Animal Science Papers and Reports vol. 22 (2004) no. 4, 667-672
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

SHORT REPORT

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

Artur Jóźwik, Emilia Bagnicka, Anna Śliwa-Jóźwik, Nina Strzałkowska, Krzysztof Słoniewski, Józef Krzyżewski, Adam Kołątaj

Polish Academy of Sciences Institute of Genetics and Animal Breeding, Jastrzębiec, 055-52 Wólka Kosowska, Poland

(Received October 25, 2004; accepted December 7, 2004)

Aminopeptidases play a crucial role in animals' metabolism. Their activity in degrading proteins and peptides is also important in milk processing industry.
Activity of AlaAP, LeuAP and ArgAP was determined in whole milk of Polish Red cows (mean yield 4200 kg per 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 typical of seasons: silages, hay, and concentrates during A/W, or grazing and concentrates during S/S. The activity of all three enzymes was found significantly higher in milk of cows at the end of S/S than at the end of A/W season.

KEY WORDS: aminopeptidases /cow / feeding season / milk

Aminopeptidases take part in maintaining cell homeostasis and are responsible for turnover of proteins, among others in their enzymatic hydrolysis. Changes in

*Supported by the State Committee for Scientific Research (KBN), grant 2 PO6Z 05826.

aminopeptidases activity reflect the disturbances in homeostatis and indicate the development of sickness [Schomburg *et al.* 2000, Somers *et al.* 2003]. Moreover, aminopetidases are crucial in degradation of proteins and peptides in milk [Murray *et al.* 1996, Izawa *et al.* 1997]. Infection of mammary gland (*mastitis)* leads to increase in the somatic cell count and the proteolytic potential of milk [Barbano 1988]. Higher activity of aminopeptidases was observed in cow's milk from infected mammary glands [Larsen *et al.* 2004]. A number of proteolytic enzymes have been known for many years to be present in milk of healthy cows. Although in many cases the physiological functions they have in milk still remain unclear, their presence there is of considerable importance for the dairy industry [Gagnaire *et al.* 2001].

Increased activity of aminopeptidases may lead to degradation of milk proteins and thus affect the milk nutritional value and technological properties. In this report results are presented of a preliminary attempt at estimating the effect of winter *vs* summer feeding on activity of three aminopeptidases in the whole milk of cows.

Material and methods

Polish Red cows were used maintained at the Polish Academy of Sciences Experimental Farm for Ecological Agriculture and Preserve Animal Breeding, Popielno, yielding on the average 4200 kg milk cow/lactation.

Only cows with healthy udders were considered.

During autumn/winter (A/W) season 23 cows were maintained on the typical winter ration (corn silage, grass silage and meadow hay + concentrates with mineral-vitamin premix). During spring/summer (S/S) season a typical summer feeding was applied (grazing on grass pasture + concentrates with mineral-vitamin premix). Becouse of some health problems four cows during S/S season received an antibiotic treatment and were excluded from further investigations. This caused that over S/S season the number of cows was reduced to 19.

At the end of each of two seasons milk samples were taken once from each cow (at the end of A/W and S/S in May and in September, respectively).

For statistical evaluation four parity classes were considered including lactations I, II, III, and later.

The activities of alanine aminopeptidase (AlaAP – EC 3.4.11.2), leucine aminopeptidase (LeuAP – EC 3.4.11.1), and arginyl aminopeptidase (ArgAP – EC 3.4.11.6) were assayed according to McDonald and Barrett [1986] with L-Alanine β-naphtylamide, L-Leucine β-naphtylamide and L-Arginine β-naphtylamide, as substrates, respectively. The enzyme activities were measured after incubation at 37°C and expressed in nMol/mg of total protein/hour. The total protein content of whole milk was measured routinelly with MilkoScan 104A/B apparatus.

Moreover, to express the enzymes activities the true protein content of milk was determined by the method described by Krawczyński and Osiński [1967] with bovine serum albumin as standard.

The data were evaluated using GLM procedure of SAS v. 8e [SAS Institute, 1999-2000]. The following statistical model was used:

$$y_{ijk} = \mu + S_i + P_j + S \times P_{ij} + b_1(x_1 - DD)_{ijk} + b_2(x_2 - MY)_{ijk} + e_{ijk}$$

where:

y_{ijk} – activity of enzyme;
μ – overall mean;
S_i – fixed effect of season;
P_j – fixed effect of parity;
$S \times P_{ij}$ – fixed effect of interaction season × parity;
DD – mean number of days-in-milk from calving to sampling;
b_1 – linear regression coefficient on days-in-milk;
MY – mean milk yield;
b_2 – linear regression coefficient on daily milk yield;
e_{ijk} – random error.

Results and discussion

Table 1 summarizes the results of variance analysis of effect of season and other factors on traits investigated. Highly significant effect of season was found on the activity of all aminopeptidases, while parity, and mean daily milk yield showed no such effect.

