Changes of protein content and its fractions in bovine milk from different breeds subject to somatic cell count

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ABSTRACT

Global milk production is undeniably dominated by 2 dairy breeds recognized worldwide: Holstein-Friesian and Jersey. A third breed, Simmental, serves as a dual-purpose breed. The objective of the present research was to establish potential changes in the fractional components of bovine milk protein (mainly whey) in relation to the health status of a dairy cow's mammary glands, which is closely determined by somatic cell count (SCC). The milk of 3 breeds was studied: Polish Holstein-Friesian (Black and Red-White varieties), Simmental, and Jersey. The cows were housed in freestall barns and fed according to the total mixed ration feeding system for both winter and summer periods. Milk samples were collected individually from each cow twice a year, in the winter and summer seasons. A total of 1,822 milk samples were evaluated (946 in winter and 876 in summer). The milk was examined for SCC, crude protein, casein, and whey fraction proteins (α -lactalbumin, β -lactoglobulin, lactoferrin, BSA, and lysozyme). The research material for each breed was split into 4 groups based on SCC (group I: $\leq 100,000$ cells/mL; group II: 101,000 to 400,000 cells/mL; group III: 401,000 to 500,000 cells/mL; and group IV: 501,000 to 1,000,000 cells/mL). It was found that an increase of SCC promulgated a progressive decline in the daily yield of milk, which was significantly true for the Polish Holstein-Friesian. The level of crude protein decreased slightly as SCC increased, and casein concentration ($r = -0.591$) also followed this trend of decline. Elevation of SCC produced a decrease of major albumins (i.e., α -lactalbumin and β -lactoglobulin). However, SCC increase induced an increase in the immunoactive proteins (lactoferrin and lysozyme) as well as BSA. The interactions of a breed with increased SCC, which can be measured based on the BSA content of the milk, has indicated various levels of susceptibility to the increase in different breeds. This is confirmed by different values of correlation coefficients for these relationships: 0.71 in

the Holstein-Friesian, 0.58 in Simmental, and 0.47 in the Jersey cows. Holstein-Friesian cows are more sensitive to mammary gland infections causing a greater decline of their daily milk yields, which, in turn, is reflected in an increase of the negative value of the correlation coefficients between SCC and milk efficiency (-0.24). In the other 2 breeds, the correlations were also negative, but substantially lower (-0.12 and -0.15).

Key words: milk protein, breed, somatic cell count

INTRODUCTION

Somatic cell count is commonly recognized as an indicator of bovine udder health, milk quality, and the technological usability of the milk (Smith, 2002; Piccinini et al., 2006). It facilitates early detection of mastitis with subclinical manifestations (Malinowski, 2001; Schukken et al., 2003; ten Napel et al., 2009). According to several professionals, a bovine quarter producing milk with an SCC $>200,000$ cells/mL shows the symptoms of subclinical mastitis (Urech et al., 1999; Smith, 2002; Pyörälä, 2003). Malinowski (2001) reports that in Poland, in the late 20th century, 30% of the dairy cow population was diagnosed with clinical mastitis, and 40% of the cows produced milk with pathological changes characteristic of the subclinical form. Regulation (EC) No. 853/2004 of The European Parliament and of The Council of 29 April 2004 on the specific hygiene rules for food of animal origin, states that raw cow's milk should not contain more than 400,000 somatic cells/mL.

Milk is a major source of valuable protein comprising 2 protein fractions: casein and whey protein. The former is a determinant of the technological usability of milk, whereas the latter is an indicator of the positive health value of milk for humans. Milk proteins affect, among other things, the digestive, immune, cardiovascular, and nervous systems and play a significant role in reducing the risk of the occurrence of different civilization (e.g., Western) diseases (Liu et al., 2007; Król et al., 2008; Smithers, 2008).

Out of numerous dairy breeds contributing to global milk production (estimates imply the presence of more

Received March 2, 2010.

Accepted October 31, 2010.

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than 100), 2 dairy breeds are of primary importance: Holstein-Friesian and Jersey. A third breed, Simmental, serves as a dual-purpose breed. The objective of the present work was to determine potential changes in protein and its fractional content in milk from cows of different breeds in relation to bovine udder health status indicated by SCC.

MATERIALS AND METHODS

Materials

The studies were conducted throughout 3 successive years (2006–2008) on milk samples collected from 3 different breeds of dairy cow—Polish Holstein-Friesian (Black and Red-White varieties), Simmental, and Jersey. The cows were managed under an intensive system in freestall barns. An animal feeding system established over both the winter and summer season, was based on TMR (corn silage, haylage, and feed concentrate). Milk samples were collected individually from each cow during trial milking, which occurred twice a year, once in the winter period and once in the summer. A total of 1,822 milk samples were examined (winter: 946 and summer: 876 samples). Data concerning daily milk performance were obtained from animal records kept by the Polish Federation of Cattle Breeders and Dairy Farmers.

