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BIOACTIVE PROTEIN CONTENT IN MILK FROM LOCAL BREEDS OF COWS INCLUDED IN THE GENETIC RESOURCES CONSERVATION PROGRAMME

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Abstract

The objective of the present study was to evaluate the protein fraction of milk from four local breeds of cows in terms of the level of biologically active substances and production season. The research included a total of 640 milk samples obtained from four local cow breeds, i.e. Polish Red (204), Whitebacked (198), Polish Black-and-White (156) and Polish Red-and-White (82). The cows of these breeds are kept predominantly in south-eastern Poland. The study was performed in two production seasons associated with different feeding regimes, i.e. summer and winter. Each milk sample was analysed for the content of total protein, casein and some whey proteins, i.e. alpha-lactoglobulin, beta-lactoglobulin, lactoferrin and serum albumin. Compared to Holstein-Friesian cows raised under intensive conditions, the milk from local cow breeds was found to be a richer source of functional whey proteins, especially in the summer season when animals graze on pasture. Due to increasing consumer interest in health-promoting foods, this milk should be used by local dairies to manufacture special dairy products (rich in biologically active substances), concentrates and whey protein preparations.

Key words: whey proteins, cow's milk, local breeds

Modern consumers look for products whose ingredients have a positive effect on the human organism, thus promoting and improving health or reducing the risk of lifestyle diseases. Milk and milk products are known as a unique source of high-quality components with beneficial effects on health. Once absorbed, casein peptides have potential to exert numerous biological effects in the human organism, i.e. they may play a crucial role in the transport and absorption of certain minerals, bind toxins, possess immunomodulatory effects, and behave as opioid antagonists or opioid receptor agonists (Phelan et al., 2009). Due to their biological activity, a serious concern has also been raised regarding whey proteins that exhibit a diverse array of nonspecific, i.e. antiinflammatory, bacteriostatic, antioxidant, opioid and anticancer

properties (Chatterton et al., 2006; Król et al., 2008; Pan et al., 2006). In Poland, most milk is obtained from Polish Black-and-White Holstein-Friesian cows raised under intensive conditions. In some regions of Poland (mountain, submontane and water-logged areas), the topography does not favour intensive or dairy farming. In these regions, cows of local breeds such as Polish Red, Whitebacked, Polish Black-and-White and Polish Red-and-White, compete with high-producing and more efficient breeds. Polish local breeds of cows represent the dual-purpose type. They are characterized by a similar milk yield, from 3913 kg for Polish Red to 4735 kg for Polish Red-and-White (PFCBDF, 2010). Local breeds of cows adapt very well to harsh environmental conditions. Interestingly, these cows are raised in the regions of high nature value that have a high proportion of grasslands. Due to scarce number and genetic distinctiveness of these breeds, the animals are included in the genetic resources conservation programme (Litwińczuk et al., 2006). Taking into consideration the current trends, extensive studies on milk obtained from the local breeds of cows have recently been undertaken.

The objective of the present study was to evaluate the protein fraction of milk from four local breeds of cows in terms of the level of biologically active substances and production season.

Material and methods

Material

The research included a total of 640 milk samples obtained from four local breeds of cows, i.e. Polish Red (204), Whitebacked (198), Polish Black-and-White (156) and Polish Red-and-White (82). The cows of these breeds are kept predominantly in south-eastern Poland. Milk samples from Polish Red and Polish Red-and-White cows were taken at the farms situated in the Gorlice district (the Beskid Średni), and those from Whitebacked and Polish Black-and-White cows at the farms in the Bug and Biebrza River catchment area (the Lubelskie and Podlaskie provinces). These small-sized farms housed all the breeds under study in conventional tie-stall barns. The study was performed in two production seasons associated with different feeding regimes, i.e. summer (June-July) and winter (December-January). The summer feeding system was based on green fodder comprising grasses and legumes supplemented by hay or straw, while winter feeding was based on haylage with fodder beet added to the diet of Whitebacked and Polish Black-and-White cows. In all the farms, feed ration was supplemented with feed concentrate. The control group was constituted by Polish Black-and-White Holstein-Friesian cows (244 samples) maintained in intensive milk production farms. Irrespective of the season, the cows were fed using a TMR system (maize silage, haylage, feed concentrate). Cows were in lactations 1 to 4. Milk samples were collected between 100 and 200 days of lactation.