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

Effect	Trait			
	AlaAP	LeuAP	ArgAP	Total protein (%)
Season of feeding	26.26***	20.4***	8.51**	11.71**
Parity	1.12	1.93	0.27	0.57
Interaction season × parity	7.01***	4.17*	0.27	0.27
Daily milk yield	0.74	2.51	0.04	2.37
Days in milk	3.77	12.30**	1.11	2.07
R^2	0.63	0.57	0.36	0.40

* P≤0.05; **P≤0.01; ***P≤0.001.

Figure 1 shows a significantly higher activity of all investigated whole milk aminopeptidases in S/S than in A/S season. Total protein content of milk was also significantly higher in S/S season.

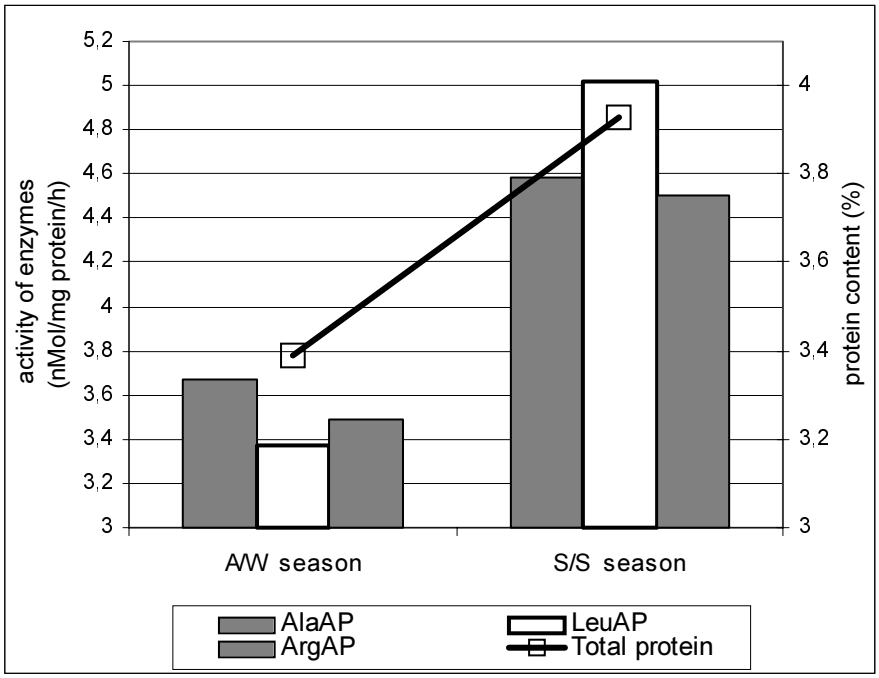

Fig. 1. Aminopeptidases' activity (nMol/mg of protein/h) and total protein content (%) of milk yielded by cows at the end of autumn/winter (A/W) or spring/summer (S/S) feeding. In all cases means for A/W differ significantly from those for S/S at $P \leq 0.01$.

Aminopeptidases remove N-terminal amino acids from oligopeptides and proteins, preferentially alanine, leucine, arginine, and fenyloanaline [McDonald and Barett 1986, Śliwa-Jóźwik *et al.* 2004] and show high potential in removing the bitterness of milk by hydrolysis of polypeptides [Saha and Hayashi 2001]. Aromatic amino acids, which cause bitter taste of milk and its products, may be removed from hydrolysed bitter peptides [Izawa *et al.* 1997]. Cheeses made of aminopeptidase-deficient milk were found stiffer and more bitter [Saha and Hayashi 2001]. High activity of aminopeptidases is necessary when strains of lactic acid bacteria are used for cheese-making [Prost and Chamba 1994].

The results presented here show significantly higher activity of three aminopeptidases in milk of cows maintained on pasture than in those kept indoors during winter. We anticipate that milk obtained during summer time may have better nutritional, technological and taste parametres than that yielded in winter. Possibility of removing certain amino acids from milk may lead to better use of milk and its products by consumers, especially those with allergies and gastrointestinal problems. Thus, milk obtained during summer feeding season may positively affect the consumer's health.

Season was found to affect both protein content and aminopeptidases activity of milk. We suppose that activity of aminopeptidases has an impact on nutritional value and technological properties of milk and quality of milk products.

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Aktywność wybranych aminopeptydaz 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 AlaAP, LeuAP, ArgAP w pełnym mleku krów w dwóch porach roku (sezonach żywienia) – jesienno-zimowej i wiosenno-letniej (odpowiednio A/W i S/S).

Wymienione enzymy pełnią ważną rolę w procesach katabolicznych, odcinając poszczególne aminokwasy od białek i peptydów. Aktywność ich określono w mleku krów rasy cp o przeciętnej wydajności około 4200 kg mleka w laktacji, raz pod koniec sezonu jesienno-zimowego i raz pod koniec sezonu wiosenno-letniego (odpowiednio 23 i 19 krów).

W pełnym mleku krów pozyskiwanym w końcu okresu żywienia letniego aktywność wszystkich badanych aminopeptydaz, jak również zawartość białka w mleku okazały się wysokoistotnie wyższe niż w mleku z końca okresu żywienia zimowego.