naukowe Przeglądu Hodowlanego. 2004. 72 (1). P. 185–193 (in Polish).

8. Brzozowski P., Ludwiczuk K., Zdziarski K. Liczba komórek somatycznych w mleku krów objętych oceną użytkowości mlecznej w Polsce Centralnej. Zeszyty Naukowe Przeglądu Hodowlanego. 1999. 44. P. 83–90 (in Polish).

9. Cheng J. B., Wang J. Q., Bu D. P., Liu G. L., Zhang C. G., Wei H. Y., Zhou L. Y., Wang J. Z. Factors affecting the lactoferrin concentration in bovine milk. Journal of Dairy Science. 2008. 91(3). P. 970–976.

10. Fox, P. F. Indigenous enzymes in milk: VI other enzymes. In P. F. Fox (Ed.). Advanced dairy chemistry–1: Proteins. Barking, UK: Elsevier Science Publishers Ltd. 1992. P. 339–367.

11. Hagiwara K., Kataoka S., Yamanaka H., Kirisawa R., Iwai H. Detection of cytokines in bovine colostrum. Veterinary Immunology and Immunopathology. 2000. 76. P. 183–190.

12. Hagiwara S., Kawai K., Anri A., Nagahata H. Lactoferrin concentration in milk from normal and subclinical mastitis cows. The Journal of Veterinary Medical Science. 2003. 65. P. 319–323.

13. Hagiwara K., Yamanaka H., Hisaeda K., Taharaguchi S., Kirisawa R., Iwai H. Concentrations of IL-6 in Serum and Whey from Healthy and Mastitic Cows. Veterinary Research Communications. 2001. 25. P. 99–108.

14. Hamann J. Relationship between somatic cell

count and milk composition. Bulletin of the International Dairy Federation. 2002. 379. P. 56–59.

15. Harmon R. J., Schanbacher F. L., Ferguson L. C., Smith K. L. Concentration of lactoferrin in milk of normal lactating cows and changes occurring during mastitis. American Journal of Veterinary Research. 1975. 36. P. 1001–1007.

16. Jakiel M., Jesiołkiewicz E., Ptak E. Zależność między zawartością komórek somatycznych a cechami wydajności mlecznej w mleku krów rasy PHF odmiany czarno-białej. Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego. 2011. 7(1). P. 9–17 (in Polish).

17. Kawai K., Nagahata H., Lee N.-Y., Anri A., Shimazaki K. Effect of Infusing Lactoferrin Hydrolysate into Bovine Mammary Glands with Subclinical Mastitis. Veterinary Research Communications. 2003. 27. P. 539–548.

18. Laemmli U. K. Cleavage of structural proteins during the assembly of head bacteriophage T4. Nature. 1970. 227. P. 680–685.

19. Leman J. Bioaktywne białka serwatki. Przegląd Mleczarski. 1995. 3. P. 65–68 (in Polish).

20. Lindmark-Mansson, H., Svensson, U., Paulsson, M., Alden, G., Frank, B., & Johnsson, G. Influence of milk components, somatic cells and supplemental zinc on milk processability. International Dairy Journal. 2000. 10. P. 423–433.

21. Litwińczuk Z., Król J., Brodziak A., Barłowska J. Changes of protein content and its fractions in bovine milk from different breeds subject to somatic cell count. Journal of Dairy Science. 2011. 94 (2). P. 684–691.

22. Maas J. A. The effect of stage of lactation on milk protein composition. Journal of Dairy Science. 2002. 85 (1). 157 p.

23. Malinowski E., Kłosowska A., Smulski S. Zmiany stężeń biologicznie aktywnych składników mleka krowiego w skutek mastitis. Medycyna Weterynaryjna. 2008. 64 (1). P. 14–19 (in Polish).

24. Ostersen S., Foldager J., Hermansen J. E. Effects of stage of lactation, milk protein genotype and body condition at calving on protein composition and renneting properties of bovine milk. Journal of Dairy Research. 1997. 64. P. 207–219.

25. Parameswaran N., Patial S. Tumor Necrosis Factor-α Signaling in Macrophages. Critical Reviews in Eukaryotic Gene Expression. 2010. 20. P. 87–103.

26. Petrovski K.R., Trajcev M., Buneski G. A review of the factors affecting the costs of bovine mastitis. Journal of the South African Veterinary Association. 2006. 77. P. 53–60.

27. Philpot W.N., Nickerson S.C. Mastitis: counter attack. A strategy to combat mastitis. Luisiana State University Agricultural Center. 1991. Published by Babson Bros. Co.

28. PN–A–86002:1999. Mleko surowe do skupu. Wymagania i badania. Raw milk - Requirements and tests.

29. Reklewska B., Bernatowicz E., Ryniewicz Z., Pinto R.R., Zdziarski K. Preliminary observations on the Echinacea induced lactoferrin production in goats. Animal Science Papers and Reports. 2004. 22 (1). P. 17–25.

30. Singh S.U., Singh A. V., Singh R., Sandbu K. S., Singh P. K., Sokal J. S., Gupta U. K., Vihan U. S. Evaluation of highly sensitive indigenous milk Elisa kit with fecal culture, milk culture and fecal-PCR for the diagnosis of bovine Johne's disease (BJD) in India. Comparative Immunology, Microbiology & Infectious Diseases. 2007. 30. P. 175–186.

31. Sharif A., Muhammad G. Somatic Cell Count As An Indicator Of Udder Health Status Under Modern Dairy Production: A Review. Pakistan Veterinary Journal. 2008. 28 (4). P. 194–200.

32. Sharma N., Singh N. K., Bhadwal M. S. Relationship of Somatic Cell Count and Mastitis: An Overview. Asian-Australasian Journal of Animal Sciences. 2011. 24 (3). P. 429–438.

33. Statsoft Inc. 2009. STATISTICA (data analysis software system), Version 9.0. www.statsoft.com

34. Steijns, J. M., van Hooijdonk, A. C. M. Occurrence, structure, biochemical properties technological characteristics of lactoferrin. British Journal of Nutrition. 2000. 84. P. S11–S17.

35. Szwajkowska M., Wolanciuk A., Barłowska J., Król J., Litwińczuk Z. Bovine milk proteins as the source of bioactive peptides influencing the consumers' immune system – a review. Animal Science Papers and Reports. 2011. 29 (4). P. 269–280.

36. Świderski F., Waszkiewicz-Robak B. Peptydy i białka jako bioaktywne składniki żywności funkcjonalnej. Przemysł Spożywczy. 2000. 11. P. 41–44 (in Polish).

37. Wojdak-Maksymiec K., Mikolajczyk K. Interactions between *TNF-α*, *LTF* and *mLYZ* Gene Variants in Determining Somatic Cell Count in Jersey Cows. Pakistan Veterinary Journal. 2012. 32(4). P. 477–482.

38. Vorland, L. H. Lactoferrin: A multifunctional glycoprotein. ActaPathologica, Microbiologica et Immunologica Scandinavica. 1999. 107. P. 971–981.