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Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

The effect of breed and the feeding system on the activity of glycosidases in cow's milk*

Artur Jóźwik[1], Nina Strzałkowska[1,], Paulina Lipińska[1], Maria Markiewicz-Kęszycka[2], Małgorzata Łysek-Gładysińska[3], Barbara Wróblewska[4], Iwona Stanisławska[5]**

[1] Institute of Genetics and Animal Breeding PAS in Jastrzębiec, Postępu 36A, 05-552 Magdalenka, Poland

[2] School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland

[3] Department of Cell Biology and Electron Microscopy, Institute of Biology, Jan Kochanowski University in Kielce, Świętokrzyska 15, 25-406 Kielce, Poland

[4] Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima10, 10-748 Olsztyn, Poland

[5] College of Rehabilitation in Warsaw, Kasprzaka 49, 01-234 Warsaw

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The aim of the study was to estimate the influence of the breeding and feeding system on the glycosidase activities in the cow's milk.
Experiment was carried out on three breed milking cow. Cows were divided into two groups: 1st-maintained at winter (TMR-system); 2nd-summer feeding system (pasture and TMR-system). Activities of β-glucuronidase, β-glucosidase, β-galactosidase, hexosaminidase, α-glucosidase and mannosidase in milk were estimated. Activities of all examined glycosidases in whole milk were significantly higher during summer season in milk cow. Differences in β-Gal and α-Glu activity ($P \leq 0.01$), in all samples between feeding seasons were observed. Negative correlations between estimated enzymes and milk yield traits (fat, protein and casein) after summer feeding were

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**Corresponding author: n.strzalkowska@ighz.pl

observed, that may indicate an increase derivative processes in high-yielding dairy cows. Those results show that change of feeding systems in dairy cows leads to increase of enzyme activities in milk which influences on intolerances of lactose in milk.

KEY WORDS: glycosidases / intolerance lactose / lysosomal enzymes / milk

Milk is one of the essential products in the human diet, rich in nutritive components [Jóźwik *et al.* 2010b, Metera *et al.* 2010]. Milk is a complex mixture composition of reflects the activities of distinct secretion and transport processes of the mammary gland and mirrors the differing nutritional requirements of mammalian neonates [Jóźwik *et al.* 2010a, Strzałkowska *et al.* 2009a,c]. The newly discovered milk proteins including a glycoprotein designated glycolactin with, as well as glycohydrolases, e.g. α-glucosidase, β-glucosidase and β-glucuronidase appear to play a role in health protection of human, particularly in case of HIV and other virus infection [Vijayalakshmi *et al.* 1997, Wang *et al.* 2000, Hickey 2012, Vieira *et al.* 2013]

Many people experience gastrointestinal disorders after ingestion of milk or milk products. The clinical manifestation can be caused by cow's milk proteins allergy and lactose intolerance. There are two different diseases with different pathogenesis. In Poland about 18-20% of population suffers from lactose intolerance. In these cases hydrolysates without lactose are prescribed to use. However, the routine exclusion lactose in children with CMA is unjustifiable [Wróblewska, et al., 2007]. It is known that majority of those people suffered β-glycosidase deficiency during the first or second decade of their lives [Turnbull 2000, Casellas *et al.* 2010, Brown-Esters *et al.* 2012]. The milk and dairy products with hydrolysed-lactose and containing higher activity of glycosidases are recommended as milk substitutes for those lactose-intolerant individuals. There have been many studies of the hydrolysis of lactose in the milk [Miller and Brand 1980, Panesar *et al.* 2006, Sener *et al.* 2006, Wróblewska, et al., 2007]. However, only a few of these studies investigated the production of oligosaccharides through a transglycosylation reaction during the hydrolysis of lactose in milk [Chen *et al.* 2002, Karasová *et al.* 2002, Hickey 2012]. Galactooligosaccharides can be produced from the milk lactose through enzymatic transglycosylation reactions of such glycosidases as β-galactosidase, α-glucosidase, β-glucosidase. We suppose that activity of these enzymes may influence the chemical composition, technological properties, nutritional value and the quality of the milk, mainly with respect to the taste. The similar effect was determined as a result of the cross-linking reaction of cow's milk proteins with microbial transglutaminase. The obtained results proved significant impact of enzymatic processing on the sensory quality and decrease of immunoreactivity of the yoghurt samples [Wróblewska, *et al*. 2011].

