

Changes in the bioactive protein concentrations in the bovine colostrum of Jersey and Polish Holstein–Friesian cows

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Abstract: The aim of this study was to determine changes in the bioactive protein concentrations in the bovine colostrum of Jersey and Polish Holstein–Friesian (PHF) cows. Double colostrum samples were collected from 16 Jersey and PHF cows, during 8 successive milking runs after calving. A total of 256 colostrum samples were analyzed to determine the pH, density, percentage share, and content of selected bioactive proteins, including the percentage share of casein fractions (α_s -, β -, γ -, and κ -) in the total casein, and the concentrations of α -lactalbumin (α -LA), β -lactoglobulin (β -LG), lactoferrin (LF), interleukin-1 β , interleukin-6, and tumor necrosis factor- α . The concentrations of the studied bioactive proteins in bovine colostrum decreased over time after calving, along with changes in the proximate composition of the colostrum. Differences were also noted in the proportions of casein fractions in the colostrum samples collected after successive milking runs: the share of α s-casein increased, the share of β -casein remained stable, and the share of κ -casein decreased. The first milking colostrum was characterized by different protein concentrations. The rate of changes in the protein content of the colostrum in subsequent milkings was affected by the breed. In the Jersey cows, a higher percentage share of κ -casein and higher levels of α -LA, β -LG, and LF in the first-milking colostrum were accompanied by a rapid decrease in their concentrations in subsequent milkings, which points to a correlation between the protein content of the colostrum and the rate of changes in the protein concentrations at subsequent milkings, thus indicating that colostrum is the best source of functional proteins.

Key words: Colostrum, breed, bioactive proteins, lactoferrin, cytokines, casein fractions

1. Introduction

Bioactive food ingredients have been widely studied recently. Bioactive compounds are defined as food components, which in addition to their high nutritional value are involved in various regulatory processes in the human body (1). The protein fraction in bovine colostrum comprises bioactive components such as major milk proteins, hormones, growth factors, and cytokines. According to Pakkanen and Aalto (2), the most important bioactive components of colostrum include growth factors and antimicrobial compounds. Due to the presence of large amounts of bioactive compounds, colostrum has a beneficial influence on newborn calves during the first days after calving and over the entire period of its secretion (3,4). Colostrum composition, similar to milk composition, may be affected by both genetic and environmental factors (5–8), yet the effects of those factors remain poorly investigated. In view of the above, the objective of this study was to determine changes in the bioactive protein concentrations in the colostrum of 2 different cattle breeds.

2. Materials and methods

2.1. Animals and sampling

The experimental materials comprised double colostrum samples collected from 8 Jersey cows and 8 Polish Holstein–Friesian (PHF) cows during 8 successive milkings after calving. The cows were in the same barn and were the same age. The first milking was carried out within 1 h after calving and subsequent milkings took place at 12-h intervals. The pooled colostrum was stirred and a representative sample was taken. A total of 256 colostrum samples were collected. The colostrum (100 mL) was poured into plastic containers and was transported at 4 °C to the laboratory of the Department of Cattle Breeding and Milk Quality Evaluation.

2.2. Colostrum quality

Fresh milk samples were assayed for density, which is an indicator of the sample quality, using a DMA 35N digital density meter (Anton Paar, Graz, Austria) and for pH to determine the suitability for further analyses and proximate chemical composition, i.e. the percentage

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content of protein, fat, lactose, dry matter, and nonfat solids (Milcoscan 133B, Foss Electric, Hillerød, Denmark). After this, the samples were immediately frozen at $-20\text{ }^{\circ}\text{C}$ for subsequent analysis.

2.3. Percentage share and content of bioactive proteins

The analysis involved the determination of the percentage share and content of selected bioactive proteins, including the percentage share of casein fractions (α_s -, β -, γ -, and κ -) in the total casein and the concentrations of α -lactalbumin (α -LA), β -lactoglobulin (β -LG), lactoferrin (LF), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α). The percentage shares of α_s -, β -, γ -, and κ -casein and the concentrations of α -LA and β -LG were determined by protein electrophoresis in 14.5% polyacrylamide gel in the presence of sodium dodecyl sulfate, using the Mini-PROTEAN 3 cell system, as described by Laemmli (9). The percentage shares of casein fractions, α -LA, and β -LG, were estimated densitometrically with the use of Quantity One 4.6 software (Bio-Rad, Hercules, CA, USA). The contents of LF, IL-1 β , IL-6, and TNF- α were determined by the "sandwich" enzyme-linked immunosorbent assay (ELISA) technique, which measures the amount of protein between 2 layers of antibodies. The LF concentrations were determined using the commercial Bovine Lactoferrin ELISA Quantification Kit (Bethyl Laboratories Inc., Montgomery, TX, USA). Commercial kits were also used to determine the interleukins (IL-1 β , IL-6)

and TNF- α (Bovine IL-1 β Screening Set and Bovine IL-6 Screening Set, Thermo Scientific Inc., Waltham, MA, USA; Bovine TNF- α DuoSet, R&D Systems Inc., Minneapolis, MN, USA). The samples were analyzed according to the protocols supplied with the kits.

