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Correlations between the content of selected whey proteins in cow milk

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The aim of the study was to determine the correlations between the concentrations of major whey proteins in cow milk. A total of 2,278 milk samples from Polish Holstein-Friesian (Black-and-White and Red-and-White varieties), Simmental and Jersey cows were analysed. In each sample the content of major whey proteins, i.e. α-lactalbumin, β-lactoglobulin, bovine serum albumin, lactoferrin and lysozyme were determined by the RP-HPLC method. Matrix scatter plots were prepared to determine the correlations between the concentrations of individual whey proteins. In the vast majority of cases a significant relationship was found between the content of individual whey proteins. Taking into account the production season and breed of cow, highly significant (p=0.001) negative correlation coefficients were obtained for the content of α-lactalbumin and bovine serum albumin, for α-lactalbumin and lysozyme, for β-lactoglobulin and bovine serum albumin, and for β-lactoglobulin and lysozyme. Positive correlations were observed for the concentrations of α-lactalbumin with β-lactoglobulin, lactoferrin and lysozyme, as well as for bovine serum albumin with lysozyme.

KEY WORDS: cow milk / α-lactalbumin / β-lactoglobulin / bovine serum albumin / correlation

The proteins present in milk influence its nutritional and health-promoting value as well as its suitability for processing. Recent reports on the multi-faceted, positive effect of components of the protein fraction of milk, particularly whey proteins, on both the newborn suckling and human beings, have led to an increased interest in these proteins. In cow milk they account for 20-25% of proteins, of which 75% are albumins, i.e. α-lactalbumin, β-lactoglobulin and bovine serum albumin (BSA). Most of these proteins are essential for the proper development of the suckling, influencing the digestive, circulatory and nervous systems. Whey proteins also include those having antibacterial, antiviral, antifungal and antiparasitic properties, i.e. lactoferrin, lysozyme, lactoperoxidase or immunoglobulin [6, 9, 16]. Their content in milk for the suckling and in the human diet is one of the factors determining a proper immune response in the organism. This is because immunoglobulins are responsible for specific humoral immunity [3]. Lactoferrin is a fundamental element of the innate, non-specific immune system of humans and other mammals [8]. Moreover, whey proteins are a source of essential amino acids and are increasingly used to enrich baby food, dietetic preparations or high-protein preparations for convalescents and athletes. They have also found application in pharmacology and cosmetology [4, 5, 18]. Thus it is important to learn the relationships between the concentrations of these proteins.

The objective of the study was to determine the correlations between the content of the main whey proteins in cow milk.

Material and methods

The study was conducted on 2,278 samples of milk from cows of three breeds raised for dairy purposes in Poland: two varieties of Polish Holstein-Friesians, i.e. Black-and-White (789 samples) and Red-and-White (486), Jersey (768) and Simmental (235). The cows were housed in free-stall barns and fed according to a TMR (Total Mixed Ration) system. The feed ration consisted of bulky feed (maize silage, haylage and hay), concentrates, and mineral and vitamin supplements. The daily yield of the cows was as follows: Polish Black-and-White Holstein-Friesian – 27.1 kg, Polish Red-and-White Holstein-Friesian – 22.8 kg, Jersey – 20.3 kg, and Simmental – 21.5 kg. The milk samples were collected individually from each cow during test-day milking in two seasons, spring/summer (May--July) and autumn/winter (December-February). The cows were in middle of their second to fourth lactations (between days 120 and 200 in milk).

Only milk samples in which the somatic cell count did not exceed 400,000/ml were used in the study. SCC was determined in a Somacount 150 apparatus (Bentley Instruments, USA). The milk samples were stored at –24ºC until further analysis.