In the literature, there is only some information concerning the influence of feeding on the activity of lysosomal enzymes in the cow's milk. Thus, the aim of this study was to estimate the influence breed and the feeding system on the activity of glycosidases in the whole cow's milk.

Material and methods

Animals and feeding procedures

The studies were conducted on three cows breed: Polish Holstein-Friesian, Montbeliarde and Jersey cows (n=20 in each group) from the commercial dairy farm in Wielkopolska region in Poland. Milk samples were taken once at the end of April, and at the end of September. Only cows with healthy udders according to actual Polish Standard for raw milk were considered.

During autumn/winter (A/W) season all cows were maintained on the typical winter ration (corn silage, grass silage and meadow hay + concentrates with mineral and vitamin premix). During spring/summer (S/S) season a typical summer feeding was applied (grazing on grass pasture + concentrates with mineral-vitamin premix).

Biochemical analysis

Using substrates from SIGMA-ALDRICH Co., the activities of β-glucuronidase (BGRD EC 3.2.1.31), α-glucosidase (α-Glu EC 3.2.1.20), β-glucosidase (β-Glu EC 3.2.1.21), β-galactosidase (β-Gal EC 3.2.1.23), β-N-acetylhexosaminidase (HEX EC 3.2.1.52) and mannosidase (MAN EC 3.2.1.24), were assayed according to Barrett and Heath [1972] with, p-Nitrophenyl-*β-D-glucuronide,* p-Nitrophenyl-α-D-glucopyranoside, p-Nitrophenyl-*β*-D-glucopyranoside and p-Nitrophenyl-*β*-D-galactopyranoside, N-acetyl-β-D-glucosaminide, p-Nitrophenyl-*β*-D-mannopyranoside respective substrates. The chemical composition of milk was determined using IR-spectrophotometer Milkoscan™ FT2 (Foss, Hillerod, Denmark).

The enzyme activities were measured after incubation at 37°C and expressed in nmol/mg of total protein/h. To express the enzyme activities, protein content of milk was determined by the method cited by Krawczyński and Osiński [1967] with bovine serum albumin as standard. The research obtained acceptance of the Local Ethics Commission for Experimentation with animals No 27/2009.

Statistical analysis

All of the data were analysed by the least-squares mean method using the GLM procedures in SAS, Version 9.1 for MS Windows (SAS, SAS/STAT 2002-2003) using the model which includes fixed effect of the interaction breed × date of sampling.

Results and discussion

The results of milk composition for all breeds are presented in Table 1. No statistically significant differences in milk composition were found between the analysed breeds. The greatest percentage contents of fat and lactose were recorded in milk coming from Jersey cows in both investigated feeding periods. In turn, statistically highly significant differences ($P \leq 0.01$) were shown in the chemical composition of milk in relation to the feeding period. The greatest changes were observed in milk

Table 1. Physico-chemical characteristics of milk during spring/summer (S/S) and autumn/winter (A/W) season in all estimate cow' breed

