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Whey protein content and fatty acids profile in milk of cows used in intensive and conventional production systems with regard to stage of lactation

Aneta BRODZIAK[1], Jolanta KRÓL[2,*], Zygmunt LITWIŃCZUK[1]

[1]Department of Breeding and Protection of Genetic Resources of Cattle, University of Life Sciences, Lublin, Poland
[2]Department of Commodity Science and Processing of Raw Animal Materials, University of Life Sciences, Lublin, Poland

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Abstract: The aim of this study was to evaluate the content of whey proteins and fatty acids profile in milk of cows used in intensive and conventional systems with regard to stage of lactation. For 1846 milk samples the content of total protein, fat and selected whey proteins, and fatty acids profile were analyzed. It was found that stage of lactation had a significant influence on the content of whey proteins in milk of cows from the two production systems. The biggest significant ($P \leq 0.01$) differences were observed in lactoferrin concentration, especially in milk of cows used in the conventional production system. It needs to be emphasized that milk from the conventional system was a richer source of both whey proteins and unsaturated fatty acids, and also conjugated linoleic acid (CLA). Higher concentrations of lactoferrin and polyunsaturated fatty acids, including CLA, in milk obtained from cows kept in the traditional system may foster the use of this raw material as a source of biologically active ingredients in the industry.

Key words: Milk, albumins, lactoferrin, conjugated linoleic acid, total mixed ration, conventional system

1. Introduction

Milk contains many biologically active substances, with protein and fat fractions being the richest sources. The content of total protein in milk equals on average 3.2%, including about 20% of whey proteins. Mainly these are albumins (about 75%), i.e. β-lactoglobulin (β-LG), α-lactalbumin (α-LA), and bovine serum albumin (BSA); and bacteriostatic substances, i.e. immunoglobulin, lactoferrin, lactoperoxidase, and lysozyme. These substances affect the digestive, immune, circulatory, and nervous systems and reduce the risk of many human diseases (1,2). In turn, milk fat contains approximately 500 fatty acids, some of which have bioactive properties (especially very long chain omega-3 fatty acids) (3). Conjugated linoleic acid (CLA) arouses the greatest interest. The amount and the quality of milk produced by cows, including health-promoting ingredients, depend on many factors such as, among others, the size and composition of the feeding dose, breed, season of production, health condition of animals, and stage of lactation (4–7). The great majority of Polish farms that produce milk use the conventional feeding system, which is based mainly on roughages produced on farms, most frequently provided ad libitum, with the use of grasslands in the spring–autumn season (8). In recent years, more and more farms are set up with a focus on intensive milk production. These farms use a single, complete system of feeding (total mixed ration, TMR, or portion mixed ration, PMR) throughout the whole year. Compared with the conventional system, this system requires more financing, but it guarantees the optimal coverage of the nutritional needs of cows, thus maximizing their genetic potential for milk production (9). The intensive production of milk allows for maximizing productivity of cows and concentrations of basic milk components at the cost of minimizing the content of biologically active substances and worsening technological parameters of milk (5,7,10–12). Stage of lactation is the main physiological factor that affects productivity of cows and milk composition. In the course of lactation production of milk reduces while the content of its main components increases.

The aim of this study was to evaluate the content of whey proteins and fatty acids profile in milk obtained from the intensive and conventional systems in subsequent stages of lactation.

2. Materials and methods

The research material consisted of 1846 milk samples, including 992 samples taken from 234 cows maintained on farms using intensive technology of milk production

