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SHARE OF SELECTED PROTEIN FRACTIONS IN THE MILK OF POLISH HOLSTEIN-FRIESIAN COWS DEPENDING ON AGE, PERFORMANCE AND STAGE OF LACTATION

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ABSTRACT

Milk is composed of more than 400 fatty acids, approximately 100 proteins and peptides, lactose, vitamins and minerals. These components have a high bioavailability and health-promoting properties to humans. They also influence technological usefulness of milk for processing. The aim of the study was to determine the influence of cow's age, level of productivity and lactation phase on milk composition with particular emphasis on the share of protein fractions and lactoferrin in milk of Polish Holstein-Friesian cows. Milk samples were collected from primiparous (P; n = 60) and multiparous (M; n = 60) cows in three phases of lactation (I – 30–60 days; II – 90–120 days; III – 210–240 days) with differentiation on cows with low (L; < 30 kg/milking) and high (H; > 30 kg/milking) performance. Lower ($P \leq 0.01$) level of α-casein was found in milk from cows ML I and MH I comparing with primiparous cows in this phase of lactation. Milk from cows PH III was characterised by lower ($P \leq 0.05$) share of α-lactalbumin comparing with milk from cows in I stage of lactation in all groups. It was observed that in subsequent phases of lactation milk was characterized by higher content of lactoferrin. In general, in multiparous cows somatic cell count (SCC) was higher comparing with primiparous cows. The obtained results can be explain by the greater number of infections of the mammary gland in older cows so the level of antibacterial components increases as well as the fact that with lactation phases mammary gland becomes more and more susceptible.

Key words: protein fractions, age, yield, lactation stage, bovine milk

INTRODUCTION

Milk is composed of more than 400 fatty acids, approximately 100 proteins and peptides, lactose, vitamins and minerals. These components have a high bioavailability and health-promoting properties to humans. They also influence technological usefulness of milk for processing [Barłowska et al. 2006, Hussain et al. 2013, Pecka et al. 2013]. Caseins are carriers of Ca, Cu, Zn, and other ions which presence determines rennet activity in cheese production [Bernabucci et al. 2002]. Milk with higher amount of κ-casein and β-lactoglobulin is desirable for fermented milk products or cheese production [Ng-Kwai-Hang 1998, Brophy et al. 2003, Kuczyńska et al. 2011]. A change of 0.1 $g \cdot L^{-1}$ in casein level in milk results in a change of cheese making performance of 0.27–0.28 $kg \cdot 100\ L^{-1}$ [Mariani 1992]. One of the whey proteins is serum albumin (SA), which gets into milk from bloodstream of the cow. It was observed that its content in milk increases during infection of the mammary gland. Its function is not fully understood, it is only known that it binds fatty acids and other small molecules [Walzem et al. 2002]. Another milk protein, lactoferrin, has as the main function the transport of iron, but it also shows bacteriostatic, bactericidal, anti-viral, anti-parasitic and immunomodulatory properties [Alderova et al. 2008]. It is observed that the amount of these proteins in milk may vary depending on milking performance, lactation stage and cow's age [Barłowska et al. 2006, Cheng et al. 2008].

The aim of the study was to determine the influence of cow's age, level of productivity and lactation phase on milk composition with particular emphasis on the

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share of protein fractions and lactoferrin in milk of Polish Holstein-Friesian cows.

MATERIAL AND METHODS

All milk samples (100 ml/cow) were collected the same day from primiparous (P; n = 60) and multiparous (M; n = 60) cows in three phases of lactation (I – 30–60 days; II – 90–120 days; III – 210–240 days) with differentiation on cows with low (L; < 30 kg/milking) and high (H; > 30 kg/milking) performance. The animals were housed in an open housing system and had been fed a basal diet (total mixed ration – TMR). Their diet was formulated according to the French standard INRA [IZ-INRA 2009].