Item		Polish Holstein Friesian		Jersey		Montbeliarde	
		mean	SD	mean	SD	mean	SD
Fat (%)	S/S	4.18	0.69	4.56	0.52	3.95	0.89
	A/W	4.15	0.76	4.85	0.86	4.17	0.92
		ns		ns		ns	
Protein (%)	S/S	3.61	0.34	3.79	0.3	3.48	0.35
	A/W	3.07	0.38	2.83	0.19	3.19	0.32
		ns		**		ns	
Casein (%)	S/S	2.99	0.25	3.16	0.25	2.78	0.26
	A/W	2.50	0.31	2.29	0.23	2.64	0.33
		ns		**		ns	
Lactose (%)	S/S	4.70	0.30	4.73	0.2	4.64	0.27
	A/W	4.89	0.18	4.98	0.26	4.92	0.15
		ns		ns		ns	
Citric acid (%)	S/S	0.14	0.02	0.14	0.02	0.17	0.03
	A/W	0.15	0.03	0.18	0.04	0.18	0.03
		ns		*		ns	
FFA (mEq/l)	S/S	0.49	0.16	0.38	0.24	0.56	0.4
	A/W	0.69	0.25	0.72	0.42	0.83	0.18
		**		**		**	
Acidity (°SH)	S/S	20.50	1.35	20.69	1.38	19.38	2.71
	A/W	17.95	1.43	16.90	1.45	19.86	2.03
		ns		ns		ns	

Significant at *P<0.05 and **P<0.01 between season system (S/S – spring and summer feeding and A/W – autumn/winter feeding). ns – non-significant.

of Jersey cows. A statistically significant (P≤0.001) reduction from 3.79 to 2.83 and 3.16 to 2.29, respectively, was recorded for the percentage contents of protein and casein following A/W feeding in comparison to the S/S feeding period. At the same time after the A/W feeding contents of citric acid and FFA increased in milk of Jersey cows by 24% and 86%. Moreover, a statistically significant (P≤0.01) increase in FFA levels was found in milk of all the analysed breeds. Table 2 shows results concerning the activity of glycosidases in milk of cows from all the studied breeds. Activity of analysed enzymes in milk of individual breeds was similar and no differences were observed, except for mannosidase. A significantly higher MAN activity (P≤0.01) was recorded in milk of Montbeliarde cows in comparison to the other investigated breeds. A significant effect of the feeding period on changes in glycosidase activity was shown in milk of the examined cows. An increase was recorded in the activity of the analysed glycosidases following the S/S feeding period. A statistically highly significant increase (P<0.01) was found for β-galactosidase (β-Gal) and α-glucosidase (α-Glu) in milk of all the analysed breeds. Moreover, a statistically significant increase (P≤0.05) was recorded for β-glucosidase activity (β-Glu) in milk of Jersey and Montbeliarde cows.

Table 2. The activity (nmol/mg protein/h) of glycosidases in cow's milk

Item		Polish Holstein Friesian		Jersey		Montbeliarde	
		mean	SD	mean	SD	mean	SD
BGRD	S/S	0.20	0.01	0.21	0.03	0.19	0,02
	A/W	0.18	0.01	0.17	0.02	0.17	0,01
		ns		ns		ns	
β-Gal	S/S	1.20	0.10	1.24	0.13	1.10	0,08
	A/W	0.92	0.13	0.94	0.08	0.89	0,04
		**				**	
β-Glu	S/S	1.08	0.08	1.11	0.1	1.10	0,08
	A/W	0.91	0.09	0.88	0.08	0.9	0,04
		ns		*		*	
HEX	S/S	9.3	0.63	9.65	1.17	8.63	0,63
	A/W	7.7	0.69	8.32	2.6	7.28	0,36
		ns		ns		ns	
α-Glu	S/S	1.21	0.08	1.28	0.17	1.18	0,09
	A/W	0.95	0.09	0.99	0.1	0.96	0,06
		**		**		**	
MAN	S/S	2.53^{A}	0.16	2.61^{A}	0.25	4.90^{B}	0,48
	A/W	2.25^{A}	0.16	2.28^{A}	0.27	4.25^{B}	0,21
		ns		ns		ns	

Significant at *P<0.05 and **P<0.01 between season system (S/S – spring and summer feeding and A/W – autumn/winter feeding). ns – non-significant.
^{AB}Between breeds, in the same rows, means bearing different superscripts differ significantly at P<0.01.

