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SHORT REPORT

Activity of selected glycosidases of whole milk in cows as related to feeding season (autumn/winter *vs* spring/summer)*

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**Glycosidases affect the chemical composition and nutritive value of milk.
The activities of α-glucosidase, β-glucosidase, β-galactosidase and mannosidase were determined in whole milk of Polish Red cows (with mean milk yield 4200 kg/lactation) at the end of autumn/winter (A/W) and then at the end of spring/summer (S/S) feeding season – 23 and 19 cows, respectively. Feeding was characteristic of seasons: silages, hay and concentrates during A/W or grazing and concentrates during S/S. Activities of all glycosidases were found significantly higher in milk of cows at the end of S/S than at the end of A/W season.**

KEY WORDS: cow / feeding season / glycosidases / lysosomal enzymes / milk

Milk is a complex mixture composition reflecting activities of distinct secretion and transport processes of the mammary gland and mirrors the differing nutritional requirements of mammalian neonates [McManaman and Neville 2003, Wiederschain

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and Newburg 2001]. The milk proteins including a glycoprotein termed glycolactin, as well as glycohydrolases, e.g. α-glucosidase, β-glucosidase and β-glucuronidase appear to play a role in health protection of humans, particularly in case of HIV and other virus infection [Bolt *et al.* 1999, Wang *et al.* 2000].

Many people experience gastrointestinal disorders after ingestion of milk or milk products. It is known that majority of them suffers from β-glycosidase deficiency during the first or second decade of life. The milk and dairy products with hydrolysed lactose and higher concentration of glycosidases are recommended as milk substitutes for lactose-intolerant individuals [Chen *et al.* 2002, Splechtna *et al.* 2001]. Out of many studies of the hydrolysis of lactose in milk only few were devoted to the production of oligosaccharides through a transglycosylation reaction. Galactooligosaccharides can be produced from the milk lactose through enzymatic transglycosylation reactions with e.g. β-galactosidase, α-glucosidase, or β-glucosidase. It can be expected 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.

In the literature, no information was found on the influence of season of the year (*i.e*. winter *vs* summer feeding) on the activity of lysosomal enzymes in the cow's milk. This report presents a preliminary attempt at estimating the relationship between the typical winter or summer feeding and glycosidases activity in the whole milk of cows.

Material and methods

Polish Red cows were used, maintained at the Polish Academy of Sciences Exprimental Farm for Ecological Agriculture and Preserve Animal Breeding, Popielno. Number of cows, their dairy performance, feeding regimen during autumn/winter (A/W) and spring/summer (S/S) season, milk sampling and statistical evaluation of results are given in the report by Jóźwik *et al*. [2004] concerning the activity of aminopeptidases (in this issue).

Only cows with healthy udders were considered.

Using substrates from SIGMA-ALDRICH Co., the activities of α-glucosidase (α-Glu – EC 3.2.1.20), β-glucosidase (β-Glu – EC 3.2.1.21), and β-galactosidase (β-Gal – EC 3.2.1.23) and mannosidase (MAN – EC 3.2.1.24), were assayed according to Barrett and Heath [1972] with p-Nitrophenyl-β-D-manno-pyranoside, p-Nitrophenyl-α-D-glucopyranoside, p-Nitrophenyl-β-D-glucopyranoside and p-Nitrophenyl-β-D-galactopyranoside as respective substrates. Moreover, the lactose content of milk was routinely determined using MilkoScan 104A/B apparatus.

The enzyme activities were measured after incubation at 37°C and expressed in nmol/mg of total protein/h. To express the enzyme activities, true protein content of milk was determined by the method cited by Krawczyński and Osiński [1967] with bovine serum albumin as standard.

Results and discussion

The results of variance analysis are presented in Table 1. Significant effect of season on the activity of all glycosidases and on the level of lactose was found. Daily milk yield and days-in-milk showed significant effect neither on enzyme activity, nor on lactose content of milk

Table 1. F-values and significance of effect of feeding system and other factors on enzyme activities and lactose content in milk

Effect	Trait				
	α-Glu	β-Glu	β-Gal	MAN	lactose (%)
Season of feeding	50.51***	34.28***	25.92***	9.81**	4.21*
Parity	1.94	1.61	3.47*	0.15	3.12*
Interaction season × parity	2.64	1.62	4.09*	2.36	2.46
Daily milk yield	0.09	0.06	0.04	0.93	0.84
Days in milk	0.15	0.38	1.43	3.65	0.45
R^2	0.56	0.61	0.60	0.38	0.45

*P≤0.05; **P≤0.01; ***P≤0.001.