2.4. Statistical analysis

The results were processed statistically with the use of STATISTICA 9.0 software (StatSoft Inc., Tulsa, OK, USA) (10). Arithmetic means (\bar{X}) and standard deviations (SDs) were calculated for each parameter. One-way analysis of variance was performed in an orthogonal design. The significance of the differences between the means was estimated using the least significant difference test.

3. Results

3.1. Physical properties and chemical composition of the colostrum

The density, pH, and proximate chemical composition of the first-milking colostrum are presented in the Table. The pH values of the colostrum were lower than the pH value of normal milk, at 6.54 and 6.56 in the Jersey and PHF cows, respectively. In both cattle breeds, the colostrum density ranged from 1.047 to 1.075 g/cm³. The colostrum of the Jersey cows was characterized by significantly ($P \leq 0.05$) higher density (1.065 g/cm³) compared with the colostrum of the PHF cows (1.056 g/cm³). The first-milking colostrum of the Jersey and the PHF cows also differed

Table. Density, pH, and proximate chemical composition of the first-milking colostrum.

Specification		Jersey	PHF
Sample number number		16	16
Density (g/cm ³)	\bar{X}	1.065*	1.056
	SD	0.017	0.015
pH	\bar{X}	6.54	6.56
	SD	0.09	0.06
Fat (%)	\bar{X}	6.49	5.70
	SD	1.71	2.11
Total protein (%)	\bar{X}	17.50*	13.87
	SD	5.96	5.56
Lactose (%)	\bar{X}	3.29	3.92*
	SD	0.72	0.26
Dry matter (%)	\bar{X}	28.47	25.70
	SD	5.18	5.99
Solids nonfat (%)	\bar{X}	23.34*	20.28
	SD	5.80	5.64

Mean values followed by an asterisk in rows are significantly different at $P \leq 0.05$.

with respect to chemical composition. The colostrum of the Jersey cows had a significantly ($P \leq 0.05$) higher content of total protein and nonfat solids. In comparison with the colostrum of the PHF cows, the colostrum of the Jersey cows also had higher concentrations of fat and dry matter, but those differences were found to be statistically nonsignificant due to high variation levels.

3.2. Percentage share of casein fractions in colostrum

The percentage share of casein fractions in the colostrum of the Jersey and PHF cows, obtained during successive milkings, is shown in Figures 1 and 2. In both cattle breeds,

the share of α_s -casein increased, the share of β -casein remained stable, and the share of κ -casein decreased over time after calving. In the Jersey cows, the percentage share of α_s -casein increased rapidly on the second day after calving, as shown by significant ($P \leq 0.05$) differences in the content of this casein fraction in the colostrum obtained from the first, second, and third milkings. Starting from the third milking, differences in the α_s -casein content of the colostrum became statistically nonsignificant. In the PHF cows, the increase in the percentage share of α_s -casein was lower and the differences between the successive milkings