A greater activity of β-glucuronidase (BGRD), N-acetyl-hexosaminidase (HEX) and mannosidase (MAN) following S/S feeding was not confirmed statistically. It is of interest that a higher activity (P≤0.01) of MAN was observed in milk of Montbeliarde cows in comparison to the other analysed breeds. The chemical composition of milk is connected with the enzymatic activity of analysed glycosidases. The greatest number of statistically confirmed correlations between glycosidases and chemical composition parameters of milk after S/S feeding was found in milk of Jersey cows (Fig. 1). A comparable number of statistically significant correlations were observed in milk of Montbeliarde cows. It was shown that the percentage contents of fat, protein and casein were negatively correlated with all the investigated enzymes following S/S feeding (Fig. 1). The dependence between the percentage content of lactose and theactivity of analysed glycosidases is determined by the effect of the genetic factor, although it was only after S/S feeding. Positive correlations between lactose and glycosidases were observed in milk of Montbeliarde cows and PHF cows, except for BGRD. In turn, negative correlations were shown for the discussed parameters in milk of Jersey cows (Fig. 1). Significant correlations between FFA and glycosidases were found only in milk of PHF cows. A markedly lower number of correlations were recorded in milk of cows after A/W feeding than following S/S feeding. Statistically significant

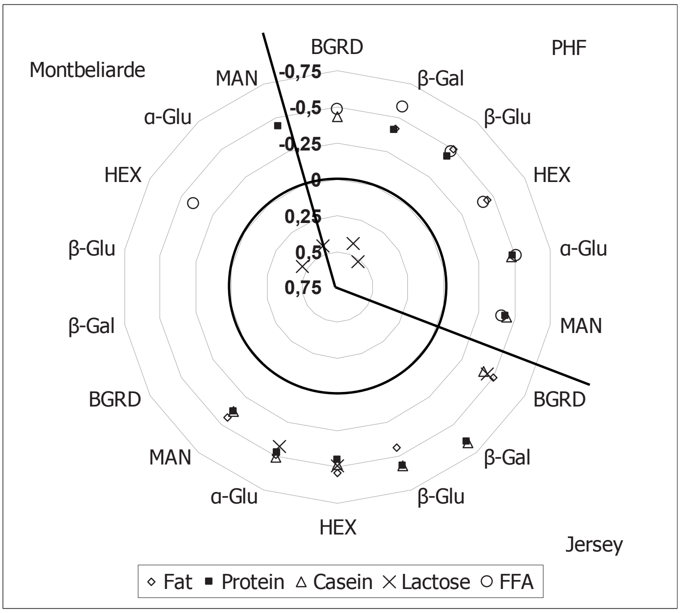

Fig. 1. Significant Pearson correlation (P<0.05) between enzymes and chemical parameters in cow's milk after spring and summer (S/S) feeding.

