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Comparison of whey proteins and lipophilic vitamins between four cow breeds maintained in intensive production system

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Abstract

The aim of the study was to determine the content of whey proteins and fat-soluble vitamins in milk samples of four cow breeds maintained in Poland, i.e. Holstein-Friesian, Montbéliarde, Jersey and Simmental. Qualitative and quantitative analyses of certain whey protein fractions, i.e. α-lactalbumin (α-La), β-lactoglobulin (β-Lg), bovine albumin serum (BSA), lactoferrin and lysozyme, and lipophilic vitamins (A, D_3 and E) were performed using a RP-HPLC method. According to the obtained results, the breed of cow significantly affected the level of whey proteins and lipophilic vitamins in milk. The lowest amounts of these nutrients were found in milk produced by the Holstein-Friesian cows. Milk of Simmental cows contained the statistically significant and highest levels of antioxidant substances, i.e. vitamins A - 0.465 mg L^{-1}, E - 1.302 mg L^{-1} and D_3 - 0.653 μg L^{-1} at $P\leq0.05$, as well as β-lactoglobulin (3.28 g L^{-1} at $P\leq0.01$). In addition, Simmental cow milk was also characterized by higher content of antimicrobial proteins - lactoferrin and lysozyme (respectively: 121.23 mg x L^{-1} at $P\leq0.01$ and 9.66 μg x L^{-1} at $P\leq0.05$) if compared with other cow breeds.

Key words: whey proteins, lipophilic vitamins, cow breeds

Introduction

Cow milk is a source not only of nutrients but also of health-promoting compounds, such as whey proteins and vitamins. Whey proteins amounts for about 20-25 % of total milk proteins, whereas 75 % of whey proteins are globulins and albumins, i.e. β-lactoglobulin (β-Lg), α-lactalbumin (α-La), bovine serum albumin (BSA) and proteose-peptone fraction (PP). The rest of whey proteins are immunoglobulins, lactoferrin, lysozyme, and lactoperoxidase. Whey proteins are an excellent source of essential amino acids and exert numerous positive effects on the human health, like reducing the risk of atherosclerosis, obesity, diabetes and cancer or even Alzheimer's disease and HIV. They also participate in binding and transport of macro-and microelements. Furthermore, whey proteins are increasingly used to enrich the food, infant formulas, dietary agents or high-protein preparations for convalescents and sportsmen. They are also used in the pharmaceutical and cosmetic industries (Król and Brodziak, 2015; Kuczyńska et al., 2012; Lindmark-Månsson et al., 2006).

Vitamins are an important group of highly active organic substances that are essential to the growth and function of the organism (Morrissey and Hill, 2009). They take part in numerous key vital processes, enhance metabolism, and improve the activity of enzymes and catalytic proteins. Although the human body requires only small quantities of vitamins, their vitamin deficiency exert a significant detrimental effect on human health. Our human

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metabolism is unable to produce them independently, or produces them in negligible quantities, so they must be regularly supplied by the diet or in the form of supplements (Zaborska et al., 2015). The market for dietary supplements has grown dynamically in the last few years. However, dietary supplements should only be used if for various reasons it is difficult to ensure a diet meeting for all the nutritional needs of the consumer (Wu et al., 2007). Moreover, unjustified use of dietary supplements may cause exceeding the "tolerable upper intake level", thereby inducing undesirable health effects (Fortmann et al., 2013; Grodstein et al., 2013; Reguła et al., 2011).

Milk and dairy products are a valuable source of vitamins, mainly fat-soluble vitamins, i.e. A, D, E and K. These vitamins exhibit a number of health-promoting properties (Kuczyńska et al., 2012; Zaborska et al., 2015). Their antioxidant effect on the decomposition of milk fat is also highly significant. The content of whey proteins and lipophilic vitamins is one of the indicators of the health-promoting quality of milk (Król et al., 2008; Kuczyńska et al., 2011; Lisak Jakopović et al., 2016; Mogensen et al., 2012).

Most of the milk produced in Poland is obtained from Polish Black-and-White Holstein-Friesian cows, and they present over 80 % of the active cow population. In 2015, the milk yield of named active population was 7,950 kg milk, with 4.07 % fat and 3.35 % protein. Montbéliarde cows had the second highest yield in that year and had 7,529 kg of milk with 3.95 % fat and 3.51 % protein (PFCBDF, 2016). In France, the milk of Montbéliarde cows is considered as for the production of high-quality cheeses. Barłowska et al. (2014) demonstrated that Montbéliarde cows, in comparison to Holstein-Friesians or Simmentals and they concluded that they are highly productive, and milk is characterized by the most favourable parameters determining suitability for cheese production, i.e. active acidity (pH value), potential acidity (°SH), thermal stability, rennet coagulation time and state of milk fat dispersion.