Chemical analysis

All the milk samples were transported to the laboratory of the Department of Commodity Science and Processing of Animal Raw Materials at the University of

Life Sciences in Lublin, Poland. Each milk sample was analysed for total protein content using an Infrared Milk Analyser (Bentley Instruments). Casein content was determined according to the AOAC Official Method 927.03 (2000).

To evaluate the content of some whey proteins, i.e. alpha-lactalbumin (alpha-LA), beta-lactoglobulin (beta-LG), lactoferrin and bovine serum albumin (BSA), whey samples from cow's milk were prepared according to Romero et al. (1996) as follows: 50 ml of raw milk was adjusted to pH 4.6 with 0.1 mol·L^{-1} HCl and allowed to stand at room temperature for about one hour for acid precipitation of caseins. Consequently, whey (7 ml) was taken from each of the samples separately and then centrifuged at 10000 rpm for 15 min. Finally, whey solutions were filtered through quality filters and then through 0.20-µm disposable sterile filters (Millipore, type GSTF). The supernatants in vials were refrigerated until further analysis and, when appropriate, injected into the chromatograph (in the amount of 20 µL).

Separation of milk whey proteins was performed by RP-HPLC method. The whole system consisted of a HPLC apparatus, a ProStar 210 pumping system, a ProStar 410 autosampler and a ProStar 325 UV-VIS detector (Varian, USA). The system was controlled by Varian Star Chromatography Workstation System ver. 6.2. The column used was Nucleosil 300-5 C18, 4.6 × 250 mm, equipped with guard column containing the same packing material (Varian, USA). The separation was carried out at 37°C using the gradient system. The mobile phase was solvent A: 90% water, 10% acetonitrile and 0.1% trifluoroacetic acid (TFA) and solvent B: 90% acetonitrile, 10% water and 0.1% TFA. The mobile phases were filtered through 0.45-µm filters (Millipore) and degassed using ultrasound. The eluate was monitored at 205 nm.

Calibration

Calibration of the chromatographic system for determination of whey proteins 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 from bovine milk (α-LA, β-LG, lactoferrin and bovine serum albumin) were purchased from Sigma (Germany). All chemicals were of HPLC analytical grade.

Data analysis

The statistical analysis included cow breed and production season and used the following linear model:

$$Y = \mu + A_i + S_j + e_{ijk}$$

where:
μ – total mean,
A_i – cow breed (i = 1…4),
S_j – production season (j = 1,2),
e_{ijk} – error.

The results obtained were analysed statistically using StatSoft Inc. Statistica ver. 6 based on two-way analysis of variance with interaction. Significance of differences was determined by Tukey's test for unequal numbers (n).

Results

It has been shown that Polish Black-and-White Holstein-Friesian cows produced double the amount of milk (more by 11.57 kg on average, $P \leq 0.01$) compared to the local breeds of cows. Milk yield of these cows ranged from 11.32 kg for Polish Black-and-White to 13.02 kg for Polish Red-and-White (Table 1). The highest content of total protein, including casein, was established in milk from Polish Red cows (3.59%; 2.64%), and the lowest in milk from Polish Black-and-White (3.27%; 2.41%) and Polish Red-and-White cows (3.26%; 2.34%) (Table 1). Milk from local breeds of cows, in comparison to milk obtained from Polish Black-and-White Holstein-Friesian cows, contained a significantly higher amount of beta-LG (by 0.56 g/l on average), whereas the difference for Polish Red and Whitebacked cows exceeded 0.60 g/l. The milk from Polish Red cows was also characterized by the highest alpha-LA content. Meanwhile, the least amount of this protein was found in milk produced under intensive conditions by Polish Black-and-White Holstein Friesian cows. The differences in alpha-LA concentration, in favour of milk from the local breeds were as follows: +0.17 g/l for Polish Red, +0.11 g/l for Whitebacked and Polish Black-and-White, and +0.06 g/l for Polish Red-and-White. Also in the case of beta-LG and alpha-LA, the milk obtained from Polish Red cows showed the highest lactoferrin content (128.7 mg/l). A slightly lower level of the protein was established in milk from Whitebacked (115.2 mg/l) and Polish Red-and-White cows (120.9 mg/l). Significantly less lactoferrin (91.4 mg/l) was determined in milk from Polish Black-and-White Holstein-Friesian cows. Another critical whey protein is bovine serum albumin (BSA). The lowest level of this protein was determined in milk from Polish Red cows (0.41 g/l). Significantly more BSA was found in the milk obtained from Polish Black-and-White (+0.06 g/l) and Whitebacked cows (+0.05 g/l).