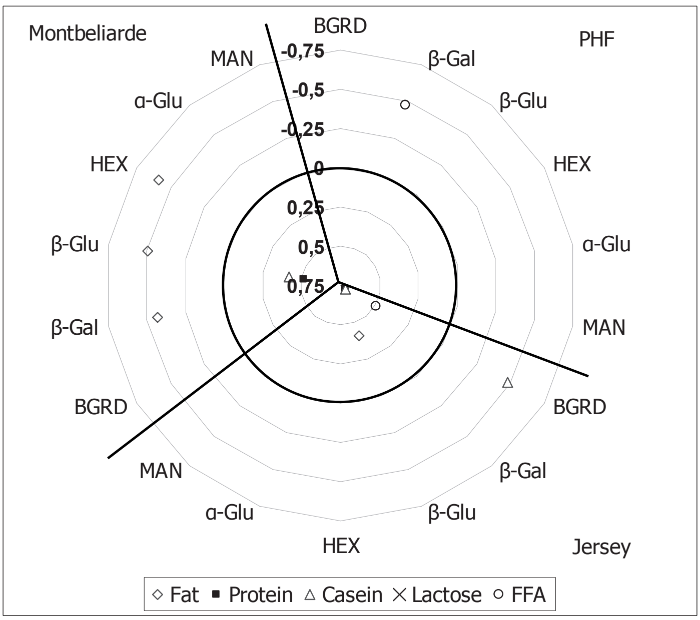

Fig. 2. Significant Pearson correlation (P<0.05) between enzymes and chemical parameters in cow's milk after autumn/winter (A/W) feeding.

correlations were found between protein and casein contents and HEX (P< 0.01) and β-Glu (P≤0.05) in Montbeliarde milk after S/S feeding (Fig. 2). It is of interest that no such correlations were observed in the other tested breeds. A similar effect was shown for acidity and β-Gal, β-Glu and HEX (P≤0.05). Following A/W feeding a greater effect of the genetic factor (breed) on correlations between the analysed enzymes and the chemical composition was observed than it was in the case of S/S feeding. No similar dependencies were shown between the investigated enzymes and the chemical composition of milk in the studied breeds following A/W feeding (Fig. 2).

Milk is a secretion of the mammalian gland and is principally composed of water, fat, protein (largely casein and whey) and carbohydrates [Jóźwik *et al*. 2010a,b, Strzałkowska *et al.* 2010, 2014]. Milk contains many types of oligosaccharides and glycoconjugates, such as glycoproteins and glycolipids, some of which are biologically active and protect against microbes, viruses, and toxins [Szwajkowska *et al.* 2011, Jóźwik *et al.* 2012a,b]. The physico-chemical composition of milk and the activity of glycosidases recorded in these analyses are determined both by genetic factors (breed) and environmental factors (feeding period). The feeding period applied in this study for dairy cows had no significant effect on the physico-chemical composition of milk produced by PHF and Montbeliarde cows, while an increase was only recorded for FFA concentrations in milk of both breeds in the A/W feeding period (Tab. 1). Milk derived from Jersey cows in comparison to that produced by PHF and Montbeliarde cows was characterised by a considerable variation in the physico-chemical composition between the S/S and A/W feeding periods. In the spring-summer (S/S) feeding period, when animals grazed in the pasture, a higher concentration was recorded for protein and casein in milk produced by cows of this breed. The increase in the concentration of the protein fraction in milk of Jersey cows in that period was connected both with genetic predispositions of this breeds to produce milk with a high concentration of the basic components, as well as feeding, which in the S/S period was characterised by a high protein level in the feed ration. Similar results were reported by Ferris *et al.* [2001] and Metera *et al*. [2010], who demonstrated that protein level in milk is increased when cows are given access to pasture. Those authors showed that higher protein and casein levels and lower contents of lactose and fat were observed in milk organic farm when compared to milk produced in the conventional farming system. Trends observed for lactose levels recorded in milk of cows coming from the analysed breeds were similar to those reported by Strzałkowska *et al.* [2009b, 2010]. Lactose is the most dominant soluble glycan structure present in bovine milk. Many people suffer from gastrointestinal problems due to the lactose content in milk or dairy products [Brown-Esters *et al.* 2012, Selvarajan and Mohanasrinivasan 2015]. Intestinal digestion of lactose involves breakdown of lactose into glucose and galactose by membrane bound lactase, located on the brush border of the small intestine. The resulting monosaccharides are absorbed into the portal circulation. In people with lactose maldigestion, a portion of lactose is not digested in the small intestine; it passes into the large intestine where it is fermented by colonic microflora [Wilson