* Correspondence: jolanta.krol@up.lublin.pl

and 854 samples taken from 191 cows from farms with the conventional production system. Milk samples were collected from cows in lactations I–III successively with the course of lactation, i.e. stage I – up to 120 days, II – from 121 to 200 days, and III – from 201 to 305 days. On the farms using intensive technology the number of cows was on average 70 heads. The average milk yield was 7150 kg. Animals were maintained in free-stall barns. The agricultural land was dominated by arable land, where mainly maize on silage was grown. Throughout the year the cows were fed a mono-diet; on the majority of farms this was in the form of a complete ration of PMR (ad libitum) that included maize silage (36.96% in the summer and 37.63% in the winter), haylage (29.33% in the summer and 31.49% in the winter), and addition of ground grain in the amount of 10%. Industrial concentrated fodder (feed concentrates and complete feed), in the amount of 24.83% in the summer and 27.14% in the winter, was delivered individually in a feed station according to daily milk yield. The energy value (feed unit for lactation, UFL) was averagely 20.1 and protein level (PDI, in grams of protein truly digestible in the small intestine) was 1910 g. However, farms with the conventional production system had a semicommercial character, and the average size of the cow herds was 15 cows with milk yield per lactation of 4200 kg. The structure of the land was dominated by permanent pasture; corn was not grown. Feed from permanent grassland accounted for about 80% of cow diets, i.e. 58.07% from pasture (cows grazing throughout the day with breaks for milking and tending) and 20.35% from hay in the summer and 52.84% from haylage and 27.09% from hay in the winter. The remaining 20% of the ration was ground grain. UFL was 12.0 and PDI was 1200 g.

The energy value, expressed in terms of net energy lactation (UFL), and the protein intake (PDI) were determined based on the INRA feeding system (INRAtion 4.07 Software).

When taking the samples, the TOK test (Eng. CML – California Mastitis Test; with the use of Mastirapid reagent) was performed to eliminate animals with inflammation of the udder. The study was conducted only on milk samples in which the somatic cell count did not exceed 400,000/mL (determined with a Somacount 150 apparatus, Bentley Instruments). The collected samples were divided into three groups with regard to stage of lactation.

Contents of total protein (Infrared Milk Analyzer, Bentley Instruments) and whey proteins, i.e. α-lactalbumin, β-lactoglobulin, bovine serum albumin, lactoferrin, and lysozyme, were determined by RP-HPLC method. In order to evaluate the content of certain whey proteins, all samples were prepared according to Romero et al. (13). Protein separation was performed with the liquid chromatography ProStar 210 model and UV-Vis ProStar 325 detector (Varian, USA). The measurements were carried out using the water/acetonitrile mobile phase with gradient elution and column Nucleosil 300-5 C18 (Varian) of 250 mm in length and 4.6 mm in diameter. The mobile phase was solvent A (90% water, 10% acetonitrile) and solvent B (90% acetonitrile, 10% water), purchased from Sigma (Germany). The solvents were filtered through 0.45-μm filters (Millipore, USA) and degassed using the ultrasounds. Total analysis time of 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. On the grounds of the obtained chromatograms, using the Star 6.2 Chromatography Workstation (Varian), the qualitative and quantitative identification of each substance was performed, followed by concentration determination. 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 injection solutions of the standards (20 μL). Purified proteins, i.e. α-LA (≥85%), β-LG (90%), bovine albumin serum (≥96%), and lactoferrin (90%), all from bovine milk, as well lysozyme (95%) from hen egg white, were purchased from Sigma (Germany). All chemicals were of HPLC analytical grade.

Additionally, in a representative number of milk samples, i.e. 320 (193 from the intensive system and 127 from the conventional system), fatty acid profile was analyzed (14,15). A Varian CG 3900 gas chromatograph with a flame-ionization detector and CP 7420 capillary column (length: 100 m) was used. These analyses included the following groups of fatty acids: SFA – saturated fatty acids, including SFAsmc – short and medium chain saturated fatty acids, including C4:0 to C14:0 acids, and SFAlc – long chain saturated fatty acids, including C15:0 to C18:0 acids; UFA – unsaturated fatty acids, including monounsaturated fatty acids (MUFA – C10:1, C14:1, C15:1, C16:1, C17:1, and C18:1) and polyunsaturated fatty acids (PUFA – C18:2, C18:3, and CLA). Percentage of fatty acid content was calculated using Star GC Workstation Version 5.5 (Varian), based on retention times of fatty acid methyl esters.

The data on daily yield of cows that were subject to the study were taken from the breeding documentation conducted by the Polish Federation of Cattle Breeders and Dairy Farmers.