High milk yield is also characteristic of the Jersey breeds (6,212 kg milk) and Simmental (6,075 kg milk) breeds. The Jersey breed is the third largest population of dairy cattle in the world. Cows of this breed are becoming increasingly popular due to their relatively high milk yield and high resistance to mastitis (Perišić et al., 2009). The milk of these cows is considered an excellent raw material for cheese production (Barłowska et al., 2014, Sturaro et al., 2012). According to Litwińczuk et al. (2011), Simmental and Jersey cows are more resistant to udder infections than Holstein-Friesians, which is reflected in the quality of the produced milk.

Currently the vast majority of farmers raising cows of high-yielding breeds have decided to introduce intensive milk production systems, with free-stall housing and total or partially mixed rations (TMR or PMR). Such system guarantee considerably higher milk yield, but may also be associated with milk of inferior chemical composition and reduced suitability for processing (Barłowska et al., 2014, Litwińczuk et al., 2015). According to Bobe et al. (2007), genetic selection of cows for high milk yield leads to a decrease in fat and protein content in the milk. However, genetic selection has no clear effect on the content of fatty acids. There is lack of information about the degree to which it affects the health-promoting value of milk, including content of lipophilic vitamins and whey proteins. The aim of this study was to determine the content of whey proteins and fat-soluble vitamins in the milk of four cow breeds kept in Poland, respectively Holstein-Friesian, Montbéliarde, Jersey and Simmental.

Materials and methods

Sampling

The research was performed at three farms. Samples from the first farm were from the Holstein-Friesian and Montbéliarde cows. From the second farm, samples from Jersey cows were taken and from the third farm Simmental cow milk. Farms were located in south-eastern Poland. Within the breed approximately 100 animals were kept in each herd. Samples were taken twice during the summer season. The selected cows were in the middle period (between 30th and 120th day) of their second, third or fourth lactation. Thereby, only milk samples in which the somatic cell count (SCC) did not exceed 400,000 mL^{-1} were included in the study (SCC was determined using a Somacount 150 apparatus, Bentley Instruments). In total, 250 milk samples analysed, respectively 66 samples from

Holstein-Friesian cows, 56 from Montbéliarde, 64 from Jersey and 64 from Simmental cows. All of the farms were included in the Milk Recording runs by the Polish Federation of Cattle Breeders and Dairy Farmers. The animals were kept in an intensive system at all farms. The cows of all breeds were kept on the farms in a free stall system, and were fed with a total mix ratio (TMR). Table 1 shows the type of feed and composition of ration. From total 48.35 kg was 51.70 % maize silage, 41.36 % haylage, 6.20 % grain meal, 0.41 % vitamin-mineral premix, 0.21 % post-extraction soya meal and 0.10 % post-extraction rapeseed meal. Daily cow's rations were balanced according to the Institut National de la Recherche Agronomique (INRA) feeding system (INRAtion 4.07 Software).

Sample analysis

Basic chemical composition, i.e. dry matter, protein, fat and lactose content, in each milk samples were analysed by Infrared Milk Analyzer (Bentley Instruments, USA). Active acidity (pH value) was measured by the Pionneer 65 pH meter (Radiometer Analytical), titratable acidity (°SH) - by titration method according to PN-68/A-86122, and density - using a lactodensimeter. In order to evaluate the content of the certain whey proteins, i.e. α-lactalbumin (α-La), β-lactoglobulin (β-LG), bovine albumin serum (BSA), lactoferrin and lysozyme, a RP-HPLC method was used. All samples were prepared according to Romero et al. (1996) with modifications (Brodziak et al., 2012). Protein separation was performed by a liquid chromatograph 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 mobile phase was solvent A (90 % water, 10 % acetonitrile) and solvent B (90 % acetonitrile, 10 % water), purchased from Sigma-Aldrich (Germany). 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. Purified proteins, i.e. alpha-La (≥ 85 %), beta-Lg (90 %), bovine albumin serum (≥96 %) and lactoferrin (90 %) all from bovine milk, as well lysozyme (95 %) from hen egg whites, were purchased from Sigma-Aldrich (Germany). 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 followed by their concentration determination. Calibration of the chromatographic system for whey proteins determination was carried out by the external standard method.

