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Effect of the feeding system and the production season on the protein fraction content in milk

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Abstract

The objective of the present research was to analyse the protein fraction content in milk, with particular regard to whey proteins, in dependence on the cows' feeding system (group 1 - extensive, 2 - semi-intensive, 3 - intensive) and production season (spring-summer and autumn-winter). Chemical analysis of the fodder was the base for calculation of energy and protein coverage of nutritional dose. A total of 1,133 milk samples were evaluated (550 in winter and 583 in summer). The milk samples were examined for the somatic cell count (SCC), the basic chemical composition, casein and whey proteins: α-lactalbumin, β-lactoglobulin, bovine serum albumin (BSA), lactoferrin and lysozyme. Higher content of crude protein, including casein, was noticed in milk obtained from cows coming from semi-intensive and intensive farms. However, milk taken from cows fed according to the group 1, which was based on fodder from permanent grasslands, had the highest concentration of major whey proteins - both in the summer (pasture and hay) and winter (hay and haylage) seasons. With the increase of silage and industrial fodder in the feed ration, the content of whey proteins - mainly β-lactoglobulin and lactoferrin - decreased, which was confirmed by the obtained negative correlation coefficients.

Key words: **whey proteins, extensive system, intensive system, correlations**

Introduction

Quantity and quality of milk produced by cows, including the content of various fractions of proteins, depends on many factors, including the size and the composition of the feed ration, breed, season of production, animal health or stage of lactation (Król et al., 2017; Litwińczuk et al., 2011; Nahar et al., 2007; Turner et al., 2007). In Poland the great majority of dairy farms use a traditional system of feeding which is based on pasture forage. In comparison to total ration systems (TMR - Total Mixed Ration and PMR - Partially Mixed Ration) this system requires less financial expenditures but does not result in high productivity of cows. Numerous studies point to higher concentration of biologically active substances in the fat fraction of milk coming from cows fed in the traditional way, i.e. using the pasture (Carroll et al., 2006; Morales-Almaráz et al., 2011; Vranjes et al., 2010). According to Loor et al. (2003), the introduction of an 8-hour grazing daily at TMR diet significantly improves the health-promoting properties of milk. Barłowska and Litwińczuk (2006) indicated that milk obtained from the grazing cows was also characterised by better technological parameters (shorter time of curd formation and higher content of big-sized fat molecules (>10 μm)), especially for a cheese production. However, the effect of feeding system on the content of certain compounds in the protein fraction of milk is not well elucidated and described as in case of the fat fraction. The content of the total proteins in milk equals on average 3.2 %, including approximately 20 % of whey proteins. These are mainly albumins (approximately 75 %), i.e. β-lactoglobulin (β-LG), α-lactalbumin (α-LA) and bovine serum albumin (BSA). Whey proteins also include bacteriostatic substances, i.e. immunoglobulins, lactoferrin, lactoperoxidase and lysozyme. Whey proteins exhibit numerous effects on the human body, such as anti-inflammatory, bacteriostatic and antioxidant actions, which indicate their ability to prevent the occurrence of civilizational diseases like atherosclerosis, diabetes, obesity, cancer or Alzheimer's disease (Bhat and Bhat, 2011; Król et al., 2011). They are also a source of essential amino acids and are increasingly used to enrich baby foods, dietary medications or protein specimens for convalescents and athletes. Whey proteins also found their application in pharmacology and cosmetology (Graf et al., 2011; Sukkar and Iorio, 2011).

The aim of the work was to analyse the protein fraction content in milk, with particular regard to whey proteins, in dependence on the cows' feeding system and the production season..

Materials and methods

Animals and feeding system

The study involved 25 farms from the area of Eastern Poland keeping Polish Holstein-Friesian, Whitebacked, Polish Black and White and Polish Red and White cows. Farms selected for the study were divided into three groups according to the feeding system which was used (Table 1), i.e. group 1 with an extensive system - 8 farms; 2 (a semi-intensive system) - 8 farms and 3 (an intensive system) - 9 farms. On the farms from groups 1 and 2 all breeds of cows were kept. However, in group 3 Polish Holstein-Friesian and crossbreeds of Polish Holstein-Friesian with analysed breeds were maintained.