Protein, fat, lactose and dry matter percentages were determined in fresh milk using Infrared Milk Analyzer 150 (Bentley Instruments Inc., Chaska, MN, USA), as well as somatic cell count using Somacount apparatus (Bentley Instruments Inc., Chaska, MN, USA). The share of protein fractions was determined by electrophoresis according to the method by Laemmli [1970] on polyacrylamide gel in presence of sodium dodecyl sulfate (SDS-PAGE). Milk samples were defatted by centrifugation (3.000 rpm for 5 min. in 4°C), then fat was removed and samples were deprived of salt by dialysis. Next, proteins were denatured (addition of 2% SDS) and samples were incubated at 100°C for 5 min. To break disulfide bonds, a reducing agent was added to the samples: 5% β-mercaptoethanol and 0.0625 M buffer (pH 6.75). The density of the samples was increased by adding glycerol and to obtain colour – bromophenol blue (0.25%) was added. The prepared samples were loaded on polyacrylamide gel consisting of 4% thickening gel, and 12% separation gel. Electrophoresis was carried out for 5 hours. Subsequently, gels were coloured with Coomassie Brilliant Blue dye. The obtained coloured gels were scanned and analysed using BioRad 6 software (Bio-Rad Laboratories, Hercules, CA, USA). Lactoferrin concentration was determined in defatted milk samples using ELISA kit (Bovine Lactoferrin ELISA Kit, Bethyl Laboratories, Montgomey, TX, USA) according to producer's instructions. Milk samples were diluted 1:2,000 and a standard curve was generated. Each sample was run in duplicate. The absorbance of samples in 96-well plate was read using Synergy TM BioTek plate reader (BioTek, Winooski, VT, USA) at 450 nm wavelengths.

The results were statistically analysed using MANOVA in Statistica 9.0 software (StatSoft Polska, Cracow, Poland). Significance of differences was determined using Duncan's test.

RESULTS

Fat content in milk was relatively low (3.35–4.36%) (Table 1). Statistically ($P \leq 0.05$) higher share of fat was found in milk from cows ML III comparing with milk from cows PH III. Higher ($P \leq 0.01$) protein percentage was detected in milk from cows PL III comparing with

Table 1. Milk composition (mean ±SD) of primiparous (P) and multiparous (M) Polish Holstein-Frisian cows (n = 120) depending on cow's performance and lactation phase

Tabela1. Skład mleka (średnia ±SD) krów pierwiastek (P) i wieloródek (M) rasy polskiej holsztyńsko-fryzyjskiej (n = 120) w zależności od ich wydajności i fazy laktacji

Cow's age Wiek krów	Performance Wydajność	Lactation phase Faza laktacji	Composition of milk, % – Skład mleka, %				
			fat tłuszcz	protein białko	lactose laktoza	dry matter sucha masa	non-fat dry matter sucha masa beztłuszczowa
P	L	I	4.36^{B} ±0.85	3.20^{B} ±0.20	5.23^{Bc} ±0.09	13.14^{B} ±0.78	9.04^{bc} ±0.29
		II	3.59^{b} ±0.76	3.11^{B} ±0.24	5.09^{Bbc} ±0.13	12.42^{B} ±0.66	8.83^{b} ±0.37
		III	3.60 ±0.48	3.37^{A} ±0.28	5.01 ±0.16	12.72^{b} ±0.81	9.12^{Acc} ±0.44
	H	I	3.64 ±0.63	2.87^{B} ±0.08	5.15^{Bc} ±0.38	12.26^{B} ±0.80	8.63^{BC} ±0.41
		II	3.42 ±0.62	2.97^{B} ±0.19	5.04^{B} ±0.07	12.05^{B} ±0.64	8.63^{b} ±0.20
		III	3.54^{b} ±0.88	3.43^{A} ±0.15	5.04^{B} ±0.19	12.64^{b} ±0.98	9.10^{Acac} ±0.28
M	L	I	3.71^{b} ±0.44	2.99^{B} ±0.16	5.12^{Bc} ±0.18	12.43^{B} ±0.62	8.72^{B} ±0.28
		II	3.71^{B} ±0.53	3.01^{B} ±0.26	5.02^{Bbc} ±0.12	12.38^{B} ±0.80	8.66^{b} ±0.33
		III	3.67^{Aa} ±0.61	3.48^{A} ±0.22	4.98 ±0.08	12.82^{A} ±0.69	9.15^{Cc} ±0.25
	H	I	3.64^{Bb} ±1.11	2.76^{B} ±0.18	5.12^{ab} ±0.13	12.12^{Ba} ±1.00	8.48^{B} ±0.27
		II	3.35^{Bb} ±0.77	2.86^{B} ±0.09	5.06^{a} ±0.16	11.91^{Ba} ±0.87	8.56^{B} ±0.15
		III	3.61 ±1.05	2.90 ±1.24	4.90^{A} ±0.57	12.02^{B} ±2.74	8.42^{ab} ±2.05

L – low performance (<30 kg), H – high performance (>30 kg); I lactation phase (30–60 days), II lactation phase (90–120 days), III lactation phase (210–240 days); lowercase letters indicate statistically significant differences at the level of P ≤ 0.05 in columns, uppercase letters indicate statistically significant differences at the level of P ≤ 0.01 in columns.