The concentrations of selected whey proteins, i.e. α-lactalbumin, β-lactoglobulin, lactoferrin, bovine serum albumin and lysozyme, were determined by reversed-phase high-performance liquid chromatography. All samples were prepared according to a method developed by Romero et al. [14] with modifications by Brodziak et al. [1]. After thawing the samples were skimmed and brought to a pH of 4.6 to induce acid precipitation of caseins. Then the separated whey was centrifuged and filtered. The whey samples prepared in this manner were subjected to chromatographic analysis. Protein separation was carried out in a ProStar 210 liquid chromatograph with a ProStar 325 UV-Vis detector (Varian, USA). In all cases separation was carried out using an acetonitrile/water mobile phase (Sigma, Germany) in a gradient and a Nucleosil 300-5 C18 column (Varian, USA), 250 mm in length and 4.6 mm in diameter. A single sample was analysed for 35 minutes at λ=205 nm. Reference substances were analysed in identical conditions using standard solutions of purified proteins, i.e. α-lactalbumin (≥85%), β-lactoglobulin (90%), bovine serum albumin (≥96%) and lactoferrin (90%), all obtained from milk proteins, as well as lysozyme (95%) obtained from chicken egg albumen (Sigma, Germany). Qualitative identification of individual substances was based on analysis of retention times read from individual chromatograms in Star 6.2 Chromatography Workstation (Varian, USA). Quantitative analysis was performed by the external standard method.

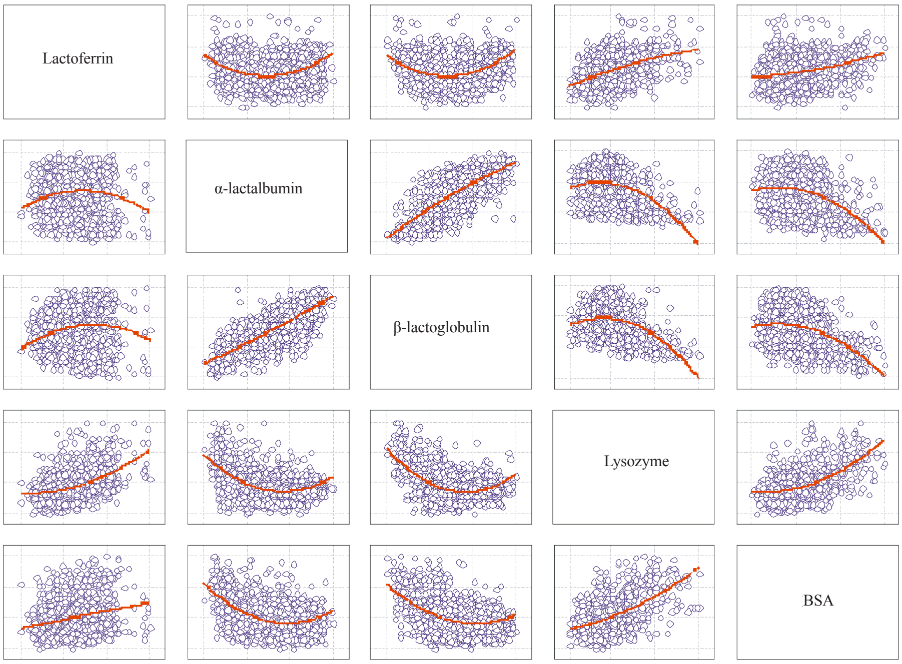
Statistical analysis of the results was carried out using StatSoft Inc. Statistica software v. 10. Scatter plots (with a polynomial fit) were used to determine correlations between variables, i.e. individual whey proteins. Pearson's correlation coefficients (r) and coefficients of determination (R^2) were given. Results with a significance level of $p \leq 0.05$ were recognized as significant.

Results and discussion

Scatter plots were made to determine the correlations between the concentrations of the whey proteins analysed (Figs. 1-7). On this basis, in conjunction with Pearson's correlation coefficients and coefficients of determination, the strength and type of relationship between variables was determined. In the vast majority of cases significant ($p=0.001$) correlations were obtained between the concentrations of individual whey proteins. It should be noted that when the production season and the breed of cow were taken into account, significant ($p=0.001$) negative values were obtained for the correlation coefficients between the content of α-lactalbumin and BSA, α-lactalbumin and lysozyme, β-lactoglobulin and BSA and β-lactoglobulin and lysozyme. The correlations between the concentrations of α-lactalbumin and β-lactoglobulin, lactoferrin and lysozyme, and BSA and lysozyme were positive.