The analysed cow population produced more milk in the summer season than in the winter season. The differences between the seasons (in favour of the summer) in the milk yield of each breed were as follows: Whitebacked – 4.42 kg, Polish Red – 1.86 kg, Polish Black-and-White – 1.22 kg, Polish Red-and-White – 3.21 kg, Polish Black-and-White Holstein-Friesian – 3.45 kg. There was also a significant effect of the season on the content of the analysed proteins. A higher concentration of protein, including casein, was determined in the milk obtained in the winter season. It ranged from 3.26% (including 2.32% for casein) in milk from Polish Red-and-White cows to 3.66% (including 2.66% for casein) in Polish Red cows. In the summer, the crude protein content of milk was lower by an average of 0.20% and casein level by 0.08%. Interestingly, the highest values were determined in milk from Polish Red cows (3.50% of protein, including 2.63% of casein). During the grazing period, the milk obtained from cows of the local breeds showed a higher concentration of lactoferrin, alpha-LA and beta-LG. The level of these proteins averaged 129.2 mg/l, 1.18 g/l and 3.60 g/l, respectively. In the winter season, depending on the breed, milk contained from 14.5 to 33.7 mg/l less lactoferrin, from 0.13 to 0.19 g/l less alpha-LA and from 0.16 up to 0.33 g/l less beta-LG. In the case of milk from Polish Holstein-Friesians, there were slight differences between the seasons in whey protein content that reached 13.7 mg/l for lactoferrin, 0.05 g/l for alpha-LA and 0.06 g/l for beta-LG

Table 1. Daily yield and content of the protein fraction of some milk components in cows of the analysed breeds with regard to production season

Item		Whitebacked			Polish Red			Polish Black-and-White			Polish Red-and-White			Holstein-Friesian		
		summer n = 101	winter n = 97	average for breed	summer n = 114	winter n = 90	average for breed	summer n = 86	winter n = 70	average for breed	summer n = 44	winter n = 38	average for breed	summer n = 136	winter n = 108	average for breed
Daily yield (kg)	x	14.30*	9.88*	12.06 A	12.93	11.07	12.32 A	11.72	10.50	11.32 A	14.43*	11.22*	13.02 A	25.62	22.17	23.75 B
	SE	0.67	0.61	0.71	0.59	0.68	0.79	0.66	0.65	0.89	0.73	0.60	0.77	0.51	0.67	0.61
Total protein (%)	x	3.24*	3.49*	3.34 BC	3.50	3.66	3.59 C	3.16*	3.43*	3.27 AB	3.17*	3.36*	3.26 A	3.30*	3.51*	3.41 BC
	SE	0.14	0.19	0.15	0.16	0.25	0.21	0.16	0.15	0.16	0.29	0.24	0.26	0.27	0.35	0.32
Casein (%)	x	2.50	2.62	2.56 b	2.63	2.66	2.64 b	2.37	2.45	2.41 ab	2.24	2.42	2.34 a	2.54	2.64	2.60 b
	SE	0.25	0.24	0.25	0.16	0.25	0.21	0.17	0.08	0.13	0.17	0.29	0.24	0.25	0.14	0.20
Alpha-LA (g/l)	x	1.18*	1.04*	1.09 BC	1.23	1.10	1.15 C	1.19*	1.00*	1.09 BC	1.12*	0.98*	1.04 AB	1.00	0.95	0.98 A
	SE	0.05	0.10	0.08	0.02	0.11	0.06	0.08	0.03	0.05	0.14	0.24	0.19	0.23	0.12	0.18
Beta-LG (g/l)	x	3.62	3.46	3.57 B	3.74**	3.49**	3.60 B	3.54**	3.21**	3.40 B	3.51**	3.27**	3.38 B	2.96	2.90	2.93 A
	SE	0.16	0.28	0.25	0.08	0.16	0.13	0.08	0.05	0.07	0.06	0.12	0.10	0.27	0.35	0.29
Lactoferrin (mg/l)	x	127.3	105.7	115.2 BC	140.9*	113.1*	128.7 C	114.3	99.8	105.9 AB	134.2*	100.5*	120.9 BC	100.4	86.7	91.4 A
	SE	14.45	26.62	21.01	18.11	15.33	17.35	24.57	14.81	19.37	23.68	14.70	19.22	28.49	12.69	21.09
BSA (g/l)	x	0.39*	0.50*	0.46 b	0.39	0.42	0.41 a	0.41*	0.54*	0.47 b	0.42	0.44	0.43 ab	0.43	0.44	0.44 ab
	SE	0.03	0.02	0.03	0.09	0.07	0.08	0.03	0.08	0.06	0.08	0.10	0.09	0.11	0.06	0.09