L – niska wydajność (<30 kg), H – wysoka wydajność (>30 kg); I faza laktacji (30–60 dni), II faza laktacji (90–120 dni), III faza laktacji (210–240 dni); małe litery wskazują różnice statystycznie istotne przy poziomie P ≤ 0,05 w kolumnach, duże litery wskazują różnice statystycznie istotne przy poziomie P ≤ 0,01 w kolumnach.

cows in I and II lactation phase independently to age and performance. In milk from multiparous cows, an increase of protein level was observed during lactation; but this augmentation was yet lower about 5% comparing with primiparous cows. In milk from all cows, increase of protein level and decrease of fat and lactose contents were observed between I and III lactation stage. This may be due to changes in energetic balance in feed ration. A positive relationship between protein and dry matter levels was observed. In milk from cows ML III, ($P \leq 0.01$) level of dry matter was higher comparing with milk from cows in I and II lactation independently to age and performance, which may be due to lower milk production in this lactation stage. Milk from primiparous cows was characterised by higher percentage of lactose and non-fat dry matter comparing with milk from multiparous cows, independently to lactation phase and performance.

SCC in milk from primiparous cows was 143.06 thousand per ml in average, while in milk from multiparous cows it was 182.00 thousand per ml in average. Higher ($P \leq 0.01$) SCC was found in milk from cows MH II comparing with milk from cows PH (all stages of lactation). In addition, SCC of cows MH II and MH III was significantly ($P \leq 0.01$) higher comparing with cows PH II and PH III (Table 2).

Molecular forms of polypeptide chains under influence of electric field migrate in gel and are subject to separation from the arrangement according to decreasing mass (Fig. 1). Results of SDS-PAGE separation are presented in Table 3. In milk from cows ML III, MH III, and PH III, higher level of α-casein was found comparing with milk from cows in I stage of lactation, independently to age and performance. Lower ($P \leq 0.01$) level of this protein was also found in milk from cows ML I and MH I comparing with primiparous cows in this phase of lactation. No statistical differences were observed in κ-casein levels among groups. An increase of α-casein along with a decrease of κ-casein level was observed in III stage of lactation compared with I stage, independently to age and performance. Level of β-casein was higher in milk from cows with higher milking performance juxtaposed to low producing cows, independently to age and lactation phase. Relation of SCC with protein fractions was also analysed. In all cases, a reverse relation between SCC and protein fraction was observed.

Higher SA content in milk from cows PH II, comparing with milk from cows in the same age and with the same milking performance in I and III lactation period, was found. However, SA level decreased with lactation phases in milk from multiparous cows. A similar relation was noticed for α-lactalbumin and lactose in milk from both, primiparous and multiparous cows. Milk from cows PH III was characterised by lower ($P \leq 0.05$) share of α-lactalbumin comparing with milk from cows in I stage of lactation in all groups.

Authors observed that in subsequent phases of lactation milk was characterized by a higher content of lactoferrin (Fig. 2). Its highest level was recorded in the milk of cows MH III (240.68 $\mu g \cdot mL^{-1}$); while milk from cows PL I was characterized by the lowest lactoferrin concentration (74.47 $\mu g \cdot mL^{-1}$).