Farms belonging to group 1 had a semi commercial character, and the average size of cow herd was 15 ± 5 cows. The land structure was dominated by permanent pasture; maize was not grown. Feed from the permanent grassland accounted for about 80 % of the cow diet - i.e. 58.1 % from pasture (cows grazing throughout the day with breaks for milking and tending) and 20.4 % from hay in the summer. However, this ration for the winter season was 52.8 % from haylage and 27.1 % from hay. The remaining 20 % of the ration was ground grain. The mean milk production per lactation and a herd was 4,300 kg.

The semi-intensive milk production system characterised farms classified into group 2. The average number of cows in the herd was 28±10 heads, and permanent grassland in the structure of land accounted for a half of the area. A whole day (from morning to evening milking) pasturing was used. The share of the fodder from pasture was 52 % (pasture - 31.1 %, hay - 2.3 % and haylage - 18.6 %) in the summer season but in the winter only 38 % (hay - 7.0 % and silage - 31.2 %). The share of the maize silage was 22.2 % in the summer and in the winter it was the primary fodder in ration (37.2 %). Concentrated feed, as in group 1, covered about 20 % of nutritional requirements of cows but with an addition of industrial fodder (14.2 % in the summer and 7.8 % in the winter). Generally, they were complete feed and extracted meals. The mean milk production per lactation for herd was 6,100 kg.

Farms in group 3 had a typical intensive type. The number of cows was on average 70±15 heads. Structure of 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, i.e. in the majority of farms in a form of complete ration of PMR (*ad libitum*) which included: maize silage (37.0 % in the summer and 37.6 % in the winter), haylage (29.3 % in the summer and 31.5 % 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 24.8 % in the summer and 27.1 % in the winter, were delivered individually in a feed station according to daily milk yield. The mean milk production per lactation for herd was 8,000 kg.

TABLE 1. Mean (± SD) composition, energy and protein coverage of nutritional dose in dependence on feeding system and production season

Type of fodder		Group of farms								
		1 n=8			2 n=8			3 n=9		
		Energy	Protein	Coverage of dose	Energy	Protein	Coverage of dose	Energy	Protein	Coverage of dose
		UFL	PDI (g)	%	UFL	PDI (g)	%	UFL	PDI (g)	%
Summer season	Pasture	6.9	701	58.1	418	437	31.1	-	-	-
		0.3	165	5.1	1.1	127	9.1	-	-	-
	Maize silage	-	-	-	3.6	250	22.2	7.1	487	37.0
		-	-	-	1.4	91	8.0	1.2	91	2.7
	Hay	2.5	268	20.4	0.3	34	2.3	-	-	-
		1.0	123	7.4	0.5	55	4.2	-	-	-
	Haylage	-	-	-	21.3	306.3	18.6	40.1	621	29.3
		-	-	-	9.2	126.3	6.9	9.9	82	4.4
	Filling farmed	2.6	229	21.6	1.7	139	11.6	1.6	129	8.9
		1.7	53	4.6	1.5	124	10.1	1.0	86	6.5
	Filling industrial	-	-	-	2.4	333	14.2	4.8	651	24.8
		-	-	-	1.7	212	8.6	1.4	175	7.0
	Total	12.0	1198.8	100	15.8	1501.9	100	19.1	1889.6	100
		1.1	271.6		2.3	287.6		2.7	185.2	
	% of requirement coverage	98.5	121.5		99.1	105.7		106.1	105.9	
		5.2	12.7		4.0	6.9		6.9	3.3	
Winter season	Maize silage	-	-	-	4.9	360	37.2	7.6	513	37.6
		-	-	-	0.8	68	2.6	0.6	48	2.2
	Hay	3.0	321	27.1	0.8	86	7.0	-	-	-
		0.9	118	9.3	0.6	66	5.5	-	-	-
	Haylage	5.9	630	52.8	4.1	458	31.2	6.3	640	31.5
		4.1	169	12.1	0.6	94	4.6	0.9	67	3.7
	Filling farmed	2.3	201	20.1	2.2	182	16.7	0.7	60	3.7
		0.6	58	4.3	0.6	48	3.2	1.0	80	5.0
	Filling industrial	-	-	-	1.1	249	7.8	5.5	708	27.1
		-	-	-	0.6	131	4.1	0.9	98	4.2
	Total	11.2	1154.0	100	13.1	1338.3	100	20.1	1923.5	100
		1.4	212.8		1.7	249.5		1.2	112.1	
	% of requirement coverage	89.7	111.5		98.7	101.6		106.6	103.3	
		2.5	5.9		8.1	7.2		2.9	3.8	