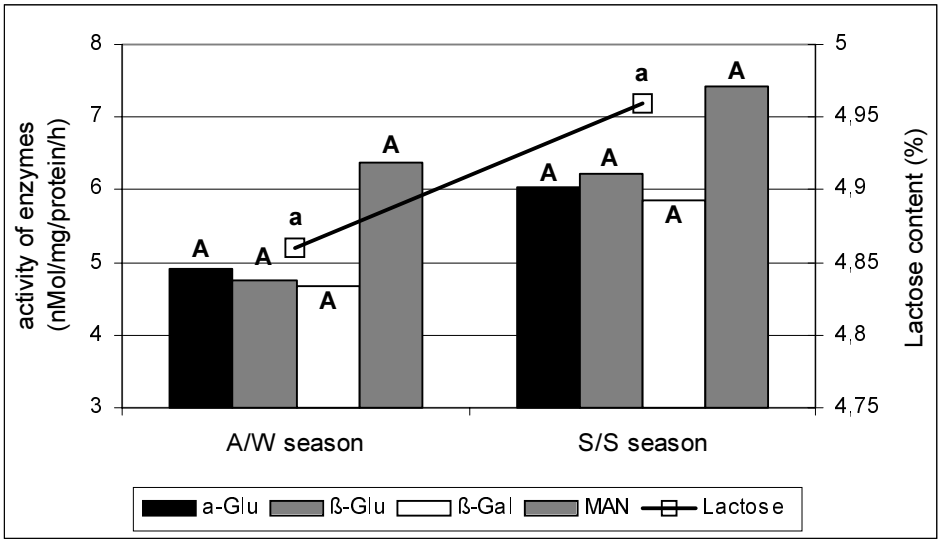

Fig 1. Glycosidases' activity (nMol/mg protein/h) and lactose content (%) of milk yielded by cows at the end of autumn/winter (A/W) or spring/summer (S/S) feeding.
[aA] Means for all enzyme activities at the end of A/W differ significantly from those found at the end of S/S at P≤0.01, while means for lactose content are significantly different at P≤0.05.

All enzyme activities and content of lactose in A/W appeared significantly lower than in S/S season (Fig. 1). Higher activity of milk glycosidases of cows maintained on pasture was probably connected with more intensive catabolic processes of glycoconjugates and oligosaccharides which are biologically active. One of the most important oligosaccharydes is lactose. Milk oligosaccharides are absorbed in the small intestine and then hydrolysed by lysosomal glycosidases [Messer and Urashima 2002]. It is well known, that many people suffer from gastrointestinal problems due do the lactose content of milk or milk products. Human infants can develop an intolerance response to lactose accompanied with symptoms similar to certain protein allergies [Gryboski 1991]. Glycosidases hydrolyse lactose into glucose and galactose – two monosaccharides, which are easier metabolizable than lactose. Milk products containing hydrolysed lactose can be consumed by lactose-intolerant people [Tanriseven and Dogan 2002].

We suspect that the higher activities of α-Glu, β-Glu, β-Gal and MAN might lead to higher degradation processes of lactose, content of which was found significantly higher in the milk during the summer than during the winter feeding (Fig. 1). Thus, milk obtained during summer feeding may have positive effect on consumers' health. Feeding system (winter *vs* summer) not only affected the chemical composition of milk, but also the activity of lysosomal glycosidases.

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Aktywność wybranych glikozydaz w pełnym mleku krów po jesienno-zimowym i wiosenno-letnim okresie żywienia

S t r e s z c z e n i e

Celem badań było określenie aktywności α-glukozydazy, β-glukozydazy, β-galaktozydazy i mannozydazy w pełnym mleku krów w dwóch porach roku (sezonach żywienia) – jesienno-zimowej i wiosenno-letniej (odpowiednio A/W i S/S). Wymienione enzymy mogą wpływać na skład chemiczny i wartość odżywczą mleka. Badania przeprowadzono na krowach rasy pc o przeciętnej wydajności około 4200 kg mleka/laktację. Stwierdzono istotnie wyższą aktywność wszystkich badanych enzymów w pełnym mleku krów po okresie żywienia letniego niż po okresie żywienia zimowego.