a, b, A, B, C – differences between the averages for breed; a, b – differences significant at P≤0.05; A, B, C – differences significant at P≤0.01.
*, ** differences between seasons within breed; * differences significant at P≤0.05; ** differences significant at P≤0.01.

in favour of the summer season. In the winter season, higher concentrations of serum albumin and significant seasonal differences were noted in milk from Polish Black-and-White (+0.13 g/l) and Whitebacked cows (+0.11 g/l).

Table 2. Results of two-way variance analysis for daily yield and milk protein fraction of some components (P values)

Factor	Daily yield (kg)	Total protein (%)	Casein (%)	Alpha-LA (g/l)	Beta-LG (g/l)	Lactoferrin (mg/l)	BSA (g/l)
Breed	0.000	0.003	0.000	0.002	0.000	0.000	0.039
Production season	0.038	0.040	0.151	0.041	0.002	0.036	0.042
Breed and production season interaction	0.000	0.383	0.000	0.211	0.820	0.199	0.238

The final results of two-way analysis of variance (summarized in Table 2) indicate a significant effect of breed on the content of all the parameters analysed. Milk casein level was the only parameter on which production season had no effect. Daily yield and casein concentration were significantly correlated with cow breed and production season.

Discussion

This study shows that the milk from Polish Red cows has a high content of total protein, including casein. Our results are consistent with those reported previously by Barłowska (2007) and Król et al. (2007). Compared to our research, Feleńczak et al. (2000) revealed that the milk from Polish Red cows contained less crude protein (3.31–3.38%, depending on the kappa-casein genotype) and had a similar casein concentration (2.56–2.67%). Low protein levels were determined in a Brazilian study (Botaro et al., 2008) performed on the milk of cows of two local breeds, i.e. Girlando and Holstein. The content of protein, including casein, was 3.07–3.15%, 2.06–2.12%; and 3.12–3.15%, 2.09–2.13%, respectively. In a study by Wedholm et al. (2006), the milk from cows of two breeds raised in Sweden (Red-and-White and Holstein) showed high casein concentration (2.76%) with 3.39% crude protein.

The analysis of whey protein content demonstrated that the milk obtained from local breeds of cows, especially Polish Red, is the most valuable source of these proteins. The major whey proteins are beta-lactoglobulin, which mainly exhibits antioxidant and anticancer activity as well as retinol-binding capacity (Król et al., 2008; Liu et al., 2007), and alpha-lactalbumin, a protein that regulates milk secretion and has a role in transport of calcium, zinc and manganese ions (Chatterton et al., 2006; Strohmaier, 2004). In 9 Swedish farms, Lindmark-Månsson et al. (2003) obtained comparable results to ours, with the closest content of these proteins noted in milk from the local breeds. The analysed milk contained an average of 3.5 g/l (2.9–4.5 g/l) beta-LG and 1.0 g/l (1.0–1.1 g/l) alpha-LA. Another Swedish study (Wedholm et al., 2006) established a considerably higher level of beta-LG, i.e. 5.5 g/kg. Using Black-

and-White cows with different proportions of Holstein-Friesian genes, Reklewska et al. (2003) showed that concentration of major milk whey proteins was 3.04–3.74 g/l for beta-LG and 1.11–1.37 g/l for alpha-LA depending on feeding regime.