Group of farms according to feeding system: 1 - an extensive system, 2 - a semi-intensive system, 3 - an intensive system
UFL - feed unit for lactation
PDI - protein digested in the small intestine

TABLE 2. Mean (± SD) content and daily yield of basic milk components in the summer and the winter seasons

Specification			No. of samples		Milk yield		Fat		Crude protein		Casein		Lactose		Dry matter	
			S	W	S	W	S	W	S	W	S	W	S	W	S	W
Group of farms	1	Content (%)	86	84	-	-	3.95^{A}	4.04	3.28^{A}	3.34	2.45^{A}	2.43^{a}	4.69^{AB}	4.75	12.54^{A}	12.78^{a}
					-	-	0.60	0.49	0.31	0.36	0.33	0.36	0.27	0.25	0.80	0.73
		Yield (kg)			16.05^{A}	14.38^{A}	0.66^{A}	0.58^{A}	0.57^{A}	0.48^{A}	0.42^{A}	0.35^{A}	0.82^{A}	0.70^{A}	2.17^{A}	1.85^{A}
					4.35	5.74	0.23	0.25	0.19	0.19	0.15	0.15	0.26	0.29	0.70	0.76
	2	Content (%)	235	220	-	-	3.98^{A**}	4.27**	3.28^{A**}	3.47**	2.51^{AB}	2.50^{a}	4.61^{A*}	4.76*	12.59^{A**}	13.15^{b**}
					-	-	0.62	0.56	0.39	0.36	0.35	0.41	0.27	0.32	0.86	0.81
		Yield (kg)			21.11^{B**}	17.31^{B**}	0.93^{B**}	0.74^{B**}	0.77^{B**}	0.60^{B**}	0.58^{B*}	0.43^{B*}	1.07^{B*}	0.85^{B*}	2.93^{B**}	2.31^{B**}
					6.20	6.99	0.34	0.31	0.24	0.24	0.19	0.18	0.34	0.36	0.94	0.94
	3	Content (%)	229	279	-	-	4.53^{B**}	4.28**	3.56^{B}	3.51	2.66^{B}	2.62^{b}	4.77^{B}	4.74	13.52^{B**}	13.18^{b**}
					-	-	0.59	0.60	0.41	0.46	0.42	0.40	0.24	0.32	0.90	0.99
		Yield (kg)			27.33^{C}	28.25^{C}	1.16^{C}	1.24^{C}	0.90^{C**}	1.02^{C**}	0.67^{C**}	0.76^{C**}	1.21^{C*}	1.37^{C*}	3.44^{C**}	3.82^{C**}
					6.82	7.15	0.39	0.30	0.29	0.24	0.24	0.18	0.38	0.35	1.09	0.87
Interaction					P-value											
Production season x feeding system					0.001		0.001		0.001		0.008		0.001		0.001	