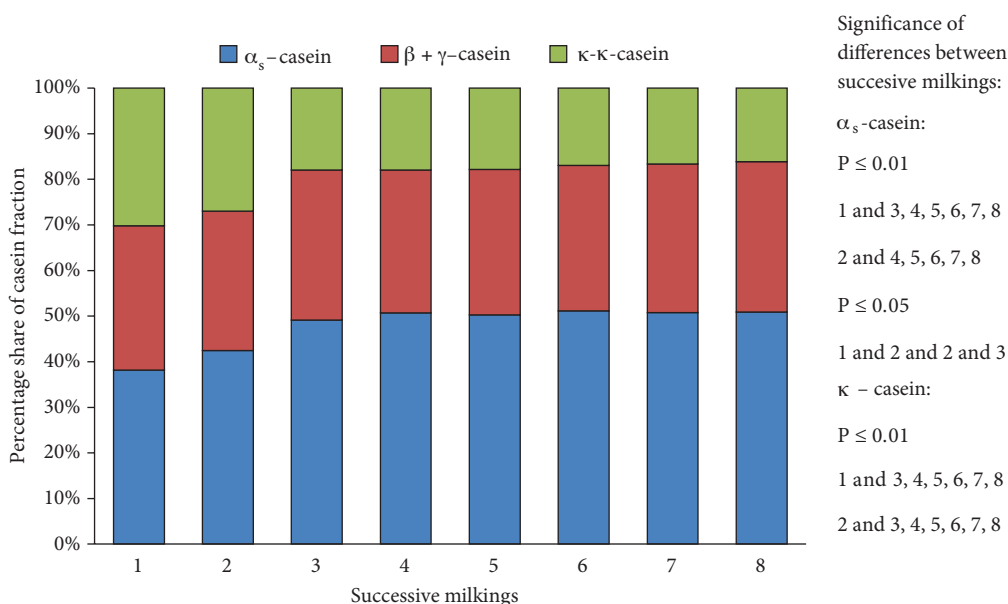


Figure 1. Changes in the percentage share of casein fractions in Jersey cow colostrum.

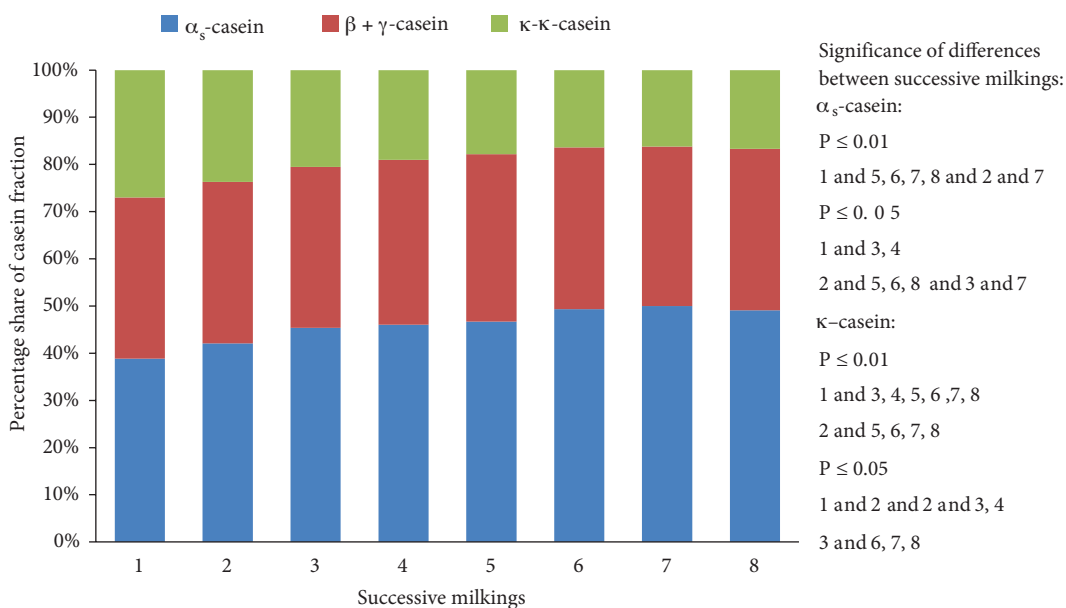


Figure 2. Changes in the percentage share of casein fractions in PHF cow colostrum.

were statistically nonsignificant. The percentage share of κ -casein in the first-milking colostrum was higher in the Jersey cows (30.21%) than in the PHF cows (26.99%). The rate of changes in the κ -casein content of the colostrum was similar in both breeds. The highest decrease in the percentage share of κ -casein in the total casein was observed between the first and second milkings after calving, and the noted difference was statistically significant ($P \leq 0.05$). Despite considerable differences in the percentage share of κ -casein in the first-milking colostrum between the studied cattle breeds, the share of this casein fraction in the last analyzed colostrum samples of the Jersey and PHF cows was comparable, at 16.12% and 16.68%, respectively. The percentage share of the $\beta + \gamma$ -casein colostrum was higher in the Jersey cows (34.40% on average) than in the PHF cows (31.98% on average). There were no differences in the share of this casein fraction between the successive milkings. Starting from the sixth milking, no variations were found in the percentage share of the casein fractions in the total casein contained in the colostrum, and the casein values noted in this milking run were comparable with those determined for milk casein.