Sampling and Measurements

All milk samples were transported to the laboratory in the Department of Commodity Science and Processing of Animal Raw Materials, University of Life Sciences, in Lublin (Poland). Each milk sample was analyzed for SCC using flow cytometry technology and a Somacount 150 apparatus (Bentley Instruments Inc., Chaska, MN), CP level by using an infrared milk analyzer (Bentley Instruments Inc.), casein percentage according to AOAC (2000; method 998.06), and whey fraction protein content by reverse-phase HPLC.

To evaluate the content of the whey proteins α -LA, β -LG, BSA, lactoferrin, and lysozyme, all samples were prepared according to Romero et al. (1996) as follows: 50 mL of raw milk was adjusted to pH 4.6 with 0.1 mol/L HCl and allowed to stand at room temperature for about 1 h to allow for acid precipitation of caseins. Then, whey (7 mL) was taken from each of the samples separately and centrifuged at $14,500 \times g$ for 15 min. Finally, whey solutions were filtered through paper filter discs (diameter: 125 mm, density: 65 g/m², grade: 3 h (Munktell, Bärenstein, Germany) and 0.20- μ m disposable sterile filters (Millipore type GSTF, Millipore, Billerica, MA). The supernatants in vials were kept

refrigerated until further analysis and were injected into the chromatograph at the suitable time (in the amount of 20 μ L). Protein separation was performed on a liquid chromatography ProStar 210 model and with a UV-visible ProStar 325 detector (Varian, Palo Alto, CA). The measurements were carried out using the water:acetonitrile mobile phase at gradient elution and a Nucleosil 300-5 C18 column (Varian) of 250 mm (length) and 4.6 mm (diameter). The mobile phase was solvent A (90% water, 10% acetonitrile) and solvent B (90% acetonitrile, 10% water), purchased from Sigma (Munich, Germany). The solvents were filtered through 0.45- μ m filters (Millipore) and degassed by using ultrasound. The total analysis time for a single sample was 35 min at 205 nm wavelength with column temperature of 37°C. The analyses of reference substances were conducted under the same conditions. Based on the obtained chromatograms, using program Star 6.2 Chromatography Workstation (Varian), the qualitative and quantitative identification of each substance was performed followed by determination of each concentration.

Calibration of the chromatographic system for whey protein determination was carried out by the external standard method. For this purpose, each protein was calibrated individually by injecting solutions of the standards (20 μ L). Purified proteins [α -LA ($\geq 85\%$), β -LG (90%), BSA ($\geq 96\%$), and lactoferrin (90%) from bovine milk, and lysozyme (95%) from hen egg whites] were purchased from Sigma. All chemicals were of HPLC analytical grade.

Statistical Analysis

The research material for each breed was split into 4 groups according to SCC detected in the milk samples: group I: $\leq 100,000$ cells/mL; group II: 101,000–400,000 cells/mL; group III: 401,000–500,000 cells/mL; and group IV: 501,000–1,000,000 cells/mL. Next, to regulate the SCC distribution, the SCC data were transformed into $\log_{10}$ SCC for each sample before statistical analysis could take place. In the summer, $\log_{10}$ SCC ranged from 4.68 to 6.04, whereas in winter it ranged from 4.52 to 6.01. The intervals appeared to be similar within the breeds evaluated.

Data are given as mean $\pm$ standard error. The obtained results were analyzed by the general linear model-factorial ANOVA procedures of Statistica version 6 (2003; Statsoft Inc., Tulsa, OK). Analysis included one-way and multi-way ANOVA with interaction, using Tukey's honestly significantly different procedure. To establish relationships between experimental variables (i.e., $\log_{10}$ SCC and milk yield as well as the content of analyzed proteins), the Pearson correlation and de-

Table 1. Milk yield, CP, and casein content (means $\pm$ SE) in milk obtained from different breeds in summer and winter in relation to SCC