Table 1. Ration and its components for cows included into the study

Component of rations	%
Maize silage	51.70
Haylage	41.36
Grain meal	6.20
Post-extraction soya meal	0.21
Post-extraction rapeseed meal	0.10
Vitamin-mineral premix	0.41

RP-HPLC method was also used to determine the concentrations of fat-soluble vitamins, i.e. A, D_3 and E. All samples were prepared by the Röse-Gottlieb fat extraction method modified by Hewavitharan et al. (1996). Vitamin separation was performed by a liquid chromatograph ProStar 210 model, a UV-VIS ProStar 325 and fluorescence ProStar 363 detectors (Varian, USA). Mobile phase was a mixture of acetonitrile, dichloromethane, methanol and water for HPLC (Sigma-Aldrich, Germany). The measurements were carried out using the column PursuitXRs 3-C18 (Varian, USA) of 150 mm length and 4.6 mm diameter. The analyses of reference substances, i.e. (±)-α-tocopherol (≥97 % HPLC) - vitamin E, cholecalciferol (≥98 % HPLC) for vitamin D_3 and retinol for vitamin A (≥99 % HPLC) (Sigma-Aldrich, Germany), were conducted under the same conditions. The qualitative and quantitative identification was performed as in the case of whey proteins.

Statistical analysis

Statistical analysis of the results was performed in StatSoft Inc. Statistica software ver. 9, using one-way analysis of variance (ANOVA). Significance of differences between means for groups was determined by Fisher's NIR test, at a level of P (alpha) = 0.05 and P = 0.01. The results were presented as mean ± standard deviation.

Results and discussion

Content of basic components and selected physical properties of milk of various breeds of cows were presented in Table 2. Results shows that milk form the Jersey cows was distinguished by the highest content of dry matter (14.62 %), in that 3.94 % total protein and 5.20 % fat. It was also the most acidic. Fig.1 shows that the cow breeds had no significant effect on the content of the analysed whey proteins. However, this factor significantly (at $P \leq 0.05$) effected on the share of whey proteins (%) in total protein content of milk (Fig. 2).

The milk obtained from cows of the Polish Holstein-Friesian breed had the highest share of whey proteins in total protein, on average 1.82 percentage points (Fig. 2) higher than milk of the other breeds. At the same time above mentioned milk contained the smallest ratio of whey proteins (6.28 g L^{-1}) and had the lowest total protein content (33.8 g L^{-1}) as well. Concentration of β-lactoglobulin and α-lactalbumin was the highest in the milk from Simmental breed and amounted 3.28 g L^{-1} β-Lg ($P \leq 0.01$) and 1.11 g L^{-1} α-La ($P \leq 0.05$), respectively (Table 3). The lowest concentrations of whey proteins were observed in milk samples of the Polish Holstein-Friesian and Jersey cows. Bonfatti et al. (2011) reported that milk obtained from Simmental cows contain more β-lactoglobulin (3.66 g L^{-1}) and α-lactalbumin (1.25 g L^{-1}), with a total protein content of 38.78 g L^{-1}, what is similar to results obtained in our study. Albumins present in milk, except α-lactalbumin include bovine serum albumin (BSA), whose concentration is an indicator of the permeability of the blood-milk barrier in the mammary gland (Lindmark-Månsson et al., 2006; Litwińczuk et al., 2011). The highest level of BSA was found in the milk of the Polish Holstein-Friesians cows (0.49 g L^{-1}). Content of BSA was lower in the milk obtained from the other breeds, ranging from 0.41 to 0.44 g L^{-1} (Table 3). According to Litwińczuk et al. (2011), cows of the Simmental and Jersey breeds are more resistant to mastitis than Holstein-Friesian cows, which was to some extent confirmed by the present study. This was indicated by the highest significant BSA content in the milk of the Polish Holstein-Friesian cows. Group of authors (Barnouin et al., 2005; Detilleux, 2002) reported that Montbéliarde cows are less susceptible to mastitis than the French Holstein-Friesians. Differences in the content of antimicrobial

Table 2. Content of basic components and physical properties of milk of various breeds of cows ($\bar{x}$ ±standard deviation)