In nearly all cases analysis of the scatter plots with the line of regression reveals no linear relationship between variables. However, it is particularly worth noting the strong positive linear correlation between α-lactalbumin and β-lactoglobulin ($R^2=0.613$, $r=0.783$; Fig. 1). Similar trends were also observed when the analysis took into account the production season and breed (Fig. 2-7). In the spring/summer season, 67% ($r=0.820$ at $p=0.001$) of the observed variance in α-lactalbumin concentration was explained by the regression on β-lactoglobulin, while the corresponding figure in the autumn/winter was only 54% ($r=0.736$ at $p=0.001$; Fig. 2 and 3). Taking into account the breed of cow, the highest value for the coefficient of determination was obtained for the Simmental breed ($R^2=0.691$, $r=0.831$ at $p=0.001$; Fig. 6). In the remaining cases the relationships between the variables analysed were much better explained by curvilinear correlations, especially for the correlations between α-lactalbumin and lysozyme, β-lactoglobulin and BSA, β-lactoglobulin and lysozyme, and BSA and lysozyme. In no case, however, did lactation number, the interaction of breed and lactation number, or the interaction of breed, production season and lactation number have a significant effect on the size of correlations between the concentrations of whey proteins. For this reason the results of these analyses were not included in the study.

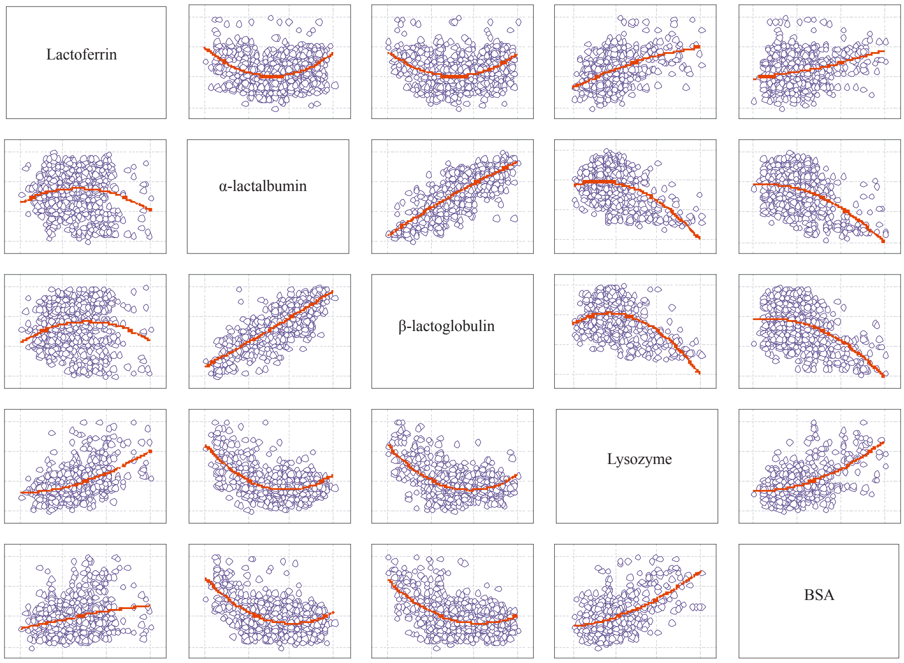
Caffin et al. [2], in an analysis of the milk of Holstein-Friesian cows, also obtained a positive correlation between the content of α-lactalbumin and that of β-lactoglobulin



Proteins	R^2	r	p
lactoferrin – α-lactalbumin	0.004	–0.061	0.010
lactoferrin – β-lactoglobulin	0.000	–0.005	0.890
lactoferrin – BSA	0.029	0.170	0.050
lactoferrin – lysozyme	0.182	0.426	0.001
α-lactalbumin– β-lactoglobulin	0.613	0.783	0.001
α-lactalbumin – BSA	0.118	–0.344	0.001
α-lactalbumin – lysozyme	0.101	–0.318	0.001
β-lactoglobulin – BSA	0.114	–0.338	0.001
β-lactoglobulin – lysozyme	0.082	–0.287	0.001
BSA – lysozyme	0.262	0.512	0.001