Breed	SCC group ¹	Samples (n)		Milk yield (kg)		CP (%)		Casein (%)	
		Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Polish Holstein-	I	92	106	27.9 $\pm$ 0.6 ^b	24.8 $\pm$ 0.5 ^b	3.61 $\pm$ 0.04 ^{ab}	3.95 $\pm$ 0.05 ^b	2.82 $\pm$ 0.05	2.95 $\pm$ 0.04 ^B
Friesian	II	53	96	27.9 $\pm$ 0.3 ^b	21.9 $\pm$ 0.7 ^{ab}	3.69 $\pm$ 0.05 ^b	3.86 $\pm$ 0.04 ^{ab}	2.78 $\pm$ 0.06	2.82 $\pm$ 0.04 ^{AB}
Black-White	III	74	48	25.6 $\pm$ 0.8 ^{ab}	20.2 $\pm$ 0.6 ^{ab}	3.50 $\pm$ 0.05 ^a	3.70 $\pm$ 0.05 ^a	2.72 $\pm$ 0.06	2.82 $\pm$ 0.04 ^{AB}
	IV	44	46	20.9 $\pm$ 0.5 ^a	19.1 $\pm$ 0.7 ^a	3.58 $\pm$ 0.04 ^{ab}	3.74 $\pm$ 0.05 ^a	2.59 $\pm$ 0.04	2.58 $\pm$ 0.04 ^A
	Total	263	296	25.6 $\pm$ 0.7	21.5 $\pm$ 0.7	3.56 $\pm$ 0.05 ^x	3.83 $\pm$ 0.06 ^x	2.72 $\pm$ 0.05	2.80 $\pm$ 0.05
Polish Holstein-	I	47	88	27.1 $\pm$ 0.8	22.3 $\pm$ 0.9 ^b	3.59 $\pm$ 0.04 ^B	3.90 $\pm$ 0.04	2.74 $\pm$ 0.03 ^b	3.02 $\pm$ 0.04 ^b
Friesian Red-	II	68	56	25.5 $\pm$ 0.8	21.4 $\pm$ 0.7 ^{ab}	3.49 $\pm$ 0.03 ^{AB}	3.82 $\pm$ 0.03	2.66 $\pm$ 0.03 ^b	2.93 $\pm$ 0.04 ^{ab}
White	III	79	66	25.3 $\pm$ 0.9	20.9 $\pm$ 0.7 ^{ab}	3.34 $\pm$ 0.05 ^A	3.75 $\pm$ 0.04	2.59 $\pm$ 0.03 ^{ab}	2.74 $\pm$ 0.05 ^{ab}
	IV	45	33	22.8 $\pm$ 0.7	18.2 $\pm$ 0.6 ^a	3.39 $\pm$ 0.03 ^{AB}	3.74 $\pm$ 0.06	2.36 $\pm$ 0.04 ^a	2.51 $\pm$ 0.05 ^a
	Total	239	243	25.0 $\pm$ 0.8	20.8 $\pm$ 0.7	3.43 $\pm$ 0.05 ^x	3.79 $\pm$ 0.05 ^x	2.61 $\pm$ 0.04	2.79 $\pm$ 0.05
Simmental	I	54	86	21.9 $\pm$ 0.8	21.5 $\pm$ 0.7	3.78 $\pm$ 0.03	3.86 $\pm$ 0.03	2.74 $\pm$ 0.04	2.88 $\pm$ 0.04
	II	48	54	21.1 $\pm$ 0.7	20.3 $\pm$ 0.6	3.75 $\pm$ 0.05	3.79 $\pm$ 0.03	2.69 $\pm$ 0.04	2.78 $\pm$ 0.05
	III	55	46	20.8 $\pm$ 0.3	20.1 $\pm$ 0.6	3.72 $\pm$ 0.03	3.70 $\pm$ 0.04	2.63 $\pm$ 0.03	2.76 $\pm$ 0.04
	IV	43	41	18.6 $\pm$ 0.8	19.6 $\pm$ 0.7	3.70 $\pm$ 0.04	3.69 $\pm$ 0.02	2.63 $\pm$ 0.04	2.67 $\pm$ 0.05
	Total	200	227	20.8 $\pm$ 0.8	20.7 $\pm$ 0.7	3.73 $\pm$ 0.05	3.74 $\pm$ 0.03	2.66 $\pm$ 0.04	2.78 $\pm$ 0.04
Jersey	I	36	62	23.7 $\pm$ 0.5	22.7 $\pm$ 0.5	4.02 $\pm$ 0.05	4.14 $\pm$ 0.04 ^a	3.14 $\pm$ 0.03 ^B	3.16 $\pm$ 0.03
	II	57	54	22.6 $\pm$ 0.4	21.9 $\pm$ 0.3	3.86 $\pm$ 0.05	4.36 $\pm$ 0.03 ^b	2.92 $\pm$ 0.04 ^{AB}	3.18 $\pm$ 0.02
	III	45	36	22.3 $\pm$ 0.7	20.0 $\pm$ 0.5	3.72 $\pm$ 0.06	4.30 $\pm$ 0.03 ^{ab}	2.78 $\pm$ 0.03 ^{AB}	3.13 $\pm$ 0.02
	IV	36	28	21.2 $\pm$ 0.4	19.0 $\pm$ 0.4	3.82 $\pm$ 0.05	4.33 $\pm$ 0.04 ^{ab}	2.66 $\pm$ 0.05 ^A	2.99 $\pm$ 0.02
	Total	174	180	22.2 $\pm$ 0.5	21.0 $\pm$ 0.4	3.85 $\pm$ 0.05 ^y	4.29 $\pm$ 0.04 ^y	2.88 $\pm$ 0.04 ^x	3.10 $\pm$ 0.03 ^x