Group of farms according to feeding system: 1 - an extensive system, 2 - a semi-intensive system, 3 - an intensive system
S - summer season, W - winter season
a, b, A, B - differences between feeding groups within season
a, b - differences significant at P <0.05; A, B - differences significant at P<0.01
*, ** - differences between seasons within feeding group
* - differences significant at P <0.01; ** – differences significant at P<0.001

Sampling and analyses

In each farm the samples of all farm fodder, i.e. green forage, silage, hay and ground grain, were taken for analyses simultaneously with the milk samples. Results of chemical analyses of the fodder were the basis to calculate the mean composition, energy and protein coverage of the nutritional dose in the INRA feeding system (INRAtion 4.07 Software). Individual theoretical ration was calculated for each cow in the INRA system in dependence on daily yield on the day of milk sampling. A total of 1,133 rations were created, in that 550 during the summer period of feeding and 583 during the winter feeding. The energy value was expressed in terms of net energy lactation (UFL - feed unit for lactation). Protein intake was given in grams of protein truly digestible in the small intestine (PDI).

All of the farms were included in the Milk Recording runs by the Polish Federation of Cattle Breeders and Dairy Farmers.

Milk samples were collected in the years 2014-2016 on each farm individually from healthy cows between 30th and 240th day of lactation, twice a year, i.e. in the summer (June-July) and winter (December-January) production seasons. Only milk samples with the somatic cell count (SCC) below 400,000 cells/mL (using a flow cytometry technology - Somacount 150 apparatus, Bentley Instruments, USA) were included into the study. The average SCC in milk obtained from different groups according to the feeding system amounted as follows: 1 - 306,000, 2 - 288,000 and 3 - 319,000 cells / mL.

In total 1,133 samples (in 550 samples from the summer and 583 from the winter) were analysed for the following parameters:

/ the basic chemical composition, i.e. content of fat, protein, lactose and dry matter - Infrared Milk Analyzer (Bentley Instruments, USA);
/ the casein percentage - according to the AOAC (2000; method 998.06);
/ the concentration of whey proteins, i.e. α-lactalbumin (α-LA), β-lactoglobulin (β-LG), bovine serum albumin (BSA), lactoferrin and lysozyme, in skimmed milk - using reverse-phase high-performance liquid chromatography (RP-HPLC). The procedure of whey protein separation was based on the method elaborated by Romero et al. (1996) with own modifications (Brodziak et al., 2012). Protein analysis was performed using 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 at gradient elution and column NUCLEOSIL 300-5 C18 (Varian, USA) of 250 mm length and 4.6 mm diameter. 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. On the grounds of the obtained chromatograms, using program Star 6.2 Chromatography Workstation (Varian, USA), the qualitative and quantitative identification of each substance was performed.

Calibration of the chromatographic system for whey proteins determination was carried out by the external standard method. For this purpose, each protein was calibrated individually by injecting 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 whites, which were purchased from Sigma (Germany) were used as standards. All chemicals were of HPLC analytical grade.

Statistical analyses

The results obtained were analysed statistically using the General Linear Model (GLM) - factorial ANOVA procedures of Statsoft Inc. Statistica ver. 8 (Statsoft Inc. 2013), on the ground of one- and two-way analysis of variance with interaction. Means and standard deviations were given for individual analysed traits. The significance of differences between means was estimated by Duncan's test (for farms) and HSD Tukey's test (for milk samples) at $P \leq 0.05$ level. The statistical analysis included the effect of feeding system and production season, using the following linear model:

$$Y_{ij} = \mu + a_i + b_j + (a_i \times b_j) + e_{ij},$$

where: Y_{ij} - dependent variable, μ - effect of total mean, a_i - effect of feeding system (i = 1..3), b_j - effect of production season (j = 1, 2), $(a_i \times b_j)$ - effect of interaction of two factors, e_{ij} - random error.

To establish the relationships between experimental variables, i.e. share of energy from each fodder in the feed ration and the analysed milk components including proteins, the Spearman's rank correlation coefficients were calculated. A value of $P < 0.05$ was considered as significant.

