The effect of feeding with linseed cake *versus* extracted rapeseed meal on the activity of lysosome's enzymes in blood serum of dairy goat

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Abstract

The aim of the study was to evaluate the activity of lysosomal enzymes from families: glycosidases such as β-galactosidase (BGAL), β-glucuronidase (BGRD), β-glucosidase (BGLU), N-acetyl-hexosaminidase (HEX), α-glucosidase (AGLU) and α-mannosidase (MAN) as well as aminopeptidases such as alanine aminopeptidase (AlaAP), leucine aminopeptidase (LeuAP), arginine aminopeptidase (ArgAP) and acid phosphatase (AcP) in serum of dairy goats fed the diet with linseed cake or rapeseed meal supplementation. The study was conducted on 16 dairy goats of the Polish White Improved (PWI) breed and lasted for 6 weeks. The goats were divided into two groups, analogous to each other, according to age of goats. The basic diet consisted of corn silage, wilted grass silage and concentrates (oats, triticale, extracted rapeseed meal), supplemented with mineral and vitamin mixtures. The diet of experimental group was supplemented with linseed cake instead of extracted rapeseed meal. The serum samples were taken twice during the experiment: three weeks after the start of the experiment and at the end of supplementation. The milk, protein and lactose yields in control group tended to decrease while in experimental group they increased in the course of the experiment in spite of the fact that the experiment was conducted after the peak of lactation. The obtained results suggested that BGRD and BGAL activities were up-regulated while the other glycosidase MAN was down-regulated after linseed cake in compare to extracted rapeseed meal supplementation. According to aminopeptidases the linseed cake supplementation caused down-regulation of LeuAP and up-regulation of ArgAP activities. The high, positive correlation was found between two aminopeptidases (ArgAP, AcP) and milk, protein and lactose content, while high and negative correlation between three glycosidases (BGRD, BGAL and MAN) and milk, protein and lactose content. Milk fat yield was highly and negatively correlated only with MAN activity.

Key words: *blood serum, lysosomal enzymes, linseed cake, extracted rapeseed meal, goat milk.*

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Introduction

Lysosomes were first described in 1955 as a biochemical entities [1] and since then, they have been a favoured candidate for a pathogenic role in connective tissue diseases [2]. Lysosomes are small organelles (0.2 to 0.5 nm diameter), performing critical functions within the human and animal body, present in all cells of animals and containing more than 40 different types of lysosomal enzymes, such as glycosidases, proteases, acid phosphatases, sulfatases and lipases, which are synthesized in the endoplasmic retic-

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ulum and modified in the Golgi apparatus [3]. The lysosomal system plays an important role in the restoration of biochemical homeostasis which can be affected by different stress factors [4]. One of the factors influencing the homeostasis of both humans and animals is their diet. The properly balanced diet (protein to energy rate) is important for ruminants. The extracted rapeseed meal is a commonly used source of protein in animal feeding. Unfortunately, the biological quality of extracted rapeseed meal is low because of high level of antinutritional or even toxic factors (glucosinolates: thioglucosides and their hydrolysis products isothiocyanates and oxazolidinethiones) [5, 6]. Linseed cake could be the rich source of protein in animal diet, however, it also contains antinutritional substances like cyanogenic glucosides, but low level [7]. Moreover, linseed cake is the richest source of fitoestrogens, being strong antioxidants which help to keep the redox processes at the optimal level. They positively influence the functioning of the reproductive system and show antitumor activity, simultaneously having a beneficial effect on cardiovascular system diseases and osteoporosis [8-11].

There are some papers on the influence of linseed cake supplementation in the cows' or goats' diets on milk quality [12-15]. On the other hand, there is much information on lysosomal enzymes function and deficiency in humans [16, http://emedicine.medscape.com/article/1182830-overview#aw2aab6b3]. However, there is limited information about the influence of linseed cake diet supplementation on lysosomal enzymes activity in animal cells.

Thus, the aim of the study was to evaluate the activity of lysosomal enzymes from families: glycosidases such as β-galactosidase (BGAL), β-glucuronidase (BGRD), β-glucosidase (BGLU), N-acetyl-hexosaminidase (HEX), α-glucosidase (AGLU) and α-mannosidase (MAN) as well as aminopeptidases such as alanine aminopeptidase (AlaAP), leucine aminopeptidase (LeuAP), arginine aminopeptidase (ArgAP) and acid phosphatase (ACP) in serum and productivity of dairy goats fed the diet with linseed cake vs. extracted rapeseed meal supplementation.