R^2 – coefficient of determination, r – correlation coefficient, p-value

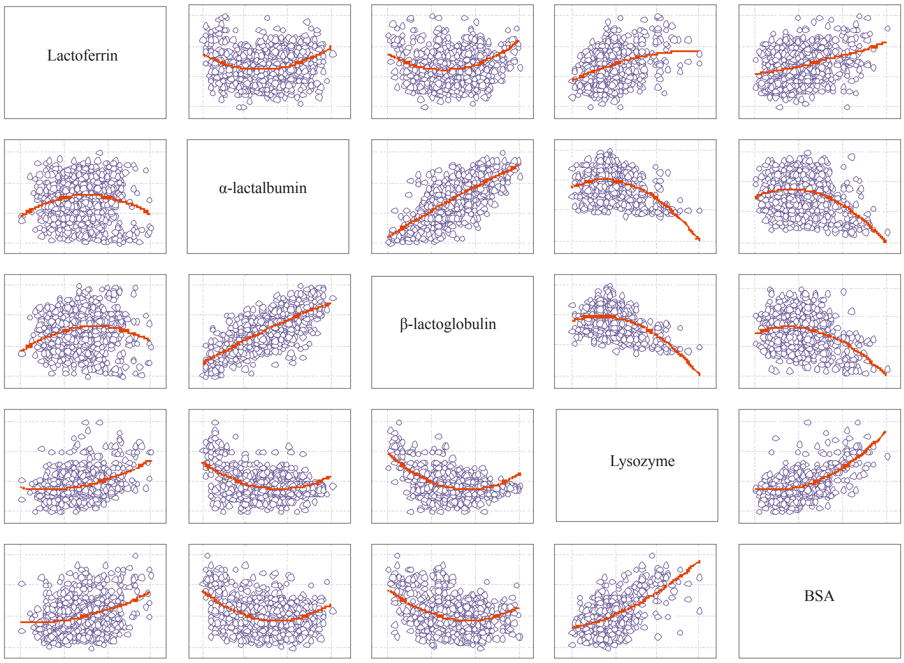
Fig. 1. Correlations between the content of whey proteins in milk



Proteins	R^2	r	p
lactoferrin – α-lactalbumin	0.000	–0.005	0.855
lactoferrin – β-lactoglobulin	0.009	–0.097	0.001
lactoferrin – BSA	0.078	0.280	0.001
lactoferrin – lysozyme	0.214	0.463	0.001
α-lactalbumin – β-lactoglobulin	0.672	0.820	0.001
α-lactalbumin – BSA	0.184	–0.429	0.001
α-lactalbumin – lysozyme	0.135	–0.368	0.001
β-lactoglobulin – BSA	0.162	–0.402	0.001
β-lactoglobulin – lysozyme	0.089	–0.299	0.001
BSA – lysozyme	0.264	0.514	0.001

R^2 – coefficient of determination, r – correlation coefficient, p-value

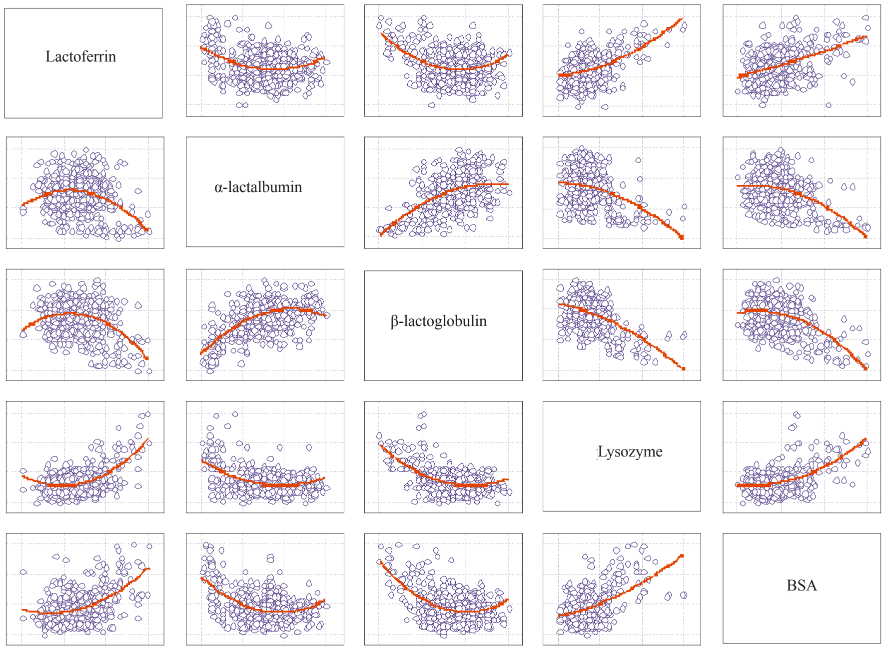
Fig. 2. Correlations between the content of whey proteins in milk obtained in the summer season