Breed of cow	n*	Total protein (%)	Fat (%)	Lactose (%)	Dry matter (%)	Acidity		Density (g mL^{-1})
						active (pH)	titratable (°SH)	
Polish Holstein-Friesian	66	3.38^{a}±0.35	4.19^{A}±0.66	4.81±0.37	13.12^{A}±0.83	6.70^{B}±0.13	6.80^{AB}±0.67	1.0302±0.013
Montbéliarde	56	3.78^{b}±0.32	4.29^{A}±0.53	4.82±0.22	13.54^{B}±0.75	6.72^{B}±0.08	6.69^{A}±0.71	1.0294±0.002
Jersey	64	3.94^{c}±0.43	5.20^{B}±0.61	4.75±0.35	14.62^{C}±0.89	6.62^{A}±0.09	7.42^{B}±0.78	1.0282±0.004
Simmental	64	3.76^{b}±0.36	4.22^{A}±0.40	4.73±0.29	13.36^{AB} ±0.69	6.69^{AB}±0.11	6.86^{AB}±0.83	1.0301±0.013

*number of samples, a, b, c - differences significant at $P \leq 0.05$; A, B, C - differences significant at $P \leq 0.01$

Table 3. Content of selected bioactive whey proteins in milk of various cow breeds s ($\bar{x}$ ±standard deviation)

Breed of cow	n*	β-Lactoglobulin (g L^{-1})	α-Lactalbumin (g L^{-1})	Bovine serum albumin (BSA) (g L^{-1})	Lactoferrin (mg L^{-1})	Lysozyme (μg L^{-1})
Polish Holstein-Friesian	66	3.01^{A}±0.26	1.03^{a}±0.25	0.49^{b}±0.12	94.01^{A}±17.15	7.38^{a}±2.11
Montbéliarde	56	3.18^{AB}±0.42	1.06^{ab}±0.28	0.44^{a}±0.08	111.89^{BC}±22.24	8.16^{ab}±2.44
Jersey	64	3.08^{A}±0.39	0.98^{a}±0.21	0.43^{a}±0.11	106.78^{B}±18.32	12.13^{c}±2.86
Simmental	64	3.28^{B}±0.45	1.11^{b}±0.34	0.41^{a}±0.09	121.23^{C}±35.47	9.66^{b}±2.59

*number of samples, a, b, c - differences significant at $P \leq 0.05$; A, B, C - differences significant at $P \leq 0.01$

proteins, i.e. lactoferrin and lysozyme, are also crucial. Milk produced by the Polish Holstein-Friesian cows was the poorest source of these antimicrobial proteins, containing on average 21% less lactoferrin and even 40 % less lysozyme than the milk of the other breeds. The milk of the Simmental cows contained the highest quantities of lactoferrin (121.23 mg L^{-1}), while the highest lysozyme content was observed in the milk of the Jersey breeds (12.13 μg L^{-1}). Lactoferrin and lysozyme play a significant role in protecting the human health, since they belong to the most important components of the non-specific immune mechanisms (Struff and Sprotte, 2008). Several authors (Brodziak et al., 2012; Król et al., 2008; Kuczyńska et al., 2012) indicated that the breed of cow influenced the health-promoting value of the milk and thus the quality of dairy products. Milk obtained from the Polish Holstein-Friesian cows was found to have the smallest quantity of biologically active substances. However, Wedholm et al. (2006), studied the milk of two Swedish cow breeds (Red-and-White and Holstein), and did not confirm that the breed affected the concentration of whey proteins. Pomiès et al. (2007) compared the milk of the Holstein-Friesian and Montbéliarde breeds and also found no influence of breed on the total content of whey proteins. It should be emphasized, however, that as in the present study, a higher quantity of whey proteins were detected in the milk samples of the Montbéliarde cows than in that of the Holstein-Friesians. Kuczyńska et al. (2012) noted a significantly ($P \leq 0.05$) higher concentrations of β-lactoglobulin in the milk of Holstein-Friesian cows than in that of the Montbéliarde breed.

According to Dolores-Perez and Calvo (1994), the concentration of β-lactoglobulin is in correlation with vitamin A content, because β-Lg is actively included in the transport of α-retinol. The present study confirmed that relationship.