DISCUSSION

Milk from primiparous cows has higher levels of lactose and non-fat dry matter comparing with milk from multiparous cows [Pecka et al. 2013]. Similar results were obtained in the presented study. Contrary to Gurmessa and Melaku [2012], who did not observe any differences in fat, non-fat dry matter, protein and lactose levels in milk between multi- and primiparous cows. However, authors found that fat content was higher at the beginning and at the end of lactation. Yet, authors did not observe any significant effect of lactation phase on non-fat dry matter, protein, lactose and ash shares in milk. Antkowiak et al. [2007] noticed that fat content in milk increased with cow's age (+0.10%), while protein and dry matter content lowered. Authors also found that fat content decreased with lactation stage (–0.35%), contrary to protein and dry matter content which increased (respectively:

Table 2. Mean ±SD of Somatic Cell Count (SCC; thousand per ml) in milk from Polish Holstein-Frisian cows (n=120) depending on cow's age, performance and lactation phase

Tabela 2. Średnia ±SD liczby komórek somatycznych (tys. · ml^{-1}) w mleku krów rasy polskiej holsztyńsko-fryzyjskiej (n=120) w zależności od wieku krów, ich wydajności i fazy laktacji

Cow's age Wiek krów	Performance Wydajność	Lactation phase – Faza laktacji		
		I	II	III
P	L	113.00^{B} ±205.91	400.70^{B} ±769.12	56.70^{B} ±50.36
	H	68.40^{B} ±64.91	173.44^{B} ±169.39	46.11^{B} ±18.88
M	L	67.75^{B} ±96.84	396.25^{B} ±621.40	147.90^{A} ±136.13
	H	134.78^{B} ±351.13	281.33^{A} ±686.25	64.00^{A} ±52.14

For explanations see Table 1.
Objaśnienia podano w tabeli 1.

Table 3. Share of selected protein fractions (mean ±SD) in milk protein from Polish Holstein-Frisian cows (n = 120) depending on cow's age, performance and lactate on phase

Tabela 3. Udział wybranych frakcji białkowych (średnia ±SD) w białku ogólnym mleka krów rasy polskiej holsztyńsko-fryzyj-skiej (n = 120) w zależności od wieku krów, ich wydajności i fazy laktacji

Cow's age Wiek krów	Performance Wydajność	Lactation phase Faza laktacji	Share of protein fractions, % – Udział frakcji białkowych, %				
			caseins – białka kazeinowe			whey proteins – białka serwatkowe	
			α-casein α -kazeina	β-casein β-kazeina	κ-casein κ-kazeina	serum albumin albumina surowicza	α-lactalbumin α-laktoalbumina
P	L	I	25.53^{B} ±3.78	19.52^{bd} ±2.59	20.01 ±4.08	13.27^{BC} ±3.10	14.93^{BCbcd} ±1.94
		II	26.53^{B} ±2.80	19.83^{bd} ±1.66	17.28 ±1.82	13.69^{BC} ±1.38	12.44^{Abc} ±3.23
		III	25.85^{B} ±2.61	17.87^{Bbc} ±2.37	16.42 ±2.07	10.67 ±4.87	10.30 ±3.41
	H	I	24.84^{B} ±3.10	20.19 ±2.01	16.49 ±0.47	11.40^{BCbc} ±1.72	14.46^{BCbd} ±2.30
		II	23.73^{B} ±4.37	20.01^{acd} ±3.74	15.68 ±4.25	12.93^{ac} ±4.22	12.31^{Abbd} ±2.40
		III	28.12^{B} ±2.21	20.46^{Bbcd} ±2.55	15.04 ±2.63	9.14^{ab} ±4.62	9.71^{ad} ±1.44
M	L	I	25.27^{Aa} ±2.16	16.15^{abc} ±1.88	18.14^{b} ±3.21	13.33^{Acbc} ±2.27	16.74^{BCcd} ±2.56
		II	25.07^{B} ±1.66	18.11^{Acd} ±2.07	18.03 ±1.78	11.49 ±2.03	15.14^{Ababc} ±2.20
		III	26.38^{B} ±3.72	18.24^{Bd} ±2.51	14.74 ±3.18	9.09 ±4.14	11.13^{C} ±2.56
	H	I	25.20^{C} ±3.78	18.70 ±2.65	18.89 ±2.83	13.28^{C} ±2.95	15.12^{Bbcd} ±2.00
		II	25.80^{BCb} ±2.74	18.57^{d} ±2.62	18.57 ±3.34	11.99^{c} ±3.33	13.47^{Bcabd} ±1.81
		III	27.01^{B} ±2.30	18.41^{Bbcd} ±2.31	15.89 ±1.89	10.55^{B} ±6.86	11.68^{Acd} ±2.27

For explanations see Table 1.
Objaśnienia podano w tabeli 1.