Proteins	R^2	r	p
lactoferrin – α-lactalbumin	0.002	0.045	0.121
lactoferrin – β-lactoglobulin	0.017	0.132	0.001
lactoferrin – BSA	0.102	0.320	0.000
lactoferrin – lysozyme	0.118	0.343	0.001
α-lactalbumin – β-lactoglobulin	0.542	0.736	0.001
α-lactalbumin – BSA	0.061	–0.247	0.001
α-lactalbumin – lysozyme	0.089	–0.298	0.001
β-lactoglobulin – BSA	0.065	–0.255	0.001
β-lactoglobulin – lysozyme	0.130	–0.360	0.001
BSA – lysozyme	0.290	0.539	0.001

R^2 – coefficient of determination, r – correlation coefficient, p-value

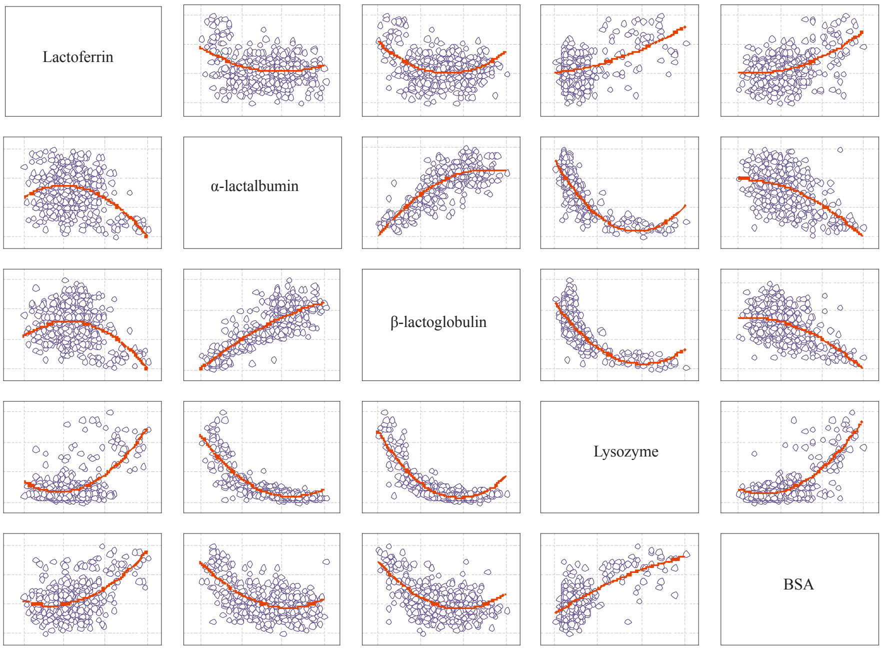
Fig. 3. Correlations between the content of whey proteins in milk obtained in the winter season



Proteins	R^2	r	p
lactoferrin – α-lactalbumin	0.037	–0.193	0.001
lactoferrin – β-lactoglobulin	0.058	–0.240	0.001
lactoferrin – BSA	0.187	0.432	0.001
lactoferrin – lysozyme	0.202	0.449	0.001
α-lactalbumin – β-lactoglobulin	0.271	0.521	0.001
α-lactalbumin – BSA	0.144	–0.380	0.001
α-lactalbumin – lysozyme	0.127	–0.356	0.001
β-lactoglobulin – BSA	0.185	–0.430	0.001
β-lactoglobulin – lysozyme	0.229	–0.479	0.001
BSA – lysozyme	0.229	0.479	0.001