Figure 1. Content of whey proteins in total protein

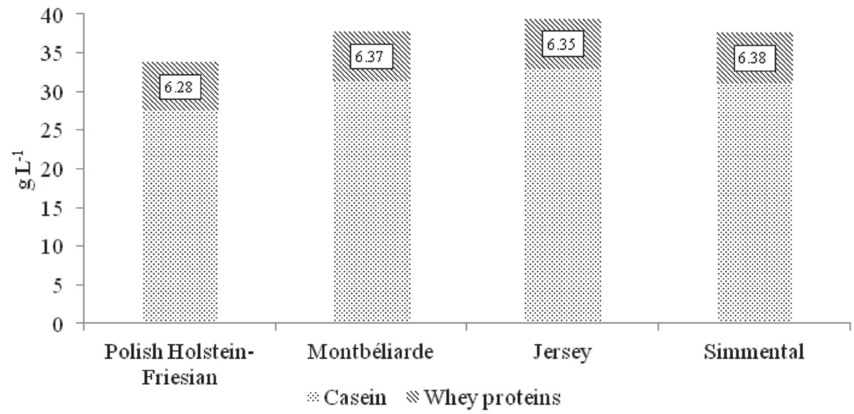
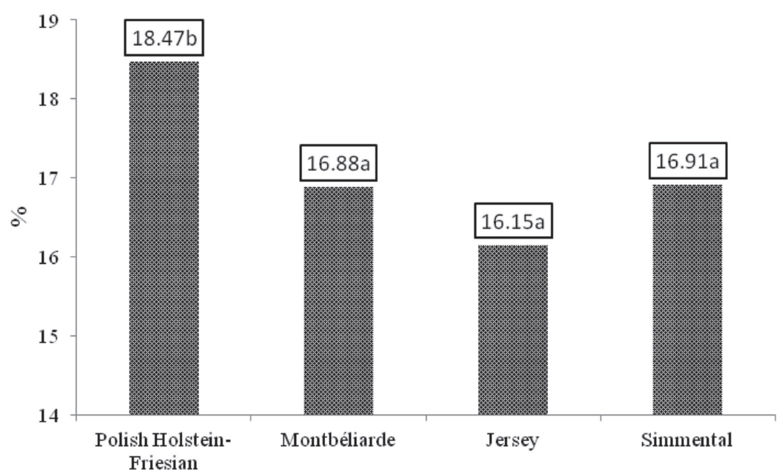


Figure 2. Share of whey proteins in total protein



a, b - differences significant at P≤0.05

The highest level of vitamin A (0.465 mg L^{-1}) was detected in milk samples of the Simmental cows, which at the same time contained the most β-lactoglobulin too. Significantly lower contents of vitamin A were observed in the milk samples containing the lowest concentrations of β-Lg, which were obtained from the Polish Holstein-Friesian and the Jersey cows. Kuczyńska et al. (2011) also reported a relationship between the content of vitamin A and β-Lg in milk.

The milk of Polish Holstein-Friesian cows also proved to be the poorest source of the other vitamins analysed (D_3 and E) and its fat content was low (41.9 g L^{-1}) too. The milk of the Jersey cows also had low content of vitamins D_3 and E, although it was distinguished by significantly (P≤0.01) higher fat content, which is approximately 20 % higher in comparison with the other breeds (Table 4). Similar differences in nutrient concentrations in milk of the Jersey and the Holstein-Friesian cows were observed by Bland et al. (2015). Significantly (P≤0.05) higher content of vitamin D_3 was detected in the milk of other tested breeds (Simmental and Montbéliarde), on average by 0.075 μg L^{-1}, with the highest value observed in milk samples of the Montbéliarde cows (0.696 μg L^{-1}). The breed also significantly (P≤0.05) affected the concentration of vitamin E, whereas higher contents were detected in milk samples produced by the Simmental and the Montbéliarde cows (Table 4). Those samples contained 0.196 and 0.137 mg L^{-1}, respectively vitamin E, while milk samples obtained from the Polish Holstein-Friesians contained approximately 0.103 mg L^{-1}, and milk from Jersey cows approximately 0.044 mg L^{-1}. Kuczyńska et al. (2012), focused on analysing milk produced by the Polish Holstein-Friesian and the Montbéliarde cows, and found no significant differences in contents of fat-soluble vitamins. Mogensen et al. (2012) reported lower content of vitamin E (on average 0.82 μg mL^{-1}) than in the present study, and showed that the breed significantly influenced its content (0.90 μg mL^{-1} of vitamin E in the milk of Holstein-Friesians and 0.51 μg mL^{-1} in Danish Red cows).