3.3. Concentrations of α -LA and β -LG

Changes in the concentrations of α -LA and β -LG in the colostrum obtained during successive milkings are illustrated in Figures 3 and 4. The first-milking colostrum of the Jersey cows contained larger amounts of α -LA (13.82 g/L) in comparison with the PHF cows (7.91 g/L). The initial α -LA content of the colostrum was higher in the Jersey cows, but then it decreased rapidly between the first and second milkings after calving, and the noted difference was statistically significant ($P \leq 0.01$). The β -LG content of the first-milking colostrum was comparable in both cattle breeds. The pattern of changes in the β -LG concentrations in the subsequent milkings was similar. From the fifth to eighth milkings, no changes were observed in the levels of α -LA and β -LG in the colostrum. In the last 4 milking runs,

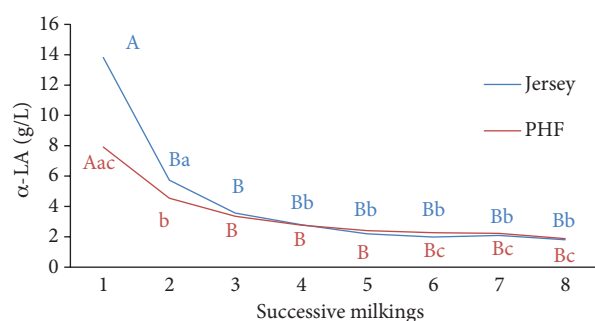
the concentrations of α -LA and β -LG were approximately 2 g/L and over 5 g/L, respectively.

3.4. LF content

Changes in the concentrations of LF in the colostrum of the Jersey and PHF cows over time after calving are illustrated in Figure 5. The LF content of the first-milking colostrum was higher in the Jersey cows compared with the PHF cows (2.65 mg/mL vs. 1.36 mg/mL), and this trend was also observed in the subsequent milkings. The first-milking colostrum of the Jersey cows contained significantly ($P \leq 0.05$) higher amounts of LF than the colostrum obtained from the second and subsequent milking runs. More rapid changes in the LF levels in the colostrum were observed in the Jersey cows in comparison with the PHF cows.

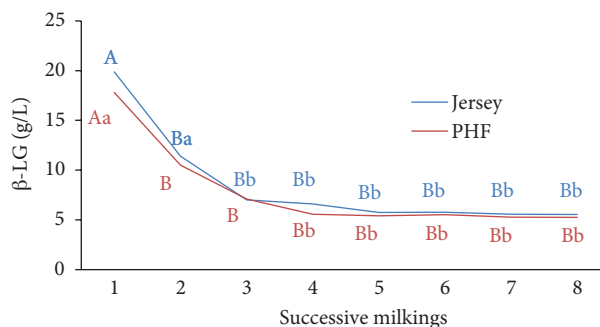
3.5. Cytokine content

Figures 6–8 show changes in the levels of IL-1 β , IL-6, and TNF- α in the colostrum obtained during the successive milkings. A decrease in the cytokine content of the colostrum, observed in the PHF cows until the sixth milking after calving, was followed by an insignificant increase noted in the last 2 analyzed samples. There were significant differences in the IL-1 β content between the colostrum samples from the successive milkings, and it was found to decrease more rapidly in the PHF cows. Moreover, in the Jersey cows, the IL-1 β content of the colostrum decreased over time after calving, and the difference between the first and eighth milkings reached 0.6 ng/mL. The noted differences were not validated by statistical analysis. The IL-6 content of the colostrum from the Jersey cows also decreased over time, and the differences in the levels of this cytokine in the colostrum from the first and second milkings and in the colostrum from the seventh and eighth milkings were significant ($P \leq 0.05$). In the PHF cows, the IL-6 content of the first-milking colostrum was significantly ($P \leq 0.01$) higher compared with the subsequent milkings, and the difference between the first and the last colostrum samples reached 1.12 ng/mL. In both of the studied cattle



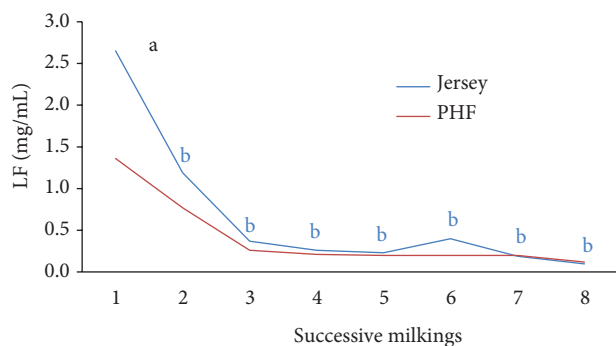
Mean values denoted by different letters are significantly different: a, b - $P < 0.05$; A, B - $P < 0.01$.