Results and discussion

According to many authors, a composition of feeding ration is a crucial factor influencing changes in the chemical composition of milk (Carroll et al., 2006; Delaby et al., 2009; Król et al., 2017; Morales-Almaráz et al., 2011; Nahar et al., 2007), but also a breed plays an important role (Brodziak et al., 2012; Król et al., 2017; Křížová et al., 2013). In every season, milk obtained from cows belonging to the farms from group 1 (Table 2) was found as the poorest source of nutrients. Therefore, it can be assumed that in this group the model did not fully cover the nutritional requirements of cows for nutrients, especially for energy. However, the animals fed the rations with maize silage and concentrated feed (group 2 and 3) produced milk with higher content of basic chemical components. Milk of cows in group 3, with regard to milk of cows in group 1, contained significantly more dry matter - from 0.40 to 0.98 % (P<0.05 and P<0.01), including fat - from 0.24 to 0.58 % (P<0.01) and crude protein - from 0.17 to 0.28 % (P<0.01), depending on the season of production. These relationships were confirmed by the higher values of positive correlation coefficients between the content of individual milk components and the share of silage and concentrated feed in the ration (Table 4). Use of the complete ration in feeding of animal from group 3 in both seasons resulted in an increase in daily milk yield as well as yield of individual milk components (Table 2). Significantly lower (P<0.01) yield achieved cows in groups 1 and 2. In comparison to group 1, even a twice higher yield of milk and its basic components were noted in group 3 in the winter season. Effect of use of more intensive feeding system (i.e. a more balanced ration) on increase of milk yield and its nutritional value was confirmed by studies of other authors (Morales-Almaráz et al., 2011; Vranjes et al., 2010; Zamani et al., 2011).

TABLE 4. Correlation coefficients (r) between share of energy in feeding dose from chosen fodder (UFL) and content of analysed milk components

	Milk components	Share of energy from the fodder					
		Pasture	Maize silage	Hay	Haylage	Filling farmed	Filling industrial
Summer season	Fat	-0.04	0.41*	-0.05	0.37*	-0.17*	0.52*
	Crude protein	-0.15*	0.19*	-0.12*	0.13*	-0.02	0.39*
	Casein	-0.03	0.13*	0.02	0.10*	-0.16*	0.31*
	Protein to fat ratio	0.04	-0.33*	0.06	-0.34*	0.19*	-0.31*
	Lactose	-0.05	0.32*	0.17*	0.31*	-0.02	0.23*
	Dry matter	-0.00	0.42*	-0.04	0.37*	-0.13*	0.55*
	α-LA	0.13*	-0.32*	0.01	-0.27	0.07	-0.25*
	β-LG	0.19*	-0.55*	0.05	-0.49*	0.23*	-0.43*
	BSA	-0.18*	0.10*	-0.19*	0.01	-0.15*	0.13*
	Lactoferrin	0.21*	-0.72*	0.10	-0.60*	0.31*	-0.50*
	Lysozyme	-0.06	-0.33*	0.05	-0.26*	0.06	-0.26*
Winter season	Fat		0.22*	-0.11	-0.15*	0.10*	0.13*
	Crude protein		0.02	-0.12	-0.08	0.09	0.18*
	Casein		0.07	-0.11*	0.12	-0.01	0.17*
	Protein to fat ratio		0.04	-0.02	0.05	0.01	0.05
	Lactose		-0.03	0.08	-0.01	0.15*	0.01
	Dry matter		0.07	-0.10	-0.14*	0.14	0.12*
	α-LA		-0.13*	0.17*	-0.09*	0.05	-0.05
	β-LG		-0.32*	0.01	-0.20*	-0.10	-0.28*
	BSA		0.17*	-0.07	0.15*	0.03	0.23*
	Lactoferrin		-0.39*	0.25*	-0.22*	0.16*	-0.52*
	Lysozyme		-0.34*	-0.13	-0.36*	0.05	-0.28*