Factor	df	Milk yield		CP		Casein	
		SS ²	P-value	SS	P-value	SS	P-value
Breed	3	310.8	0.044*	5.74	0.000***	3.46	0.011*
SCC	3	378.7	0.006**	1.69	0.028*	30.88	0.000***
Production season	1	341.6	0.200	4.41	0.000***	0.02	0.388
Breed $\times$ SCC	9	364.1	0.059	1.37	0.843	1.17	0.138
Breed $\times$ season	3	1,226.7	0.000***	5.02	0.000***	0.12	0.205
SCC $\times$ season	3	202.4	0.153	1.26	0.133	0.31	0.137
Breed $\times$ SCC $\times$ season	9	298.2	0.782	3.59	0.012*	0.58	0.269
Total		2,3735.2		129.93		80.72	

^{a,b}Means for SCC group within breed with different superscripts differ significantly; $P < 0.05$.^{A,B}Means for SCC group within breed with different superscripts differ significantly; $P < 0.01$.^xMeans for season within breed with different superscripts differ significantly; $P < 0.05$.^yMeans for season within breed with different superscripts differ significantly; $P < 0.01$.¹Group I: $\leq 100,000$ cells/mL; group II: 101,000 to 400,000 cells/mL; group III: 401,000 to 500,000 cells/mL; and group IV: 501,000 to 1,000,000 cells/mL.²SS = type III sum of squares.* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 2. Correlation (*r*) and determination (*R*²) coefficients between SCC and milk yield as well chosen milk components

Breed	Milk yield			Casein			BSA			Lactoferrin			Lysozyme		
	<i>r</i>	<i>R</i> ²		<i>r</i>	<i>R</i> ²		<i>r</i>	<i>R</i> ²		<i>r</i>	<i>R</i> ²		<i>r</i>	<i>R</i> ²	
Polish Holstein-Friesian	-0.245**	0.06		-0.573***	0.329		0.711***	0.505		0.687***	0.472		0.694***	0.482	
Simmental	-0.123	0.015		-0.691***	0.478		0.577***	0.333		0.710***	0.519		0.603***	0.364	
Jersey	-0.148	0.022		-0.723***	0.523		0.472***	0.222		0.540***	0.292		0.693***	0.481	
Total	-0.157	0.025		0.591***	0.348		0.591***	0.348		0.652***	0.425		0.632***	0.398	

P* < 0.01; *P* < 0.001.

termination coefficients were calculated. A value of *P* < 0.01 was considered significant. Moreover, multiple linear regression equations were determined by using the progression step method.

The statistical analysis included the effect of production season, breed, and SCC using the following linear model:

$$Y_{ijk} = \mu + a_i + b_j + c_k + e_{ijk},$$

where Y_{ijk} = dependent variable, μ = effect of total mean, a_i = effect of breed ($i = 1, \dots, 4$), b_j = effect of productive season ($j = 1, 2$), c_k = effect of SCC ($k = 1, \dots, 4$), and e_{ijk} = random error.

RESULTS AND DISCUSSION

Data compared in Table 1 indicate that daily yield of milk declined progressively with the increase of SCC, but significant dependences ($P < 0.05$) were observed only in Polish Holstein-Friesian (Black-White and Red-White varieties). The absence of interchangeable relationships between SCC and the daily yield of milk was confirmed by the relatively low correlation coefficients (Table 2). The *r* coefficient was from -0.123 for Simmental cows to -0.245 ($P < 0.01$) for Polish Holstein-Friesian. It should be noted that the influence of season was not indicated.

The concentration of CP tended to decline slightly as SCC increased. This was mainly associated with a significant increase of some whey proteins levels. Lindmark-Månsson et al. (2006) report that CP content does not usually change as the SCC increases to 1,000,000 cells/mL. Moreover, a seasonal influence on protein content was shown, excluding the Simmental breed (Table 1).

A downward tendency for casein concentration with increasing SCC was noted. The significant decline ($P < 0.05$) in casein content between groups I and IV was established in milk obtained from Polish Holstein-Friesian Red-White cows from the summer season (0.38%) and winter season (0.51%). A distinct negative relationship between milk casein and SCC was confirmed by a relatively high value of correlation coefficient ($r = -0.591$, $P < 0.001$; Table 2).