Material and methods

The study was conducted on 16 dairy goats of the Polish White Improved (PWI) breed. The experiment lasted from 12 to 17 week of lactation e.g. in the middle stage of lactation. The average milk yield obtained in 280 days of lactation was about 800 kg, with 3.35% of fat and 3.20% of total protein. Goats were machine milked twice a day. The animals were kept in a loose barn and fed according to the INRA feeding norms [17]. The goats were divided into two groups, analogous to each other, according to age of goats. The basic diet consisted of corn silage, wilted grass silage and concentrates (oats, triticale, extracted rapeseed meal), supplemented with mineral and vitamin mixtures. The diet of experimental group was supplemented with linseed cake instead of extracted rapeseed meal (Table 1). Water was available *ad libitum*. All animals were free from mastitis. According to the results of ELISA test (IDEXX CAEV/MVV Total Ab Screening Test) all goats included in the study were free from caprine arthritis-encephalitis virus infection. The serum samples were taken twice during experiment: three weeks after the start of the experiment and at the end of supplementation. Milk yield was recorded during morning milking and its parameters (fat, protein, lactose content and yield, somatic cell count, urea content) were established.

In the lysosomal fractions of blood serum the activities of alanyl-aminopeptidase (AlaAP – E.C. 3.4.11.2); leucyl-aminopeptidase (LeuAP – E.C. 3.4.11.1); arginyl-aminopeptidase (ArgAP – E.C. 3.4.11.6); acid phosphatase (AcP – E.C. 3.1.3.2); α-glucuronidase (BGRD – E.C. 3.2.1.31); β-galactosidase (BGAL – E.C. 3.2.1.23); β-glucosidase (BGLU – E.C. 3.2.1.21); N-acetyl-β-glucosaminidase (HEX – E.C. 3.2.1.30), α-glucosidase (AGLU E.C. 3.2.1.20) and mannosidase (MAN, EC 3.2.1.25) were determined. The activity of AcP, BGRD, BGAL, BGLU, AGLU, MAN and HEX were measured as 4-nitrophenyl derivatives at 420 nm according to Barrett and Heath's [18] micromethod. The activity of AlaAP, LeuAP and ArgAP were measured as Fast Blue BB salt (4-benzoyloamino-2, 5-diethoxybenzenediazinium chloride) derivatives at 540 nm by the method of McDonald and Barrett [19]. In the blood serum the protein concentration was measured by the modified Total Protein-Biuret Method [20].

The statistical evaluation of results was performed with the CORR and GLM procedures of SAS Version 9.1 for Windows [21], using the model including a fixed effect of time of milk collection and effect of diet interaction.

All procedures involving animals were performed in accordance with the Guiding Principles for the Care and Use of Research Animals, and were approved by the Local Ethics Commission (Permission No. 48/2005).

Table 1. Ingredients of the diets for goats

Ingredients (% in DM)	Diet	
	Control	Experimental
Corn silage	29.7	30.7
Meadow hay	29.2	30.1
Oats (grain)	18.7	19.3
Triticale (grain)	11.2	–
Extracted rapeseed meal*	11.2	–
Linseed cake**	–	19.9

**Chemical composition (% of DM): crude protein 36.5; ether extract 2.4; crude fiber 12.5; crude ash 7.2*
***Chemical composition (% of DM): crude protein 30.9; ether extract 9.2; crude fiber 7.8; crude ash 6.9*

Results and discussion

The linseed cake supplementation had a positive impact on milk, protein and lactose yield in comparison with extracted rapeseed meal supplementation (Table 2). The differences were observed both between control and experimental groups and within experimental group between the times of sampling. The milk yield in control group tended to decrease while in experimental group increased during experiment in spite of the fact that the experiment was conducted after the peak of lactation. These results are in opposition to many studies summarizing by Petit [13], who concluded that the effect of linseed seed supplementation in the dairy cows diet had no or little effect on milk yield. Moreover, Bernard *et al*. [22] presented similar results from the study on dairy goats. However, similar to other studies [13, 22] we did not obtain differences in fat, protein and lactose content and urea level between both groups. In our study the somatic cell count (SCC) in milk was lower in experimental than in control group after 6 weeks of supplementation.

There were differences in activity of three from six studied glycosidases: BGAL, MAN and BGRD and in activity of two from four studied aminopeptidases: LeuAP, ArgAP between control and experimental group, especially after 6 weeks of supplementation. The high, positive correlation was found between two aminopeptidases (ArgAP, AcP) and milk, protein and lactose content, while high negative correlation between three glycosidases (BGRD, BGAL and MAN) and milk, protein and lactose content (Table 3). Milk fat yield was high and negative correlated only with MAN

Table 2. The effect of linseed cake supplementation on lysosome enzymes activity in blood serum