Figure 3. Changes in the α -LA content of colostrum obtained during 8 successive milkings.



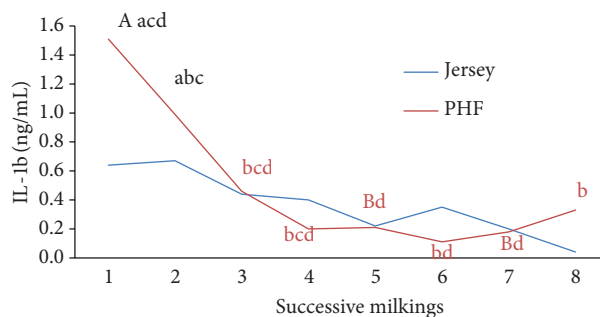
Mean values denoted by different letters are significantly different: a, b - $P < 0.05$; A, B - $P < 0.01$.

Figure 4. Changes in the β -LG content of colostrum obtained during 8 successive milkings.



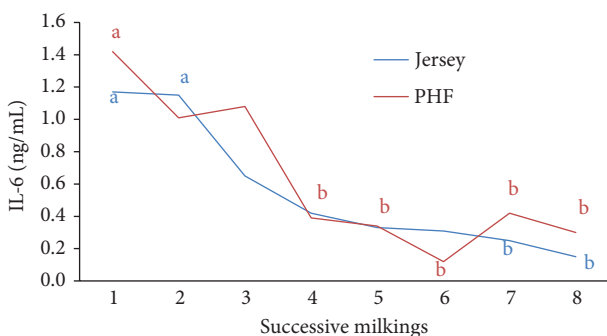
Mean values denoted by different letters are significantly different: a, b - $P < 0.05$.

Figure 5. Changes in the LF content of colostrum obtained during 8 successive milkings.



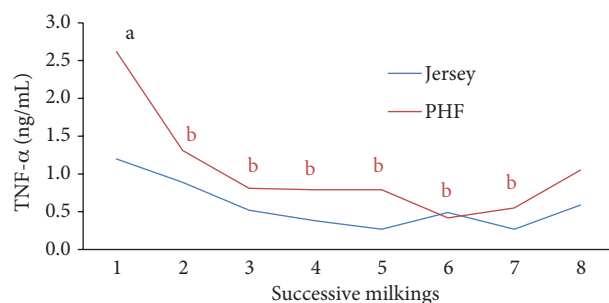
Mean values denoted by different letters are significantly different: a, b - $P < 0.05$; A, B - $P < 0.01$.

Figure 6. Changes in the IL-1 β content of colostrum obtained during 8 successive milkings.



Mean values denoted by different letters are significantly different: a, b - $P < 0.05$.

Figure 7. Changes in the IL-6 content of colostrum obtained during 8 successive milkings.



Mean values denoted by different letters are significantly different: a, b - $P < 0.05$; A, B - $P < 0.01$.

Figure 8. Changes in the TNF- α content of colostrum obtained during 8 successive milkings.

breeds, the TNF- α content of the colostrum was found to decrease until the sixth milking after calving and to increase slightly in the last 2 analyzed samples. Significant ($P \leq 0.05$) differences were noted only with regard to the first-milking colostrum of the PHF cows, which contained larger amounts of TNF- α than the colostrum samples from the subsequent milkings, except for the eighth milking.

4. Discussion

4.1. Physical properties and chemical composition of the colostrum

The pH values of the colostrum were lower than the pH value of normal milk, which is consistent with the findings of Klimeš et al. (11) and Wielgosz-Groth and Groth (12), who noted a pH increase over time after calving, yet the colostrum pH values in the cited experiments were lower than those in the present study. In both of the cattle breeds, the colostrum density ranged from 1.047 to 1.075 g/cm³, which, according to Fleenor and Stott (13), testifies to its high quality. Results similar to our chemical

composition of the first-milking colostrum were reported by other authors (7,8,14,15). Szulc and Zachwieja (15) demonstrated that the composition of bovine colostrum may vary widely depending on the cow's age, the housing system, the health status of the animals, and the time and method of milking. Kuczaj et al. (14) compared the chemical composition of the colostrum in red-and-white and black-and-white cattle and found that the colostrum of the black-and-white cows was characterized by a higher density and higher total protein content. According to the above authors, low-yielding dairy cattle breeds and beef breeds have higher concentrations of colostrum components. This hypothesis has been validated in the present study, since the colostrum of the Jersey cows had a higher content of major components than that of the PHF cows. From the environmental factors, farm size may reflect on the colostrum quality. According to Kehoe et al. (4), differences in the colostrum nutrient concentrations were found between small and large farms. Larger farms reported longer times of milking colostrum after calving, a

higher somatic cell count, and a lower nutrient content of the colostrum compared with small farms (less than 100 cows). The farm from our study was small according to Kehoe et al. (4).