By examining whey protein concentrations in the milk, it was found that an increase in SCC decreased the content of major albumins (i.e., α -LA and β -LG) by a small degree. Differences in α -LA levels between groups I and IV in the winter period (Table 3) ranged, subject to the breed, from 0.07 g/L (Jersey) to 0.21 g/L (Simmental), whereas β -LG contents ranged from 0.17 g/L (Black-White) to 0.36 g/L (Simmental). In the summer season the differences in α -LA levels ranged

Table 3. Main albumin content (means $\pm$ SE) in milk obtained from different breeds in summer and winter in relation to SCC

Breed	SCC group ¹	α -LA (g/L)		β -LG (g/L)		BSA (g/L)	
		Summer	Winter	Summer	Winter	Summer	Winter
Polish Holstein-Friesian Black-White	I	1.10 $\pm$ 0.02	1.08 $\pm$ 0.02	3.37 $\pm$ 0.05 ^B	3.05 $\pm$ 0.08	0.46 $\pm$ 0.03 ^A	0.52 $\pm$ 0.02 ^a
	II	1.13 $\pm$ 0.01	0.98 $\pm$ 0.02	3.43 $\pm$ 0.06 ^{AB}	2.99 $\pm$ 0.07	0.54 $\pm$ 0.02 ^{AB}	0.56 $\pm$ 0.02 ^{ab}
	III	1.05 $\pm$ 0.02	0.99 $\pm$ 0.01	3.02 $\pm$ 0.04 ^{AB}	2.99 $\pm$ 0.07	0.71 $\pm$ 0.02 ^B	0.77 $\pm$ 0.01 ^{ab}
	IV	0.99 $\pm$ 0.01	0.89 $\pm$ 0.03	2.92 $\pm$ 0.07 ^A	2.88 $\pm$ 0.07	0.79 $\pm$ 0.03 ^B	0.83 $\pm$ 0.01 ^b
	Total	1.08 $\pm$ 0.02	0.99 $\pm$ 0.02	3.20 $\pm$ 0.06	2.98 $\pm$ 0.07	0.64 $\pm$ 0.02	0.68 $\pm$ 0.02
Polish Holstein-Friesian Red-White	I	1.14 $\pm$ 0.02	0.98 $\pm$ 0.02	3.35 $\pm$ 0.08 ^B	2.94 $\pm$ 0.06	0.44 $\pm$ 0.02 ^a	0.45 $\pm$ 0.02 ^A
	II	1.11 $\pm$ 0.01	0.94 $\pm$ 0.02	3.36 $\pm$ 0.04 ^B	2.95 $\pm$ 0.05	0.56 $\pm$ 0.03 ^{ab}	0.64 $\pm$ 0.01 ^{AB}
	III	1.02 $\pm$ 0.02	0.94 $\pm$ 0.02	2.98 $\pm$ 0.04 ^{AB}	2.78 $\pm$ 0.07	0.72 $\pm$ 0.03 ^b	0.83 $\pm$ 0.02 ^B
	IV	0.94 $\pm$ 0.02	0.90 $\pm$ 0.02	2.74 $\pm$ 0.03 ^A	2.71 $\pm$ 0.05	0.80 $\pm$ 0.04 ^b	0.85 $\pm$ 0.01 ^B
	Total	1.04 $\pm$ 0.02	0.94 $\pm$ 0.02	3.07 $\pm$ 0.05	2.78 $\pm$ 0.06	0.60 $\pm$ 0.03	0.71 $\pm$ 0.01
Simmental	I	1.16 $\pm$ 0.02	1.16 $\pm$ 0.01	4.27 $\pm$ 0.10 ^B	3.38 $\pm$ 0.07 ^b	0.38 $\pm$ 0.02 ^a	0.42 $\pm$ 0.01 ^a
	II	1.19 $\pm$ 0.01	1.07 $\pm$ 0.02	4.15 $\pm$ 0.12 ^B	3.24 $\pm$ 0.09 ^{ab}	0.46 $\pm$ 0.02 ^{ab}	0.56 $\pm$ 0.01 ^{ab}
	III	1.13 $\pm$ 0.01	1.00 $\pm$ 0.02	3.66 $\pm$ 0.05 ^A	3.15 $\pm$ 0.07 ^{ab}	0.57 $\pm$ 0.03 ^b	0.52 $\pm$ 0.01 ^{ab}
	IV	1.03 $\pm$ 0.02	0.95 $\pm$ 0.02	3.57 $\pm$ 0.04 ^A	3.02 $\pm$ 0.09 ^a	0.58 $\pm$ 0.02 ^b	0.60 $\pm$ 0.01 ^b
	Total	1.13 $\pm$ 0.01	1.06 $\pm$ 0.02	4.01 $\pm$ 0.9 ^y	3.21 $\pm$ 0.08 ^y	0.49 $\pm$ 0.03	0.53 $\pm$ 0.01
Jersey	I	1.05 $\pm$ 0.02	1.01 $\pm$ 0.01	3.42 $\pm$ 0.07	2.98 $\pm$ 0.05	0.40 $\pm$ 0.03	0.48 $\pm$ 0.01 ^a
	II	1.04 $\pm$ 0.02	0.95 $\pm$ 0.01	3.43 $\pm$ 0.07	2.97 $\pm$ 0.04	0.48 $\pm$ 0.02	0.55 $\pm$ 0.02 ^{ab}
	III	1.00 $\pm$ 0.01	0.98 $\pm$ 0.02	3.09 $\pm$ 0.06	2.79 $\pm$ 0.04	0.52 $\pm$ 0.02	0.59 $\pm$ 0.01 ^{ab}
	IV	0.96 $\pm$ 0.01	0.94 $\pm$ 0.01	2.94 $\pm$ 0.06	2.69 $\pm$ 0.05	0.60 $\pm$ 0.02	0.66 $\pm$ 0.01 ^b
	Total	1.00 $\pm$ 0.02	0.97 $\pm$ 0.01	3.25 $\pm$ 0.07 ^x	2.88 $\pm$ 0.05 ^x	0.51 $\pm$ 0.02	0.60 $\pm$ 0.02