Milk of cows is a valuable source of lipophilic vitamins in the human nutrition. Therefore, the demand of an adult for vitamins analyzed in the present study was calculated in relation to the results obtained for milk samples obtained from cows of different breeds, and the doses recommended by FAO/WHO (2004) and feeding standards for Polish population (Jarosz, 2012). Unfortunately, milk produced by the Holstein-Friesian cows covers the demands for vitamin A, D_3, and E in the least. An adult would have to drink more than 1 L of that milk daily (women - 1.4 L, men - 1.7 L) to achieve the recommended daily intake for vitamin A (500 μg for women and 600 μg for men), 8.5 L in the case of vitamin D_3 (according to the world's recommendations for women and men - 5 μg per day) and more than 6 L (women - 6.8 L, men - up to 9 L) of vitamin E, basing on the world's recommendations: 7.5 mg per day for women and 10 mg for men.

It is worth noting that the much smaller amount of milk should be consumed if it was a more valuable source of these vitamins, which could be milk samples obtained from the Simmental cows, assuming that the milk would be the only source of these nutrients in the diet. Thus, for both sexes 1 L of milk would be enough to fully deliver vitamin A to the body, 7.7 L - vitamin D_3 (from Montbéliarde breed even 0.5 L less) and 6.8 L (women - 5.8 L, men - 7.7 L) for vitamin E. Apparently, we assumed that the milk would be the only source of these nutrients in the human diet.

Table 4. Content of selected fat-soluble vitamins in milk of various breeds of cows ($\bar{x}$ ±standard deviation)

Breed of cow	No.	Vitamin A (α-retinol) (mg L^{-1})	Vitamin D_3 (cholecalciferol) (μg L^{-1})	Vitamin E (α-tocopherol) (mg L^{-1})
Polish Holstein-Friesian	66	0.359^{a}±0.071	0.589^{a}±0.106	1.106^{a}±0.299
Montbéliarde	56	0.414^{ab}±0.080	0.696^{b}±0.157	1.243^{b}±0.338
Jersey	64	0.398^{a}±0.094	0.620^{ab}±0.170	1.199^{ab}±0.284
Simmental	64	0.465^{b}±0.077	0.653^{b}±0.113	1.302^{b}±0.310

a, b - differences significant at P≤0.05; A, B - differences significant at P≤0.01

Conclusion

It can be conclude that the cow breeds significantly affected the level of the whey proteins, milk fat and lipophilic vitamins in analysed milk. The poorest source of these nutrients was the milk produced by the Holstein-Friesian cows. The Simmental breed was the most favourably in terms of content of bioactive components. It contained the highest quantities of antioxidants such as vitamins A, E and D_3, as well as β-lactoglobulin, which also exhibits antioxidant properties. This milk was also characterized by the highest content of antimicrobial proteins (lactoferrin and lysozyme).

Usporedba proteina sirutke i liposolubilnih vitamina između četiri pasmine krava držane u intenzivnim sustavima proizvodnje

Cilj ovog istraživanja bio je odrediti sadržaj proteina sirutke i vitamina topljivih u mastima u uzorcima mlijeka za četiri pasmine krava koje se uzgajaju u Poljskoj, t.j. holstein-frizijske, Montbéliarde, Jersey i simentalske. Kvalitativna i kvantitativna analiza određenih frakcija proteina sirutke, odnosno α-laktalbumina (α-La), β-laktoglobulina (β-Lg), albumina goveđeg seruma (BSA), laktoferina i lizozima te liposolubilnih vitamina (A, D3 i E) provedena je pomoću metode RP-HPLC. Prema dobivenim rezultatima, pasmina krava značajno je utjecala na razinu proteina sirutke i liposolubilnih vitamina u mlijeku. Najniže količine tih hranjivih tvari utvrđene su u mlijeku kojeg su proizvele krave pasmine holstein-friesian. Mlijeko simentalskih krava sadržavalo statistički najznačajnije i najviše razine antioksidativnih tvari, odnosno vitamina A - 0,465 mg L^{-1}, E - 1,302 mg L^{-1} i D3 - 0,653 μg L^{-1} (P≤0,05), kao i β-laktoglobulin (3,28 g L^{-1}, P≤0,01). Osim toga, kravlje mlijeko simentalca također odlikuje visokim sadržajem antimikrobnih proteina - laktoferina i lizozima (odnosno: 121,23 mg L^{-1}, P≤0,01 i 9,66 μg L^{-1}, P≤0,05) u usporedbi s drugim pasminama krava.