R^2 – coefficient of determination, r – correlation coefficient, p-value

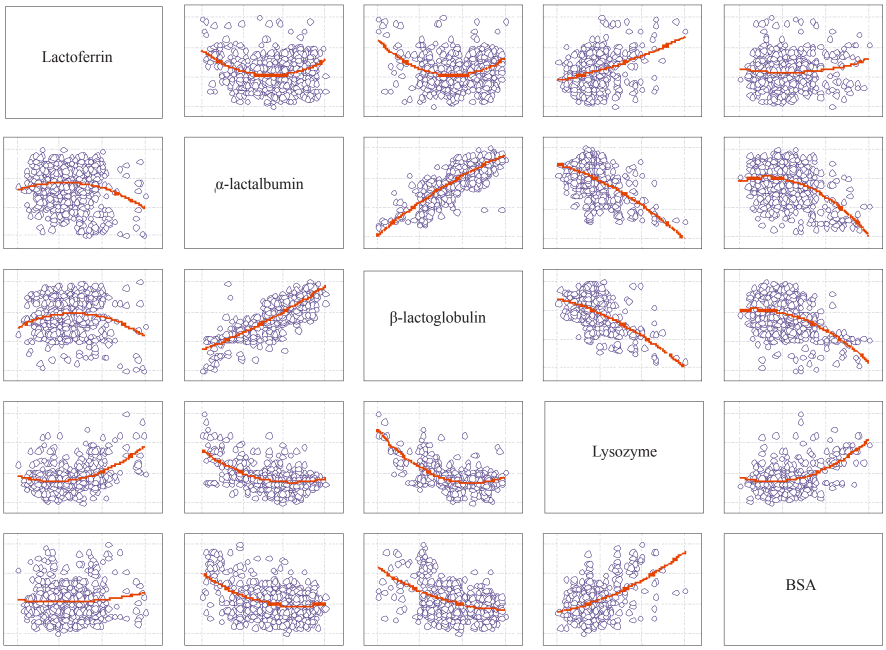
Fig. 4. Correlations between the content of whey proteins in milk obtained from the Black-and-White variety of Polish Holstein-Friesian cows



Proteins	R^2	r	p
lactoferrin – α-lactoalbumin	0.070	–0.264	0.001
lactoferrin – β-lactoglobulin	0.045	–0.212	0.001
lactoferrin – BSA	0.203	0.451	0.001
lactoferrin – lysozyme	0.209	0.457	0.001
α-lactalbumin – β-lactoglobulin	0.627	0.792	0.001
α-lactalbumin – BSA	0.274	–0.523	0.001
α-lactalbumin – lysozyme	0.566	–0.752	0.001
β-lactoglobulin – BSA	0.252	–0.502	0.001
β-lactoglobulin – lysozyme	0.491	–0.701	0.001
BSA – lysozyme	0.452	0.672	0.001

R^2 – coefficient of determination, r – correlation coefficient, p-value

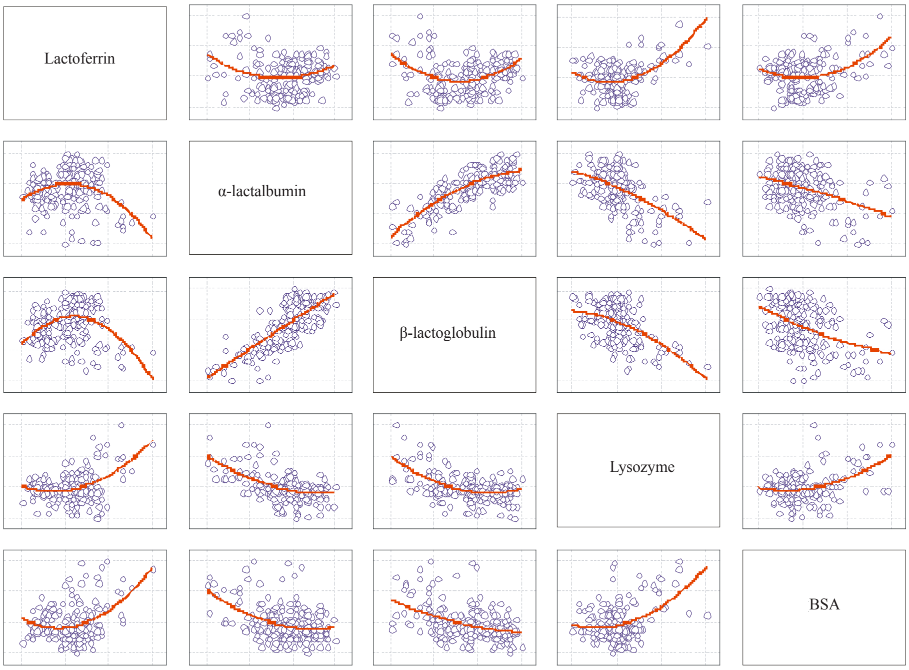
Fig. 5. Correlations between the content of whey proteins in milk obtained from Red-and-White variety of Polish Holstein-Friesian cows