Factor	df	α -LA		β -LG		BSA	
		SS ²	P-value	SS	P-value	SS	P-value
Breed	3	0.12	0.648	12.02	0.000***	0.05	0.072
SCC	3	0.25	0.019*	6.09	0.028*	14.25	0.001***
Production season	1	0.52	0.006**	12.97	0.000***	0.67	0.008**
Breed $\times$ SCC	9	0.35	0.093	7.99	0.043*	0.51	0.238
Breed $\times$ season	3	0.16	0.122	3.91	0.040*	0.10	0.065
SCC $\times$ season	3	0.23	0.153	3.10	0.033*	0.99	0.002**
Breed $\times$ SCC $\times$ season	9	0.24	0.782	4.32	0.012*	0.21	0.269
Total		18.50		257.02		42.43	

^{a,b}Means for SCC group within breed with different superscripts differ significantly; $P < 0.05$.

^{A,B}Means for SCC group within breed with different superscripts differ significantly; $P < 0.01$.

^xMeans for season within breed with different superscripts differ significantly; $P < 0.05$.

^yMeans for season within breed with different superscripts differ significantly; $P < 0.01$.

¹Group I: $\leq 100,000$ cells/mL; group II: 101,000 to 400,000 cells/mL; group III: 401,000 to 500,000 cells/mL; and group IV: 501,000 to 1,000,000 cells/mL.

²SS = type III sum of squares.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

from 0.09 g/L (Jersey) to 0.20 g/L (Red-White), and β -LG levels ranged from 0.45 g/L (Black-White) to 0.70 g/L (Simmental) at $P < 0.01$. It is noteworthy that in the case of α -LA, all differences determined within each breed proved to be statistically insignificant, whereas this was only true for some differences in β -LG. The absence of explicit relationships between SCC and content of major milk albumins is confirmed by very low, statistically insignificant correlation coefficients. Negative values of both correlations, however, indicate a direction of change and may imply that the decrease of milk proteins is likely to be higher in more advanced stages of udder disease.

The increase in SCC caused a significant increase ($P < 0.001$) in immunoactive protein (lactoferrin and lysozyme) concentrations in milk (Table 4). The milk with the highest SCC (group IV) was shown to contain the largest amount of these milk proteins. Compared with milk obtained from cows assigned to group I, the differences for the all of the examined material averaged 28.17 mg/L for lactoferrin (i.e., 34.8% value of group I) and 5.15 μ g/L (60.2%) for lysozyme. Similar dependencies were noted for BSA, whereas the highest increase in this protein between groups I and IV (nearly double) was established in milk from Polish Holstein-Friesian cows ($P < 0.01$). A substantial effect of SCC

Table 4. Main lactoferrin and lysozyme content (means $\pm$ SE) in milk obtained from different breeds in summer and winter in relation to SCC