α-LA - α-lactalbumin, β-LG - β-lactoglobulin, BSA - bovine serum albumin
* - differences significant at P <0.05

Kruczyńska (2011) stated that maize silage was fundamental to bacterial protein synthesis in the rumen and has a positive impact on the quantity of milk. Nahar et al. (2007), however, found that feeding cows with green fodder and feed concentrates increased the dry matter content in milk, including proteins and fat. A crucial component of milk, due to technological usefulness, is casein. According to Mackle et al. (1999), its concentration in milk might be affected by diet of cows, even in 10 %. Application of complete doses and feed concentrates containing protein and protected amino acids (group 3) increased the share of casein in milk in comparison to milk of cows from group 2 (by 5 % in the winter - $P<0.05$ and 6 % in the summer - $P<0.01$) and group 1 (by 8 % in the winter - $P<0.05$ and 9 % in the summer - $P<0.01$). Confirmation of these results were outcomes of some other authors (Brodziak et al. 2018; Cermanová et al., 2011; Król et al., 2008), who reported lower content of casein in milk of grazing cows.

TABLE 3. Mean (± SD) content of chosen whey proteins in analysed milk of cows in the summer and winter seasons

Specification			α-LA		β-LG		BSA		Lactoferrin		Lysozyme	
			S	W	S	W	S	W	S	W	S	W
Group of farms	1	Content (g/L)	1.08^{b}	1.00	3.54^{C**}	3.26^{B**}	0.36^{A**}	0.46^{**}	0.19^{C**}	0.12^{B**}	$7.64\ 10^{-6\ AB**}$	$12.39\ 10^{-6\ B**}$
			0.14	0.13	0.42	0.38	0.08	0.14	0.05	0.01	$3.09\ 10^{-6}$	$8.10\ 10^{-6}$
		Yield (g)	18.35^{A*}	14.39^{A*}	61.92^{A**}	46.62^{A**}	6.09^{A}	7.02^{A}	3.32^{B**}	1.76^{A**}	$123.03\ 10^{-6\ A*}$	$176.46\ 10^{-6\ a*}$
			5.20	6.44	22.29	20.69	3.10	3.80	1.38	0.80	$65.00\ 10^{-6}$	$97.0\ 10^{-6}$
	2	Content (g/L)	1.06^{b*}	0.99 *	3.34^{B**}	3.14^{AB**}	0.42^{B*}	0.48^{*}	0.15^{B**}	0.11^{B**}	$8.65\ 10^{-6\ B*}$	$10.15\ 10^{-6\ AB*}$
			0.51	0.15	0.52	0.43	0.22	0.13	0.04	0.02	$3.65\ 10^{-6}$	$8.01\ 10^{-6}$
		Yield (g)	22.95^{B**}	16.95^{AB**}	76.00^{B**}	55.27^{B**}	10.64^{B}	8.62^{A}	3.36^{B**}	1.88^{A**}	$188.12\ 10^{-6\ C}$	$179.59\ 10^{-6\ a}$
			6.40	7.56	22.54	21.74	7.71	4.33	1.37	0.86	$84.93\ 10^{-6}$	$164.19\ 10^{-6}$
	3	Content (g/L)	0.96^{a}	0.99	2.86^{A**}	3.04^{A**}	0.45^{B}	0.43	0.09^{A}	0.08^{A}	$6.31\ 10^{-6\ A}$	$7.54\ 10^{-6\ A}$
			0.13	0.12	0.33	0.25	0.17	0.09	0.02	0.01	$3.46\ 10^{-6}$	$2.41\ 10^{-6}$
		Yield (g)	26.63^{C**}	27.96^{B**}	70.15^{AB**}	84.99^{C**}	11.73^{B}	12.88^{B}	2.28^{A**}	2.35^{B**}	$169.84\ 10^{-6\ B**}$	$215.65\ 10^{-6\ b**}$
			8.44	7.48	22.36	22.13	5.34	5.05	0.82	0.96	$92.07\ 10^{-6}$	$89.07\ 10^{-6}$
Interaction												P-value
Production season x feeding system				0.007		0.001		0.001		0.001		0.001