4.2. Percentage share of the casein fractions in the colostrum

A decrease in κ -casein content and an increase in α_s -casein content, as observed in our study, was also reported by Wielgosz-Groth (16), who noted a negative correlation between these casein fractions. A decrease in the percentage share of κ -casein in the colostrum obtained during successive milkings most probably resulted from a decrease in casein glycopeptide in the κ -casein structure, as this component is only present immediately postpartum (17).

4.3. Concentrations of α -LA and β -LG

The obtained results confirm the high quality of the first-milking colostrum, attributed mostly to high globulin concentrations. As demonstrated by Szulc and Zachwieja (15), the β -LG content decreases nearly 4-fold during the first 6 days after calving, from 11.38 g/L to 3.32 g/L. The values recorded in our study were higher than those reported by the above authors, yet the noted trend is similar: the 4-fold decrease in the β -LG content of the colostrum during the first 8 milkings was identical to that the cited study. Our results support the findings of other authors (11,15,18–21) who also observed a decrease in the concentrations of major whey proteins, α -LA and β -LG, in bovine colostrum over time after calving. The absence of significant differences in the content of α -LA and β -LG in the colostrum obtained from the fifth and subsequent milkings indicates that their levels stabilized within 48 h postpartum, reaching the values reported for milk.

4.4. LF content

Higher LF concentrations in the colostrum of the Jersey cows, compared with the PHF cows, are in agreement with the findings of Tsuji et al. (22). According to the cited authors, the physiological and biochemical processes underlying variations in the LF content of the colostrum and milk in different cattle breeds should be thoroughly investigated. Our results are higher than the LF concentration findings of Kehoe et al. (4) and Yoshida et al. (23), who reported 0.82 ± 0.54 mg/mL and 0.34 ± 0.23 mg/mL, respectively. According to Kehoe et al. (4), differences may exist because of laboratory techniques; however, bovine colostrum does contain lower LF concentrations than other species. How to increase the LF concentration

in cow's milk, unfortunately, has not been clearly established, and the results obtained so far indicate only the large variability of the content based on the somatic cell count, yield, and stage of lactation (24). Among the factors influencing the content of bioactive proteins in cow's milk, Cheng et al. (25) also mentioned the daily yield, protein and lactose content, and the number of milk samples included in the study.

4.5. Cytokine content

The findings of the current study are consistent with those of Hagiwara et al. (26), who analyzed the concentrations of IL-1 β , IL-6, and TNF- α in bovine colostrum samples collected immediately postpartum and 3 and 5 days after calving. The cited authors observed a decrease in the cytokine content of the colostrum over time after calving. In a study by Goto et al. (27), the IL-1 β content of the colostrum samples collected from Holstein–Friesian cows immediately after parturition ranged from 0.5 to 5 ng/mL. In the present experiment, the IL-1 β content of the first-milking colostrum in the PHF cows also remained within this range (1.51 ± 1.45 ng/mL). Goto et al. (27) reported problems with determining the levels of IL-1 β in colostrum samples collected 3 days after parturition since they were below the detection limit (0.5 ng/mL) of the applied analytical method. No such problems were encountered in the course of the current study.

The concentrations of the studied bioactive proteins in the bovine colostrum decreased over time after calving, along with changes in the proximate composition of the colostrum. Differences were also noted in the proportions of the casein fractions in the colostrum samples collected after successive milking runs: the share of α_s -casein increased, the share of β -casein remained stable, and the share of κ -casein decreased. The first-milking colostrum was characterized by different protein concentrations. The rate of changes in the protein content of the colostrum in the subsequent milkings was affected by breed. In the Jersey cows, a higher percentage share of κ -casein and higher levels of α -LA, β -LG, and LF in the first-milking colostrum were accompanied by a rapid decrease in their concentrations in the subsequent milkings, which points to a correlation between the protein content of the colostrum and the rate of changes in the protein concentrations at subsequent milkings, thus indicating that the colostrum is the best source of functional proteins and should be fed to neonatal calves.

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