Breed	SCC group ¹	Lactoferrin (mg/L)		Lysozyme (μ g/L)	
		Summer	Winter	Summer	Winter
Polish Holstein-Friesian Black-White	I	76.63 $\pm$ 2.52	69.45 $\pm$ 0.59 ^{ab}	5.03 $\pm$ 0.20 ^a	6.79 $\pm$ 0.28 ^a
	II	74.53 $\pm$ 2.54	66.58 $\pm$ 1.17 ^a	7.49 $\pm$ 0.25 ^{ab}	7.01 $\pm$ 0.31 ^{ab}
	III	85.27 $\pm$ 3.01	77.34 $\pm$ 1.50 ^{ab}	7.91 $\pm$ 0.23 ^{ab}	7.46 $\pm$ 0.24 ^{ab}
	IV	102.43 $\pm$ 1.19	102.53 $\pm$ 0.86 ^b	11.17 $\pm$ 0.09 ^b	11.54 $\pm$ 0.41 ^b
	Total	84.72 $\pm$ 2.31	79.07 $\pm$ 1.08	7.91 $\pm$ 0.17	8.18 $\pm$ 0.26
Polish Holstein-Friesian Red-White	I	75.49 $\pm$ 3.01 ^a	79.45 $\pm$ 0.68	9.25 $\pm$ 0.14 ^a	9.21 $\pm$ 0.40
	II	80.12 $\pm$ 2.82 ^{ab}	76.64 $\pm$ 1.68	9.42 $\pm$ 0.23 ^{ab}	9.23 $\pm$ 0.61
	III	90.45 $\pm$ 1.90 ^{ab}	86.89 $\pm$ 1.75	10.81 $\pm$ 0.19 ^{ab}	9.76 $\pm$ 0.54
	IV	109.45 $\pm$ 1.92 ^b	102.65 $\pm$ 1.69	14.70 $\pm$ 0.13 ^b	12.53 $\pm$ 0.31
	Total	89.45 $\pm$ 2.43	87.54 $\pm$ 1.57	12.05 $\pm$ 0.19	11.82 $\pm$ 0.45
Simmental	I	98.45 $\pm$ 0.85 ^a	90.68 $\pm$ 2.14	8.04 $\pm$ 0.14 ^a	8.43 $\pm$ 0.22 ^a
	II	106.31 $\pm$ 1.98 ^{ab}	93.11 $\pm$ 3.10	9.76 $\pm$ 0.20 ^{ab}	9.40 $\pm$ 0.26 ^{ab}
	III	111.23 $\pm$ 1.52 ^{bc}	101.53 $\pm$ 1.62	10.42 $\pm$ 0.33 ^{ab}	10.28 $\pm$ 0.27 ^{ab}
	IV	123.93 $\pm$ 1.20 ^c	119.12 $\pm$ 2.61	12.38 $\pm$ 0.15 ^b	12.35 $\pm$ 0.25 ^b
	Total	110.91 $\pm$ 1.37	101.31 $\pm$ 2.38	10.22 $\pm$ 0.25	10.13 $\pm$ 0.26
Jersey	I	81.53 $\pm$ 1.71	75.77 $\pm$ 1.96	10.82 $\pm$ 0.10	10.92 $\pm$ 0.18
	II	82.36 $\pm$ 0.72	74.12 $\pm$ 2.14	14.21 $\pm$ 0.18	11.81 $\pm$ 0.35
	III	89.98 $\pm$ 2.18	83.02 $\pm$ 1.96	17.04 $\pm$ 0.18	16.14 $\pm$ 0.24
	IV	113.66 $\pm$ 1.81	98.97 $\pm$ 2.01	17.13 $\pm$ 0.12	17.88 $\pm$ 0.36
	Total	92.25 $\pm$ 1.56	83.07 $\pm$ 2.02	14.81 $\pm$ 0.14	14.19 $\pm$ 0.30

Factor	df	Lactoferrin		Lysozyme	
		SS ²	P-value	SS	P-value
Breed	3	16,393.00	0.004**	80.58	0.010**
SCC	3	582,835.00	0.000***	7,454.68	0.000***
Production season	1	2,643.00	0.046*	38.26	0.603
Breed $\times$ SCC	9	28,176.00	0.000***	180.25	0.553
Breed $\times$ season	3	4,827.00	0.226	54.28	0.091
SCC $\times$ season	3	3,510.00	0.995	58.68	0.779
Breed $\times$ SCC $\times$ season	9	10,462.00	0.159	227.01	0.710
Total		1,368,601.00		18,978.42	

^{a-c}Means for SCC group within breed with different superscripts differ significantly; $P < 0.05$.

¹Group I: $\leq 100,000$ cells/mL; group II: 101,000 to 400,000 cells/mL; group III: 401,000 to 500,000 cells/mL; and group IV: 501,000 to 1,000,000 cells/mL.