Traits	Group	Sampling		
		1st Ls_{mean}	2nd Ls_{mean}	SE
Milk yield [kg]	Control	1.72	1.50**	0.12
	Experimental	1.85[a]	2.25**[b]	
Protein yield [g]	Control	52.14	58.64*	4.97
	Experimental	59.31	66.88*	
Lactose yield [g]	Control	83.02	72.37**	6.09
	Experimental	86.32[a]	109.68**[b]	
SCC[#]	Control	5.20	5.51*	0.80
	Experimental	5.22	4.98*	
AlaAP	Control	1.42[A]	0.66[B]	0.12
	Experimental	1.71[A]	0.68 B	
LeuAP	Control	2.45[A]	1.57**[B]	0.20
	Experimental	2.61[A]	0.97**[B]	
ArgAP	Control	1.04	0.80**	0.17
	Experimental	1.03	1.37**	
AcP	Control	4.46	5.96	0.85
	Experimental	6.26	7.17	
BGRD	Control	0.37	0.41**	0.06
	Experimental	0.32	0.23**	
βGAL	Control	0.35[A]	0.52**[B]	0.08
	Experimental	0.45[A]	0.75**[B]	
BGLU	Control	0.21	0.32	0.05
	Experimental	0.22	0.32	
HEX	Control	1.72	1.90	0.25
	Experimental	1.78	1.91	
AGLU	Control	1.55	1.80	0.27
	Experimental	1.97	1.38	
MAN	Control	4.44	5.13**	0.43
	Experimental	4.46	3.39**	

a, b, A, B – different letter within rows indicate statistical significance at $p < 0.05$ and $p < 0.01$, respectively
***statistical significance at $p < 0.01$ in columns according to particular traits*
#SCC – milk somatic cell count
SE – standard error

Table 3. Pearson correlation between lysosomal enzymes activity and milk productivity traits

Traits	Milk yield [kg]	Fat yield [g]	Protein yield [g]	Lactose yield [g]
ArgAP	0.51*	–	0.77*	0.51*
AcP	0.44*	–	–	0.44*
BGRD	–0.47*	–	–0.48*	–0.45*
BGAL	–0.42*	–	–0.47*	–0.48*
MAN	–0.43*	–0.51*	–0.54*	–

$*p < 0.05$

activity. Subsequent data suggested that BGRD and BGAL expression was up-regulated while the other glycosidase MAN was down-regulated after linseed cake in comparison to extracted rapeseed meal supplementation. According to aminopeptidases activity the linseed cake supplementation caused down-regulation of LeuAP and up-regulation of ArgAP activity. Lysosomal enzymes are a necessary ones to biological systems as a means of utilizing sugars for energy or have a critical roles in turnover of peptides or others. The degraded macromolecular substrates can be both internalized and endogenous. When the lysosomal enzyme deficiency occurs the un-degraded substrates accumulate within the lysosomes which leads to malfunction of the organs [16]. Thus, the sufficient activities of lysosomal enzymes are crucial for functioning of many organs and whole organisms.

Generally, aminopeptidases participate in protein maturation, activation, modulation, degradation and determination of stability of protein and peptides. Leucyl-aminopeptidase catalyzes the hydrolysis leucine, while ArgAP arginine residues from the N-terminal end of various peptides and proteins [23]. In the presented study the proteolytic enzymes such as leucine aminopeptidase activity decreased while arginine aminopeptidase increased in both groups towards the end of study but in experimental group the activity of enzymes were twice as low (LeuAP) or twice as high (ArgAP) as in the control group. The increase of activity of aminopeptidases suggested an increase in protein degradation in lysosomes and testified to overabundance of protein in the diet [24]. Moreover, Burley *et al.* [25] stated the decrease in AlaAP activity when the increase in protein level in diet occur. On the other hand, the activity of particular enzymes depends on the availability of substratum. It could mean that there are lower leucine and higher arginine residues content in linseed seed cake than in extracted rapeseed meal.

Glycosidases are hydrolase enzymes which catalyze the hydrolysis of glycosidic linkages in polysaccharides and glycosylamines (proteoglycans) as well. Fronczyk [24] found the increase in glycosidases activity in animals fed the diet with low level of protein and he suggested that it is the adaptation of organisms to inconvenient environmental circumstances e.g. deficit of protein. In the presented study BGRD and MAN activity decreased while BGAL activity increased after linseed cake supplementation.

Conclusion

The urea level in milk was similar in both groups and did not exceed referential values which means that both diets were well balanced according to energy/protein ratio. However, the activity of some lysosomal enzymes differed according to the type of diet: with extracted rapeseed meal or linseed cake supplementation. There were strong relationship between some lysosomal enzymes activity and milk yield traits. Moreover, the milk, protein and lactose yield were higher, while the level of SCC was lower after linseed cake than extracted rapeseed meal supplementation. The obtained result suggests that the using of linseed cake in the goats' diet have beneficial impact on productivity and health status of animals.

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