2005, Husain 2010]. Glycosidases are enzymes that are able to hydrolyse glycosidic bonds in oligo- or polysaccharides and hetero-glycosides. They are widely distributed in all organisms, but their physiological function is not always fully recognized. In some cases they are able to catalyse the opposite direction of hydrolysis [Husain 2010, Vieira *et al.* 2013]. Enzymatic hydrolysis of lactose is an important biotechnological process, because the hydrolysed products may be consumed by lactose maldigesters. The modified hydrolysed whey product was incubated with Lactozyme to change the chemical composition of saccharides. The increased level of glucose (83.5%) in hydrolysate was observed [Wróblewska *et al*. 2007]. Glycosidases hydrolyse lactose into monosaccharides, which are more readily metabolised than lactose [Jóźwik *et al.* 2008, Husain 2010]. The most important enzyme is β-d-galactosidase (lactase) which hydrolyses lactose into glucose and galactose.

Results presented in Table 2 showed that β-Gal activity was determined by feeding. A change in the feeding system to pasture grazing resulted in a significant increase in lactase activity ($P \leq 0.01$) in milk of all analysed breeds. In our earlier studies similar dependencies were shown between the feeding system and the activity of lysosomal enzymes in milk of PC cows [Jóźwik *et al.* 2008]. Moreover, a significant increase in the activity of α-Glu ($P \leq 0.01$) and β-Glu ($P \leq 0.05$) was shown in all analysed breeds, except for PHF in the summer feeding period. The change of the feeding season had no effect on changes in the activity of BGRD, HEX and MAN in milk of all analysed breeds. Earlier studies by Jóźwik *et al.* [2008] showed a significant increase in the activity of lysosomal enzymes. Cows of the PC breed exhibited a lower lactation yield in comparison to the investigated breeds. Pasture grazing in the vegetation period may cover the nutrient requirements in cows of this breed. Perhaps the increase in the activity of the enzymes in the milk of cows was determined by their access to pasture. A higher activity of glycosidases in milk of cows kept on the pasture was probably connected with more intensive degradation processes in the case of glycoconjugates and oligosaccharides, which are biologically active. The investigated glycosidases are enzymes found in animal, bacterial and plant cells. Husain [2010] showed that galactosidases are widely distributed in plant tissues. These enzymes have been shown to be involved in a number of biological processes, including plant growth, fruit ripening and the hydrolysis of lactose. Interesting results were recorded for correlations between the investigated glycosides and the chemical composition of milk. A considerable variation was shown for these dependencies between the investigated feeding seasons. A greater number of significant correlations was shown during the grazing season than in the winter season (Fig. 1-2). Negative correlations were recorded between the analysed glycosidases, except for BGRD, and the percentage contents of fat, protein and casein for all investigated breeds during pasture grazing. Negative correlations were also observed between the activity of glycosidases and the concentration of FFA and acidity in milk of all investigated breeds, except for α-Glu and MAN in milk of Montbeliarde cows. We need to stress the interesting fact that a lower number of correlations was recorded during winter feeding. In milk of Montbeliarde cows significant positive correlations were observed between the activity of β-Glu and HEX and the percentage contents of protein and casein as well as milk acidity. Available literature provides no data on correlations between the activity of investigated enzymes and the chemical composition of cows' milk. Because no information is presently available concerning the specific identity of the glycoconjugates and oligosaccharides involved, or the degree to which they are hydrolysed, the physiological significance of estimated glycosidases in cows' milk is unclear. Panicke *et al.* [2000] showed dependencies between the activity of lysosomal enzymes in the blood of cows and contents of protein and urea in milk. Especially a high or a low content of protein in the food ration affects the lysosomal enzyme activities considerably.

We suspect that the higher activities of β-Gal, β-Glu and α-Glu might lead to enhanced degradation processes of lactose, the content of which was found to be significantly higher in the milk during the summer than during the winter feeding period. Thus, milk obtained during summer feeding may have a positive effect on consumers' health. Feeding period (winter vs. summer) not only affected the chemical composition of milk, but also the activity of lysosomal glycosidases.

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