Ključne riječi: proteini sirutke, lipofilni enzimi, pasmine krava

References

1. Barłowska, J, Litwińczuk, Z., Wolanciuk, A., Pastuszka, R. (2014): The chemical composition, cytological quality and technological suitability of the milk of three breeds of red and white cows fed in a TMR system. *Roczniki Naukowe PTZ* 10, 115-124.
2. Barnouin, J., Bord, S., Bazin, S., Chassagne, M. (2005): Dairy management practices associated with incidence rate of clinical mastitis in low somatic cell score herds in France. *Journal of Dairy Science* 88, 3700-3709. https://doi.org/10.3168/jds.S0022-0302(05)73056-3
3. Bland, J.H., Grandison, A.S., Fagan, C.C. (2015): Effect of blending Jersey and Holstein-Friesian milk on Cheddar cheese processing, composition, and quality. *Journal of Dairy Science* 98, 1-8. https://doi.org/10.3168/jds.2014-8433
4. Bobe, G., Lindberg, G.L., Freeman, A.E., Beitz, D.C. (2007): Composition of milk protein and milk fatty acids is stable for cows differing in genetic merit for milk production. *Journal of Dairy Science* 90, 3955-3960. https://doi.org/10.3168/jds.2007-0099
5. Bonfatti, V., Di Martino, G., Carnier, P. (2011): Effectiveness of mid-infrared spectroscopy for the prediction of detailed protein composition and contents of protein genetic variants of individual milk of Simmental cows. *Journal of Dairy Science* 94, 5776-5785. https://doi.org/10.3168/jds.2011-4401
6. Brodziak, A., Barłowska, J., Król, J., Litwińczuk, Z. (2012): Effect of breed and feeding system on content of selected whey proteins in cows' milk in spring-summer and autumn-winter seasons. *Annals Animal Sciences* 12, 261.269.
7. Detilleux, J.C. (2002): Genetic factors affecting susceptibility of dairy cows to udder pathogens. *Veterinary Immunology and Immunopathology* 88, 103-111. https://doi.org/10.1016/S0165-2427(02)00138-1
8. Dolores-Perez, M., Calvo, M. (1994): Interaction of β-lactoglobulin with retinol and fatty acids and its role as a possible biological function for this protein: Review. *Journal of Dairy Science* 78, 978-988. https://doi.org/10.3168/jds.S0022-0302(95)76713-3
9. FAO/WHO World Health Organization and Food and Agriculture Organization of the United Nations. (2004): Vitamin and mineral requirements in human nutrition. Second edition. Vitamin E, 94-104.
10. Fortmann, S.P., Burda, B.U., Senger, C.A., Lin, J.S., Beil, T.L., O'Connor, E., Whitlock, E.P. (2013): Vitamin, mineral, and multivitamin supplements for the primary prevention of cardiovascular disease and cancer, A Systematic Evidence Review for the U.S. Preventive Services Task Force. Evidence Syntheses, No. 108. Rockville (MD), Agency for Healthcare Research and Quality (US).
11. Grodstein, F., O'Brien, J., Kang, J.H., Dushkes, R., Cook, N.R., Okereke, O., Manson, J.E., Glynn, R.J., Buring, J.E., Gaziano, M., Sesso H.D. (2013): Long-term multivitamin supplementation and cognitive function in men: a randomized trial. *Annals of Internal Medicine*159, 806-814. https://doi.org/10.7326/0003-4819-159-12-01312170-00006

12. Hewavitharana, A.K., van Brakel, A.S., Harnett, M. (1996): Simultaneous liquid chromatographic determination of vitamins A, E and beta-carotene in common dairy foods. *International Dairy Journal* 6, 613-624. https://doi.org/10.1016/0958-6946(95)00057-7

13. Jarosz, M. (2012): Feeding standards for Polish population - amendment. Vitamins. Food and Nutrition Institute, Warsaw, 112-113.

14. Król, J., Litwińczuk, Z., Brodziak, A. (2008): Content of protein and its fractions in milk of Simental cows with regard to a rearing technology. *Annals of Animal Sciences* 1, 57-61.

15. Król, J., Brodziak, A. (2015): Milk proteins with antibacterial properties. *Problemy Higieny i Epidemiologii* 96, 399-405.

16. Kuczyńska, B., Nałęcz-Tarwacka, T., Puppel, K., Gołębiewski, M., Grodzki, H., Slósarz, J. (2011): The content of bioactive components in milk depending on cow feeding model in certified ecological farms. *Journal of Research and Applications in Agricultural Engineering* 56, 7-13. https://doi.org/10.2754/avb201281020139

17. Kuczyńska, B., Puppel, K., Gołębiewski, M., Kordyasz, M., Grodzki, H., Brzozowski, P. (2012): Comparison of fat and protein fractions of milk constituents in Montbeliarde and Polish Holstein-Friesian cows from one farm in Poland. *Acta Veterinaria Brno* 81, 139-144.