Group of farms according to feeding system: 1 - an extensive system, 2 - a semi-intensive system, 3 - an intensive system
α-LA - α-lactalbumin, β-LG - β-lactoglobulin, BSA - bovine serum albumin
S - summer season, W - winter season
a, b, A, B - differences between feeding groups within season
a, b - differences significant at $P<0.05$; A, B - differences significant at $P<0.01$
*, ** - differences between seasons within feeding group;
* - differences significant at $P<0.01$;
** - differences significant at $P<0.001$

The results presented in Table 3 indicate significant correlations between the content of the whey proteins in milk and the feeding systems of cows used. Both in the summer and winter seasons, the milk obtained from cows fed according to the model I (group 1) was characterised by the highest concentration of major whey proteins, i.e. α-LA, β-LG and lactoferrin. With the increase of silage and industrial fodder share in the ration, the content of β-LG and lactoferrin significantly ($P<0.01$) decreased. In the winter, the content of β-LG in milk of cows from group 2 decreased by 0.12 g/L, in comparison to group 1, and in group 3 - by 0.22 g/L, while for lactoferrin - 0.01 and 0.04 g/L, respectively. Greater statistically significant ($P<0.01$) differences between these two groups were observed in the summer. In group 2, the amount of β-LG was reduced by 0.20 g/L (by 6 % with regard to group 1), while in group 3 even by 0.68 g/L (by 19 %). Lactoferrin concentration decreased respectively by 0.04 g/L (less by 27 % in comparison to group 1) and 0.10 g/L in group 3 (by 53 %). These relationships were confirmed by the obtained negative correlation coefficients (Table 4). The highest values of these coefficients were stated in the summer season between lactoferrin concentration and the share of maize silage ($r=-0.72$ at $P<0.05$), haylage ($r=-0.60$ at $P<0.05$), as well industrial fodder ($r=-0.50$ at $P<0.05$). In the winter season, the obtained values were lower ($P<0.05$), i.e. $r=-0.39$, $r=-0.22$ and $r=-0.52$, respectively. For β-LG the correlations were respectively: the summer ($r = -0.55$; $r=-0.49$ and $r=-0.43$, at $P<0.05$) and the winter ($r=-0.32$; $r=-0.20$ and $r=-0.28$, at $P<0.05$). With a greater percentage of the pasture in feed ration, the contents of β-LG and lactoferrin increased in milk, which was confirmed by the obtained correlation coefficients (β-LG - $r=0.19$ and lactoferrin - $r=0.21$, at $P<0.05$) - Table 4. The higher level of functional whey proteins in milk of grazing cows was also shown by Król et al. (2008) and Mackle et al. (1999). According to them, a higher share of whey proteins in grazing cows' milk results from the higher content of bioactive components in fresh forage. Carroll et al. (2006) stated that not limited (*ad libitum*) access to the green feed (pasture) causes the increase in valuable milk components i.e. in the not saturated fatty acids and the share of the total solids. However, Turner et al. (2007) found that unrestricted access to pasture (ad libitum) did not lead to an increase in concentration of lactoferrin in milk. During the winter feeding period, the hay was the feed that had the greatest influence on lactoferrin content ($r=0.25$ at $P<0.05$). In the summer, hay supplementation led, to a lesser extent, to this protein increase in milk ($r=0.10$). Thus, it can be assumed that the decisive influence on the content of lactoferrin in milk analysed was noticed for a share of roughages derived from permanent grasslands, i.e. from pasture in the summer and hay in the winter period. The seasonal differences in the content of α-LA, β-LG and lactoferrin in milk were also obsereved. The highest level of these proteins (with regard to the feeding system, excluding group 3) was found in the summer, which corresponded well to the results of other studies (Wedholm et al., 2006; Litwińczuk et al., 2011; Brodziak et al., 2018). A larger share of industrial fodder in ration negatively affected the content of lysozyme in milk ($r=-0.26$ - summer; $r=-0.28$ - winter). Regardless of the production season, the least amount of lysozyme was obtained in milk of cows fed a total ration (group 3). There were no differences in the BSA content between the feeding systems in the winter. Its concentration was at the level of 0.43-0.48 g/L. Significant (at $P<0.05$) differences were noticed, however, in the summer. Milk of grazing cows (group 1) was characterised by the smallest concentration of BSA, which was also confirmed by the negative correlation coefficient obtained ($r=-0.18$ at $P<0.05$). Moreover, the share of industrial fodder and silage in the ration affected the growth of this protein fraction in milk. Similar tendencies were reported by Brodziak et al. (2012; 2018) and Mackle et al. (1999). Mackle et al. (1999), who showed that energy absorbed in the ration might affect the concentration of whey proteins in milk of cows, also including the serum proteins. They also noted that the content of major whey proteins, i.e. α-LA and β-LG, decreased while the level of BSA increased when the animals had no access to pasture. However, Křížová et al. (2013) obtained statistically higher content of total whey proteins in milk of cows from non-grazed herds (0.51 %) with regard to grazed animals (0.47 %).