Statistica ver. 6 (StatSoft Inc.) was used for statistical calculations. The analysis was done on the basis of the general linear model – ANOVA procedure. The significance of differences between the means was assessed with the use of Tukey's test for different counts at the levels of P (alpha) = 0.05 and P = 0.01.

3. Results and discussion

The data from Table 1 show that with each stage of lactation there was an increase in the concentration of total protein, including whey proteins. Greater changes in the content of the analyzed proteins were observed in the case of milk from the conventional production system. The raw material obtained from cows in stage III of lactation had an increase of 24.0% in total protein and an increase of 8.3% in whey proteins as compared with stage I of lactation. In the intensive production system the increase of total protein content amounted to 10.2% (from 3.43% in stage I to 3.78% in stage III) and the increase was only 2.9% (from 0.69% to 0.71%) in the case of whey proteins. A significantly ($P \leq 0.05$) higher concentration of whey proteins was observed in milk produced in the conventional system (0.75% as compared to 0.71%). It contained significantly ($P \leq 0.01$) more β-lactoglobulin (by 0.26 g L^{-1}), lactoferrin (by 14 mg L^{-1}), and lysozyme (by 0.56 μg L^{-1}), while the amount of BSA was higher (by 0.04 g L^{-1} at $P \leq 0.05$) in the case of intensive production. According to Turner et al. (4) and Mackle et al. (16), concentration of whey proteins in milk depends to a great extent on the availability of pasture forage in the feeding dose. Milk of cows that have unlimited access to pasture (ad libitum) is characterized with a greater amount of bioactive substances in the protein fraction. It can be assumed that their concentration is indirectly affected by the content of biologically active substances that are present in grass forages. The analysis of the content of particular whey proteins as regards stages of lactation showed that there was a statistically significant ($P \leq 0.05$) decrease of major albumin concentration, i.e. α-lactalbumin and β-lactoglobulin. The calculated value of these proteins contents between stages III and I of lactation shows comparable changes of concentrations of these albumins during lactation, both in the case of the conventional and the intensive production system. Heck et al. (10) and Caffin et al. (17) confirmed the decline in α-lactalbumin in milk with the course of lactation. According to Caffin et al. (17), this may be due to a decrease in milk production in late lactation, as this protein is a component of the lactose synthase complex. In our own research a decrease of milk

Table 1. Content of total protein and whey proteins in milk of cows with regard to production system of cows and stage of lactation.

Item		Intensive system				Conventional system				Factor influence
		Stage of lactation (days)			Average	Stage of lactation (days)			Average	Production system and stage of lactation
		I (≤120)	II (121–200)	III (201–305)		I (≤120)	II (121–200)	III (201–305)		
Number of samples		392	253	347		312	188	354		
Total protein (%)	mean	3.43^{A}	3.69^{B}	3.78^{C}	3.64**	3.13^{A}	3.35^{B}	3.88^{C}	3.46**	x
	SD	0.43	0.41	0.40	0.44	0.39	0.45	0.50	0.56	
Whey proteins (%)	mean	0.69	0.72	0.71	0.71*	0.72^{a}	0.75^{ab}	0.78^{b}	0.75*	ns
	SD	0.14	0.17	0.09	0.18	0.15	0.18	0.11	0.18	
β-Lactoglobulin (g L^{-1})	mean	3.19^{A}	3.24^{B}	3.20^{AB}	3.21**	3.50^{B}	3.41^{A}	3.42^{A}	3.47**	ns
	SD	0.37	0.36	0.38	0.38	0.41	0.43	0.42	0.43	
α-Lactalbumin (g L^{-1})	mean	1.06^{b}	1.07^{b}	0.98^{a}	1.02*	1.14^{B}	1.08^{A}	1.07^{A}	1.10*	ns
	SD	0.15	0.15	0.14	0.15	0.14	0.16	0.16	0.16	
Bovine serum albumin (g L^{-1})	mean	0.48	0.47	0.47	0.48*	0.44	0.46	0.45	0.45*	ns
	SD	0.13	0.13	0.11	0.13	0.10	0.13	0 .14	0.13	
Lactoferrin (mg L^{-1})	mean	103.47^{A}	110.92^{B}	112.48^{C}	109.05**	112.68^{A}	122.13^{B}	134.28^{C}	123.05**	xx
	SD	14.10	17.61	18.74	18.38	17.78	22.51	22.82	22.62	
Lysozyme (μg L^{-1})	mean	10.26	10.08	10.33	10.24**	10.60	10.76	11.01	10.80**	ns
	SD	0.96	1.03	1.10	1.07	1.01	1.23	1.28	1.22	