18. Lindmark-Månsson, H., Brånning, C., Alden, G., Paullsson, M. (2006). Relationship between somatic cell count, individual leukocyte populations and milk components in bovine udder quarter milk. *International Dairy Journal* 16, 717-727. https://doi.org/10.1016/j.idairyj.2005.07.003

19. Lisak Jakopović K., Barukčić I., Božanić R. (2016): Physiological significance, structure and isolation of α-lactalbumin. *Mljekarstvo* 66 (1), 3-11. http://dx.doi.org/10.15567/mljekarstvo.2016.0101

20. Litwińczuk, Z., Barłowksa, J., Król, J., Brodziak, A., Matwijczuk, A., Kowal, M. (2015): Chemical composition and technological suitability of milk with regard to the feeding system of cows. *Medycyna Weterynaryjna* 71, 231-235.

21. Litwińczuk, Z., Król, J., Brodziak, A., Barłowska, J. (2011): Changes of protein content and its fractions in bovine milk from different cow breeds subject to somatic cell count. *Journal of Dairy Science* 94, 684-691. https://doi.org/10.3168/jds.2010-3217

22. Mogensen, L., Kristensen, T., Søegaard, K., Jensen, S.K., Sehested, J. (2012): Alfa-tocopherol and beta-carotene in roughages and milk in organic dairy herds. *Livestock Science* 145, 44-54. https://doi.org/10.1016/j.livsci.2011.12.021

23. Morrissey, P.A., Hill, T.R. (2009): Fat-soluble vitamins and vitamin C in milk and milk products. In: Fox PF, Mc Sweeney P.L.H. (ed) Advanced Dairy Chem. 3, Springer, New York, 527-589. https://doi.org/10.1007/978-0-387-84865-5_12

24. Perišić, P., Skalicki, Z., Petrović, M.M., Bogdanović, V., Ružić-Muslić, D. (2009): Simmental cattle breed in different production systems. *Biotechnology in Animal Husbandry* 25, 315-326. https://doi.org/10.2298/BAH0906315P

25. PFCBDF - Polish Federation of Cattle Breeders and Dairy Farmers - PFHBiPM. (2016): The results of assessment of value in use of dairy cows, Warsaw.

26. PN-68/A-86122: Milk. Research methods.

27. Pomiès, D., Martin, B., Chilliard, Y., Pradel, P., Rémond, B. (2007): Once-a-day milking of Holstein and Montbéliarde cows for 7 weeks in mid-lactation. *Animal 1*, 1497-1505. https://doi.org/10.1017/S1751731107000778

28. Reguła, J., Gramza-Michałowska, A., Stachowiak, B. (2011): Participation of dietary supplements in adult nutrition. *Problemy Higieny i Epidemiologii* 92, 614-616.

29. Romero, C., Perez-Andújar, O., Olmedo, A., Jiménez, S. (1996): Detection of cow's milk in ewe's or goat's milk by HPLC. *Chromatographia* 42, 181-184. https://doi.org/10.1007/BF02269650

30. Struff, W.G., Sprotte, G. (2008): Bovine colostrum as a biologic in clinical medicine: a review - part II: Clinical studies. *International Journal of Clinical Pharmacology and Therapeutics* 46, 211-225. https://doi.org/10.5414/CPP46211

31. Sturaro, A., Tiezzi, F., Penasa, M., De Marchi, M., Cassandro, M. (2012): Study of milk coagulation properties in multibreed Italian dairy herds. *Acta Agriculturae Slovenica Suppl.* 3, 89-92.

32. Wedholm, A., Hallén, E., Larsen, L.B., Lindmark-Månsson, H., Karlsson, A.H., Allmere, T. (2006): Comparison of milk protein composition in a Swedish and a Danish dairy herd using reversed phase HPLC. *Acta Agriculturae Scandinavica*, Section A, *Animal Science* 56, 8-15. https://doi.org/10.1080/09064700600836745

33. Wu, J.H., Croft, K.D. (2007): Vitamin E metabolism. *Molecular Aspects of Medicine* 28, 437-452. https://doi.org/10.1016/j.mam.2006.12.007

34. Zaborska, A., Król, J., Brodziak, A. (2015): Vitamin E - its function and importance for human. *Food Industry* 69, 28-31.