With respect to the daily yield of individual whey proteins (Table 3), the cows in group 3 were generally characterised by the highest yield of whey proteins, although the lowest content of these components were found in their milk, which was most probably related to the greatest quantity of milk produced by the animals in this group. It should be noted that higher yields of β-LG, lactoferrin and lysozyme were found in group 2 in the summer season.

Conclusion

The significant effect of feeding system of cows on chemical composition of milk, including the whey protein content, was shown. With the increase of silage and industrial fodder share in the ration, the percentage of basic milk components increased. However, the content of β-LG and lactoferrin decreased, which was confirmed by the obtained negative correlation coefficients. Regardless of the production season, the highest concentration of the major whey proteins characterised the milk taken from cows belonging to group 1, which was based on the fodder from permanent grasslands. Therefore, it could be assumed that a decisive influence on the content of whey proteins in milk was noticed for the share of roughages derived from permanent grasslands, i.e. from pasture in the summer and hay in the winter period.

Utjecaj sustava hranidbe i sezone laktacije na udjel proteina u mlijeku

Sažetak

Cilj ovog istraživanja bio je analizirati sadržaj proteinske frakcije mlijeka, s posebnim naglaskom na protein sirutke, ovisno o sustavu hranidbe krava (grupa 1 - ekstenzivni, 2 - djelomično intenzivni, 3 - intenzivni) kao i sezoni laktacije (proljeće-ljeto i jesen-zima). Kemijske analize krmiva bile su temelj za izračun dostatnosti energije i proteina u dnevnom unosu nutrijenata. Ukupno je ispitano 1133 uzorka mlijeka (550 zimi i 583 ljeti), a određivani su ukupan broj somatskih stanica (SCC), osnovni kemijski sastav, te udjeli kazeina i proteina sirutke - α-laktalbumin, β-laktoglobulin, albumin krvnog seruma (BSA), laktoferin i lizozim. Viši udio ukupnih proteina, uključujući kazein, utvrđen je u mlijeku krava s farmi s djelomično intenzivnim i intenzivnim sustavom hranidbe. Međutim, neovisno o sezoni laktacije, najveće koncentracije proteina sirutke utvrđene su u mlijeku krava svrstanih u grupu 1, čija se hranidba temeljila na kontinuiranoj ispaši (ljeti - ispaša i sijeno; zimi - sijeno i silaža). Porastom udjela silaže i industrijskih krmiva u hranidbi, udjel proteina sirutke (prije svega β-laktoglobulina i laktoferina) je padao, što je potvrđeno i dobivenim negativnim koeficijentima korelacije.

Ključne riječi: **protein sirutke, ekstenzivni sustav, intenzivni sustav, korelacije**

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