a, b: Differences within production system between stages of lactation significant at $P \leq 0.05$; A, B, C: differences within production system significant at $P \leq 0.01$; *: differences between production systems significant at $P \leq 0.05$; **: significant at $P \leq 0.01$.
Factor influence – x: significant at $P \leq 0.05$; xx: significant at $P \leq 0.01$; ns: not significant.

production was observed during lactation from 25.2 kg in stage I to 21.2 kg in stage III in the intensive system and from 17.6 kg to 11.4 kg in the conventional system.

In the case of lactoferrin and lysozyme an increase was noticed in their contents during lactation, and for lactoferrin the changes were statistically significant ($P \leq 0.01$). Larger differences in concentrations of those proteins with the course of lactation were found in milk from the conventional system. In stage III of lactation as compared with stage I, this milk contained more lactoferrin by 21.6 mg L^{-1} (by 19.2%) and lysozyme by 0.41 μg L^{-1} (by 3.9%). In the intensive system this increase was low, accounting for 8.7% for lactoferrin and only 0.7% for lysozyme. The changes described are confirmed in the interactions obtained for proteins between the production system and stage of lactation (Table 1). A significant increase in the concentration of lactoferrin in milk with the course of lactation was also showed by Cheng et al. (18), who obtained a high correlation between content of lactoferrin and stage of lactation ($r = 0.557$). They also found a significant ($P < 0.01$) negative correlation between lactoferrin concentration and daily production of milk as well as significant ($P < 0.018$) interactions between stage of lactation and milk yield. A negative genetic correlation ($r = -0.36$) between milk yield and lactoferrin was obtained by Soyeurt et al. (19). These dependencies are confirmed in our own study. A greater increase of lactoferrin concentration in the course of lactation was observed in the milk of cows maintained in the conventional system, which is characterized by lower productivity of milk. Changes in the content of lactoferrin in the course of lactation were also observed by Król et al. (6) and Hiss et al. (20).

On the basis of the data from Table 2 it can be stated that milk produced in the conventional production system was characterized by a higher proportion of unsaturated fatty acids (UFA, MUFA, and PUFA) in milk fat (at generally lower fat contents). However, generally, significant differences ($P \leq 0.01$) were found

Table 2. Proportion of fat and fatty acids in milk (%) with regard to production system of cows and stage of lactation.

Item		Intensive system				Conventional system				Factor influence
		Stage of lactation (days)			Average	Stage of lactation (days)			Average	Production system and stage of lactation
		I (≤120)	II (121–200)	III (201-305)		I (≤120)	II (121–200)	III (201–305)		
Number of samples		60	68	65		46	40	41		
Fat (%)	mean	4.21^{A}	4.42^{B}	4.69^{C}	4.44**	4.16^{A}	4.31^{B}	4.50^{C}	4.32**	x
	SD	0.59	0.46	0.61	0.56	0.48	0.46	0.61	0.56	
SFA	mean	70.81	71.95	71.27	71.84	69.01	69.59	68.71	68.75	ns
	SD	5.02	3.80	3.09	4.38	3.85	3.31	5.20	4.01	
SFAsmc	mean	23.73	24.76	23.73	24.70	22.27	23.20	22.62	22.05	ns
	SD	4.19	3.48	3.41	3.20	4.99	4.32	5.13	5.17	
SFAlc	mean	47.07	47.18	47.54	47.22	46.74	46.40	46.08	46.71	ns
	SD	4.29	3.93	3.72	4.05	4.97	4.34	5.94	5.90	
UFA	mean	28.58	27.78	28.42	28.11*	30.64^{ab}	30.06^{a}	32.02^{b}	31.18*	x
	SD	4.89	3.73	3.10	3.52	3.85	3.31	3.96	3.67	
MUFA	mean	25.66	24.93	25.49	25.36	27.24^{ab}	26.70^{a}	28.38^{b}	27.45	ns
	SD	4.67	3.36	2.78	3.04	3.47	3.07	3.66	3.86	
PUFA	mean	2.92	2.84	2.92	2.79**	3.40	3.36	3.64	3.57**	ns
	SD	0.60	0.76	0.60	0.63	0.82	0.72	0.85	0.79	
CLA	mean	0.30	0.31	0.29	0.30**	0.59	0.52	0.65	0.60**	ns
	SD	0.08	0.07	0.06	0.05	0.43	0.35	0.45	0.45	