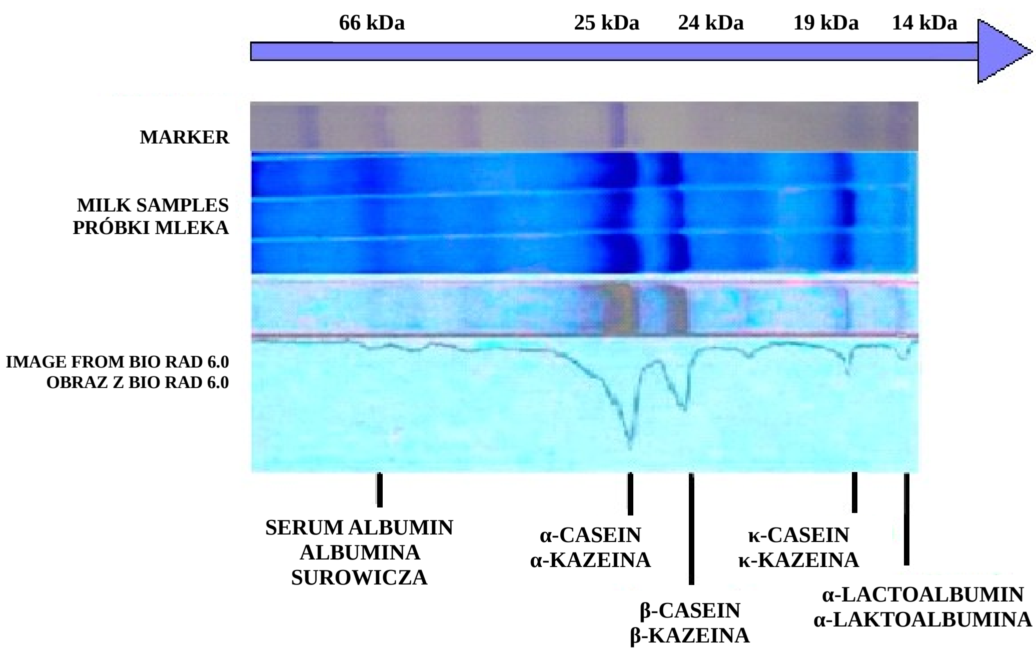

Fig. 1. Electrophoretic separation of proteins milk of cows on polyacrylamide gel with sodium dodecyl sulfate (SDS-PAGE)

Rys. 1. Obraz rozdziału elektroforetycznego frakcji białek mleka krowiego na żelu poliakrylamidowym w obec-ności siarczanu dodecylu sodu (SDS-PAGE)

+0.64% and +0.83%). In presented study, increase of protein and decrease of fat and lactose levels between I and III lactation phase were observed. Sitkowska [2008] observed that multiparous cows produced milk with more fat (+0.15%) and protein (+0.17%) comparing with primiparous cows. Fat and protein contents were higher at the beginning of lactation. Barłowska et al. [2006] showed that with lactation phases contents of fat (+0.8%), protein (+0.43%) and dry matter (+1.04%) increase, contrary to lactose level which lowers (–0.12%). Moreover, authors noticed that with lactation phases fat-protein ratio improves.

Antkowiak et al. [2007] found that SCC increases with cow's age which confirms that SCC in primiparous cows in lower than in multiparous cows, as observed in presented study. Authors explain that this phenomenon is caused by an accumulating impact of successive cases of *mastitis*. Fractions of immunologically active cells become less effective in fighting of pathogens which results in an increase of SCC after each mammary gland inflammation. Lactation phase had a likewise effect on SCC (+124 thousand per ml). Sitkowska [2008] found as well that multiparous cows produced milk with higher SCC (+0.45 SCC) than primiparous cows. It should be noted that in the analysed herd, low SCC was observed, which indicates good health of mammary gland of cows.

The content of caseins in general increases with lactation phase [Barłowska et al. 2006]. While, Hamed et al. [2012] observed negative correlation between lactation phase and age versus β-, αs1- and κ-casein share in milk. In our study, in all cows, negative interaction was also observed. Several authors point a reduction of α-, β-, κ-casein amounts in milk with high SCC [Litwińczuk et al. 2011, Hamed et al. 2012, Zielak-Steciwko et al. 2014]. Moreover, changes in α- and κ-casein amounts occurs also during bacterial inflammations of the mammary gland [Pecka-Kiełb et al. 2016]. Such association indicates that the reduction of casein level with raising SCC is probably due to higher content of proteases from somatic cells which break casein micelles [Hamed et al. 2012].