An important ingredient of milk whey fraction proves to be lactoferrin, whose average content in milk is 0.1 g/l. It is a multifunctional protein exhibiting antimicrobial, antiviral, antioxidant, antiinflammatory and cancer preventing activities. It is thought to be responsible for iron absorption and bioavailability (Pan et al., 2006; Reichel et al., 1998). A high lactoferrin content (56.10–164.12 µg/ml) was also reported by Reichel et al. (1998) who evaluated whey produced in the cheese making. Meanwhile, Lindmark-Månsson et al. (2003) obtained 90 mg/l (70–110 mg/l) of this protein in raw milk from Swedish farms and this quantity is comparable to the present findings for the milk from Polish Black-and-White Holstein-Friesian cows. A substantially lower proportion of lactoferrin (7.30–14.73 mg/l) in milk from Black-and-White cows was noted by Reklewska et al. (2003). Another crucial whey protein is bovine serum albumin (BSA), which has opioid agonist activity as well as inhibiting ACE (angiotensin converting enzyme) (Smithers, 2008). Half less BSA in milk was found in Sweden (Lindmark-Månsson et al., 2003).

Taking into consideration the influence of season, it was stated that independent of cow breed, the milk obtained in the winter season was characterized by a higher content of crude protein, including casein. However, the milk collected in the summer contained more biologically active proteins, but significant differences were noted in milk from the local breeds of cows. Barłowska (2007), Król et al. (2007) and Reklewska et al. (2003) have indicated more beneficial chemical composition of milk obtained in the autumn-winter months. Some differences in bioactive components of milk produced in winter and summer were also reported by Reklewska et al. (2003) who assessed the milk from Black-and-White cows with over 85% Holstein-Friesian genes, which were fed using a TMR system all year round. The authors also showed, similar to our research, some marked seasonal differences in the content of functional whey proteins in favour of milk from grazing cows. A higher level of these proteins was also reported by Król et al. (2007).

The results of two-way variance analysis point to a highly significant effect of breed and season on the analysed parameters. When analysing the milk from two Swedish cow breeds, i.e. Red-and-White and Holstein, Wedholm et al. (2006) did not confirm any breed influence on milk production and the level of whey proteins discussed. In turn, Barłowska (2007), who investigated the milk from cows of 7 breeds raised in Poland reported a significant effect of both factors on milk yield and level of crude protein, including casein.

In conclusion, the milk from local cow breeds, in comparison to Holstein-Friesian cows raised under intensive conditions, is a more valuable source of functional whey proteins, especially in the summer season when animals graze outdoors. Due to increasing consumer interest in health-promoting foods, this milk should be used by local dairies to manufacture special dairy products rich in biologically active substances, as well as concentrates and whey protein preparations.

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Zawartość bioaktywnych białek w mleku krów ras lokalnych objętych programem ochrony zasobów genetycznych

STRESZCZENIE

Celem pracy była ocena frakcji białkowej mleka pozyskiwanego od krów czterech ras miejscowych pod względem zawartości związków biologicznie czynnych z uwzględnieniem sezonu produkcji. Badaniami objęto ogółem 640 prób mleka pobranego od krów czterech ras lokalnych, tj. polskiej czerwonej – 204, białogrzbietej – 198, polskiej czarno-białej – 156 i polskiej czerwono-białej – 82. Krowy tych ras utrzymywane są głównie na terenie Polski południowo-wschodniej. Badania wykonano w dwóch sezonach produkcji, związanych z różnym żywieniem, tj. w okresie letnim i zimowym. W każdej próbie mleka oznaczono zawartość białka ogólnego, kazeiny oraz wybranych białek serwatkowych, tj. α-laktoalbuminy, β-laktoglobuliny, laktoferyny i albuminy serum. Stwierdzono, że mleko krów ras lokalnych, w porównaniu do krów holsztyńsko-fryzyjskich użytkowanych systemem intensywnym, jest bogatszym źródłem funkcjonalnych białek serwatkowych, zwłaszcza w sezonie letnim, kiedy zwierzęta korzystają z pastwiska. Mleko to, z uwagi na duże zainteresowanie konsumentów żywnością prozdrowotną, powinno być zatem wykorzystywane przez lokalne mleczarnie do produkcji specyficznych artykułów mleczarskich, bogatych w składniki biologicznie czynne oraz koncentratów i preparatów białek serwatkowych.