a, b: Differences within production system between stages of lactation significant at $P \leq 0.05$; A, B, C: differences within production system significant at $P \leq 0.01$; *: differences between production systems significant at $P \leq 0.05$; **: significant at $P \leq 0.01$.
Factor influence – x: significant at $P \leq 0.05$; ns: not significant.

only for the content of PUFA, for CLA. The proportion of fatty acids from the PUFA group was higher in the milk produced conventionally, where the diet base was a pasture, as compared to the intensive system. Many authors (11,12,21,22) showed that the share of pasture in animal nutrition positively affects contents in milk. This is probably also related to the specific increase in the amount of C18:3 acid, which is the only one that cannot be synthesized in the mammary gland (23). The analyzed milk taken from farms using the conventional feeding system also contained twice as much CLA (0.52%–0.65%) as compared with milk from the intensive production system (0.29%–0.31%). This low proportion of CLA in milk from the intensive system probably results from the applied feeding system, i.e. TMR. According to Bauman et al. (24), feeds used in the TMR system are characterized by a lower concentration of unsaturated fatty acids, including CLA, as compared with the conventional system (grazing). A higher content of CLA in the milk of cows fed on grass forage results from the increased load of linoleic acid (which is a CLA precursor) into the rumen. Similar results were obtained by Butler et al. (22), who found that an increase in the share of fodder in the ration reinforces its energy density. This is conducive to achieving higher productivity of cows but, simultaneously, also adversely affects the fatty acid composition of the milk, i.e. leads to an increase in the content of SFA and decrease of UFA. Bargo et al. (21) showed that the introduction of green fodders (increasing PUFA in a diet) to the TMR system reduced the content of long chain fatty acids in milk from 48.11 to 45.06 g per 100 g of fatty acids. The data from Table 2 show that in the intensive system stage of lactation did not have any statistically significant influence on the proportion of particular groups of fatty acids in milk. In the conventional system, though, the influence of stage of lactation was observed as regards the proportions of acids from the UFA and MUFA groups. Similarly to Barlowska et al. (25), in own study the highest content of CLA in milk was observed in the late stage of lactation. The interaction between production system and stage of lactation turned out to be statistically significant ($P \leq 0.05$) only in the case of total fat content and UFA share (Table 2).

In conclusion, it can be stated that stage of lactation had a significant influence on the content of whey proteins, both in the intensive and conventional production systems. The greatest changes involved lactoferrin, the concentration of which significantly increased with the course of lactation, especially in the milk of cows maintained in the conventional system. Along with the course of lactation in both production systems there was a decrease of the content of main albumins, i.e. α-lactalbumin and β-lactoglobulin, in milk. Assessing fatty acids profile, stage of lactation had the sole influence on the proportion of acids from the UFA and MUFA groups in milk obtained in the conventional system. It needs to be emphasized that the milk from the conventional production system was a richer source of both whey proteins and unsaturated fatty acids, including CLA. Higher concentrations of lactoferrin and polyunsaturated fatty acids, including CLA, in milk obtained from cows kept in the traditional system may foster the use of this raw material as a source of biologically active ingredients in the industry.

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