In our study, positive correlation between SCC and SA was observed in milk from primiparous cows. A likewise relation was found by several authors [Lieske et al. 2005, Batavani et al. 2007, Zeng et al. 2009, Litwińczuk et al. 2011]. Also Urech et al. [1999] showed increased level of proteins linked to inflammation (among other SA and lactoferrin) in milk from cows with higher SCC caused by subclinical *mastitis*. Litwińczuk et

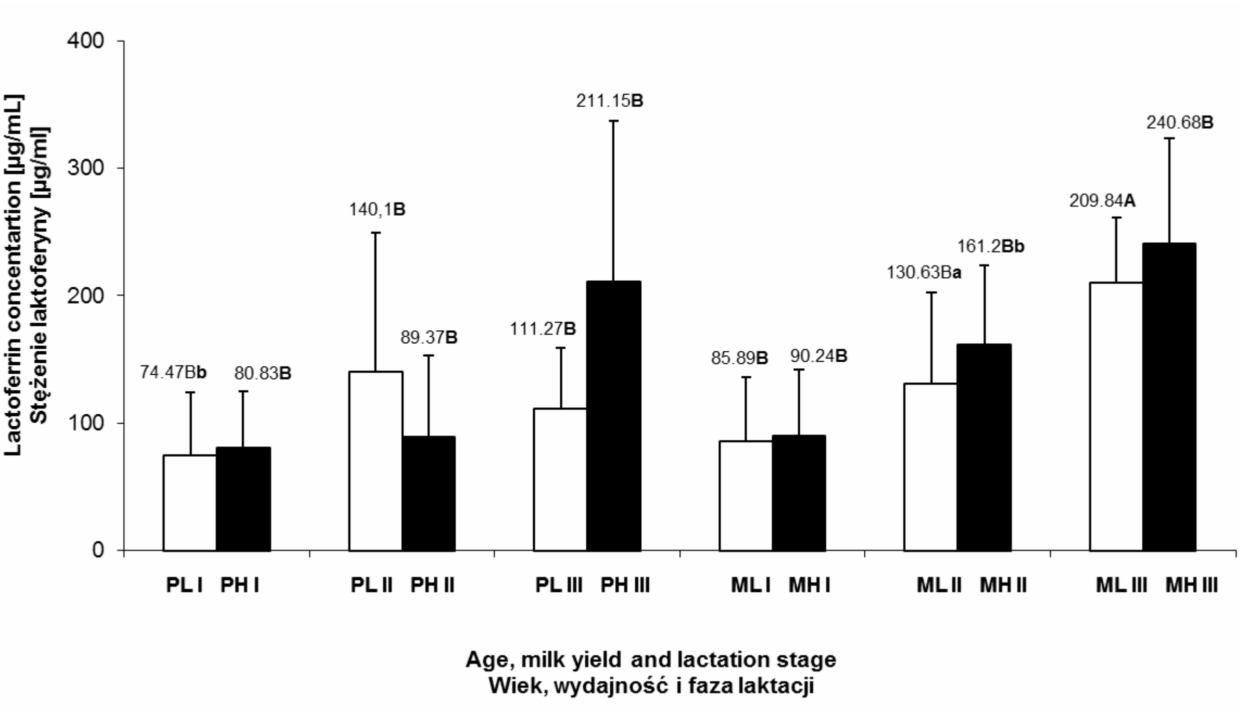

Fig. 2. Lactoferrin concentration in milk from Polish Holstein-Frisian cows (n = 120) in subsequent lactation phases depending on cow's age and performance (for explanations see Table 1)

Rys. 2. Zawartość laktoferyny w mleku krów rasy polskiej holsztyńsko-fryzyjskiej (n = 120) w kolejnych fazach laktacji w zależności od wieku krów i wydajności (objaśnienia podano w tabeli 1) al. [2011] found an opposite relationship, i.e. decrease of α-lactalbumin level with increased SCC.

In our study, SA level decreased with lactation phases in milk from multiparous cows, as well as α-lactalbumin and lactose levels in milk from both, primiparous and multiparous cows. It may be associated with the fact that α-lactalbumin is a monomeric globular whey protein included in enzymatic system of galactose transferase responsible for synthesis of lactose [Król et al. 2008].