²SS = type III sum of squares.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

on immunoactive milk proteins and BSA was confirmed by relatively high positive values of computed correlation coefficients. For lactoferrin content: $r = 0.652$ at $P < 0.001$, for lysozyme: $r = 0.632$ at $P < 0.001$, and for BSA: $r = 0.591$ at $P < 0.001$ (Table 2). Urech et al. (1999), in a study of quarter milk, indicated similar tendencies when 100,000 cells/mL was recognized as the threshold of SCC. Quarter milk obtained from clinically healthy mammary glands contained an average of 84,000 cells/mL, whereas milk from infected glands had 293,000 cells/mL ($P < 0.001$). Urech et al. (1999) found significant growth of lactoferrin concentrations (by 0.45% at $P < 0.01$) and BSA (by 0.10%) in milk from the affected udder. Similarly, Hamann (2002) defined the “gold standard” for SCC to be $\leq 100,000$ cells/mL, and counts above this point provided evidence of disturbed milk secretion that would lead to a reduced

production of daily milk, changes in its chemical composition, and a deterioration of the processing properties of the milk. Similar conclusions are presented in the studies of Piccinini et al. (2006), Berglund et al. (2007), Barłowska et al. (2009), and Forsbäck et al. (2009). Lindmark-Månsson et al. (2000, 2006) observed that an SCC of $> 5,000$ cells/mL increases lactoferrin content in milk, and a close relationship between this milk component and the status of udder health has been confirmed by very high correlation coefficients between milk lactoferrin concentration and SCC ($r = 0.962$ and $r = 0.918$ at $P < 0.001$) obtained in 2 independent studies (Lindmark-Månsson et al., 2000, 2006). A significant increase ($P < 0.05$) of lactoferrin and BSA content with concurrent progression of a mammary gland disease was also noted in goat and buffalo milk (Leitner et al., 2004; Piccinini et al., 2006).

Table 5. Results of multiple linear regressions

Equation	Coefficient ¹	
	R	R ²
Milk yield (kg) = 27.09 – 0.22 × breed – 0.13 × SCC	0.26	0.06
CP (%) = 3.39 + 0.221 × breed + 0.135 × season – 0.10 × SCC	0.30	0.09
Casein (%) = 2.94 – 0.59 × SCC + 0.085 × breed	0.62	0.39
α-LA (g/L) = 0.92 + 0.193 × season – 0.06 × SCC	0.21	0.04
β-LG (g/L) = 2.36 + 0.272 × season + 0.188 × breed – 0.11 × SCC	0.37	0.14
BSA (g/L) = 0.31 + 0.51 × SCC + 0.081 × season	0.54	0.29
Lactoferrin (mg/L) = 58.16 + 0.621 × SCC + 0.084 × season + 0.061 × breed	0.66	0.43
Lysozyme (μg/L) = 5.31 + 0.538 × SCC + 0.078 × breed	0.57	0.32

¹R = multiple correlation coefficient; R² = coefficient of determination.

The present research has revealed significant interactions ($P < 0.01$) between breed and SCC, as illustrated by BSA content in milk, that point to differences in susceptibility of the 3 breeds to an increase in SCC. This finding may be attributed to, among other things, a difference in permeability of the blood–milk barrier in the mammary gland. Probably, in Simmental and Jersey cows, SCC growth is not followed by such intensive penetration of BSA from blood to milk as was observed in Holstein-Friesian cows. This may also imply greater immunity for cows of these breeds (i.e., Simmental and Jersey) to mammary gland infections. This statement may be confirmed by different values of the calculated correlation coefficients between BSA content and the SCC in milk of cows of different breeds (Table 2). The highest dependence ($r = 0.71$ at $P < 0.001$) was obtained for Holstein-Friesian cows, whereas a noticeably lower dependence was found for Simmental cows ($r = 0.58$ at $P < 0.001$) and Jersey cows ($r = 0.47$ at $P < 0.001$). Greater sensitivity of Holstein-Friesian cows to udder infections was manifested by significantly greater decreases in their daily yield of milk. This sensitivity was also reflected in higher negative correlation coefficient values between SCC and daily yield of milk in cows of this breed (-0.24 at $P < 0.01$). In the other 2 breeds (Simmental and Jersey) under investigation, the correlations were also negative, but their values were substantially lower ($r = -0.12$ and $r = -0.15$, respectively) and statistically insignificant. These findings correspond with the earlier studies of the present researchers (Barłowska et al., 2009), who investigated a relationship between SCC and milk yield, as well as technological usability in 4 breeds [i.e., Polish Holstein-Friesian (Black and Red-White varieties), Simmental, and the Polish native cattle breed known as Whitebacks]. Significant ($P < 0.01$) interactions (breed × SCC) for daily yield of milk, contents of protein, casein, and lactose, protein/fat ratio, and rennet-induced coagulation time of milk have been found.

By using a step progressing method, the equations for multiple linear regression for dependent variables were identified (Table 5). It was stated that the regression models described precisely the lactoferrin and casein content in milk, which confirmed the relatively high R (0.66 and 0.62) and R² (0.43 and 0.39) coefficients. The models obtained for content of lysozyme and BSA should also be noted.

ACKNOWLEDGMENTS

This work was completed within the project no. N311 014 31/0649 financed by the Ministry of Science and Higher Education (Warsaw, Poland).

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