Proteins	R^2	r	p
lactoferrin – α-lactalbumin	0.008	–0.090	0.010
lactoferrin – β-lactoglobulin	0.000	–0.005	0.890
lactoferrin – BSA	0.003	0.058	0.050
lactoferrin – lysozyme	0.104	0.323	0.001
α-lactalbumin – β-lactoglobulin	0.691	0.831	0.001
α-lactalbumin – BSA	0.156	–0.395	0.001
α-lactalbumin – lysozyme	0.260	–0.510	0.001
β-lactoglobulin – BSA	0.183	–0.428	0.001
β-lactoglobulin – lysozyme	0.248	–0.498	0.001
BSA – lysozyme	0.182	0.427	0.001

R^2 – coefficient of determination, r – correlation coefficient, p-value

Fig. 6. Correlations between the content of whey proteins in milk obtained from Simmental cows



Proteins	R^2	r	p
lactoferrin – α-lactalbumin	0.003	–0.055	0.403
lactoferrin – β-lactoglobulin	0.004	0.065	0.320
lactoferrin – BSA	0.057	0.238	0.001
lactoferrin – lysozyme	0,098	0.313	0.001
α-lactalbumin – β-lactoglobulin	0.624	0.790	0.001
α-lactalbumin – BSA	0.148	–0.384	0.001
α-lactalbumin – lysozyme	0.253	–0.503	0.001
β-lactoglobulin – BSA	0.178	–0.422	0.001
β-lactoglobulin – lysozyme	0.210	–0.458	0.001
BSA – lysozyme	0.119	0.345	0.001

R^2 – coefficient of determination, r – correlation coefficient, p-value

Fig. 7. Correlations between the content of whey proteins in milk obtained from Jersey cows

(r=0.12). It should be noted that both of these proteins are albumins and have the common characteristics of this group. Mackle et al. [13] assessed the effect of energy consumed in the feed ration on the concentration of whey proteins in cow milk. The authors noted that when the cows had no access to pasture, the content of the main whey proteins, i.e. α-lactalbumin and β-lactoglobulin, increased while that of BSA decreased. According to Leitch and Wilcox [10] and Linden van der et al. [11], the antibacterial properties of lactoferrin and lysozyme synergize. In a study by Semba et al. [15], the correlation coefficient between the content of lactoferrin and lysozyme in human milk was r=0.179 at p=0.12. At the onset of inflammation of the mammary gland there is an increase in the activity of numerous antibacterial components of milk, such as lactoferrin, lysozyme, lactoperoxidase, IgG and BSA, which may be used as indicators of udder health [7, 17]. Król et al. [7] obtained a positive, high correlation coefficient (r=0.721) between the content of lactoferrin and IgG in milk, which indicates a close correlation between the content of these proteins. Litwińczuk et al. [12], in an analysis of the relationships between individual whey proteins and the somatic cell count (SCC) in milk, which reflects the health condition of the mammary gland, showed that an elevated SCC only slightly decreased the content of the main albumins, i.e. α-lactalbumin and β-lactoglobulin. However, as the somatic cell count increased there was a significant increase in the content of immunoactive proteins (lactoferrin and lysozyme) and BSA. The authors obtained the highest correlation coefficients between BSA content and SCC in the milk of Holstein-Friesian cows (r=0.711), and markedly lower coefficients for the milk of Simmental (r=0.577) and Jersey (r=0.472) cows.

To sum up, in the vast majority of cases a significant relationship was noted between the content of individual whey proteins in cow milk. Taking into account both the production season and the breed of cow, significant (p=0.001) negative correlation coefficients were obtained between α-lactalbumin and bovine serum albumin, α-lactalbumin and lysozyme, β-lactoglobulin and bovine serum albumin, and β-lactoglobulin and lysozyme. Positive correlations were found between the concentrations of α-lactalbumin and β-lactoglobulin, lactoferrin and lysozyme, and bovine serum albumin and lysozyme. The growing interest in whey proteins substantiates the need to establish the relationships between their concentrations. These proteins not only determine the nutritional value of the milk obtained, but are also indicative of the health condition of the mammary gland. For this reason monitoring of their concentrations, particularly those with antibacterial properties (lactoferrin and lysozyme) and bovine serum albumin, is recommended.

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