The increase in lactoferrin in late lactation can be explained by the increased sensitivity of mammary gland to infections, as lactoferrin is a component of innate immunity and one of the barriers that protect the mammary gland against germs. Our results are consistent with the findings of other authors. Cheng et al. [2008] stated that lactation phase is a factor significantly influencing the increase of lactoferrin level, since authors found a positive correlation between lactation stage and lactoferrin content ($r = 0.557$; $P \leq 0.001$). According to Hagiwara et al. [2003], lactoferrin content in the last lactation phase showed a tendency to rise; however, authors indicated that they did not observe a clear relationship between lactation phase and lactoferrin concentration. Numerous studies have shown a significant positive correlation between the level of lactoferrin and SCC [Hagiwara et al. 2003, Cheng et al. 2008, Litwińczuk et al. 2011, Zielak-Steciwko et al. 2014]. In turn, Cheng et al. [2008] showed a negative correlation ($r = –0.472$) between lactoferrin concentration and daily milk yield. It was also demonstrated that lactoferrin concentration in milk depends on cow's age. Hagiwara et al. [2003] showed that with cow's age, lactoferrin concentration in milk decreased ($r = –0.38$, $P \leq 0.001$). However, in our study, an opposed relationship was found which may be a consequence of a greater number of infections of the mammary gland, and ipso facto due to higher SCC in milk of multiparous comparing with primiparous cows. Also Cheng et al. [2008] demonstrated that lactoferrin concentration in milk from cows in 3rd, 4th and 5th lactations was higher than in milk from cows in 1st and 2nd lactations.

CONCLUSIONS

Analyzing the obtained results, it can be concluded that lactation phase is an important factor determining lactoferrin level, as well as κ-casein, serum albumin, and α-lactalbumin share in milk. Additionally, cow's age and lactation stage has greater influence on basic composition of milk. It can be explained by the fact that with lactation phase mammary gland is more and more susceptible to inflammations so levels of antibacterial components (lactoferrin, serum albumin, etc.) increases.

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UDZIAŁ WYBRANYCH FRAKCJI BIAŁKOWYCH ORAZ SKŁAD MLEKA KRÓW W ZALEŻNOŚCI OD WIEKU, WYDAJNOŚCI I FAZY LAKTACJI

STRESZCZENIE

Mleko składa się z ponad 400 kwasów tłuszczowych, około 100 białek i peptydów, laktozy, witamin i minerałów. Składniki te charakteryzuje wysoka biodostępność oraz właściwości prozdrowotne u ludzi. Wpływają one również na technologiczną przydatność mleka w przetwórstwie. Celem pracy było określenie wpływu wieku, wydajności oraz fazy laktacji na skład mleka ze szczególnym uwzględnieniem udziału frakcji białkowych w mleku krów rasy polskiej holsztyńsko fryzyjskiej. Materiał do badań stanowiły próby mleka pobrane od pierwiastek (P; n = 60) i wieloródek (M; n = 60) będących w różnych fazach laktacji (I – 30–60 dni; II – 90–120 dni; III – 210–240 dni), z podziałem na krowy o niskiej (L; <30 kg) i wysokiej (H; >30 kg) wydajności. Odnotowano niższy ($P \leq 0,01$) poziom α-kazeiny w mleku krów ML I i MH I w porównaniu do mleka pierwiastek w I fazie. W mleku krów PH III odnotowano niższy ($P \leq 0,05$) udział α-laktoalbuminy w porównaniu do mleka krów w I fazie laktacji we wszystkich grupach. Zaobserwowano, że mleko w kolejnych fazach laktacji charakteryzowało się wyższą zawartością laktoferyny. Liczba komórek somatycznych była wyższa u wieloródek w porównaniu do pierwiastek. Uzyskane wyniki można wyjaśnić większą liczbą zapaleń gruczołu mlekowego u starszych krów, w związku z tym wzrasta poziom składników przeciwbakteryjnych oraz faktem, że w kolejnych fazach laktacji gruczoł mlekowy staje się coraz bardziej podatny na infekcje.

Słowa kluczowe: frakcje białkowe, wiek, wydajność, faza laktacji, mleko krowie