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Influence of starvation and sex on some lysosomal enzymes activity in young dairy cattle

Abstract

The study included 177 Polish Friesian cattle with an over 75% Holstein proportion - 117 heifers and 60 young bulls 250 days old. The animals were progeny of 27 AI Holstein sires. All individuals were housed in a tie stall and fed *ad libitum* silage, hay and concentrate until the 7th month of age. From the 7th to the 8th month of age the animals of both sexes received an *ad libitum* full concentrate diet. Almost in the all cases the activities of lysosomal enzymes were higher in heifers (except NAG, BGAL, AP). The 48 h starvation did not influence AAP, LAP, AP, LL, BGLU activity in bulls and BGRD, BGAL, BGLU and NAG in heifers.

Key Words: cattle, lysosomal enzymes, blood serum

Zusammenfassung

Titel der Arbeit: **Einfluss des Hungers und Geschlechtes auf die lysosomale Enzymaktivität bei Jungrindern**

Die Untersuchungen erfolgten an 117 Färsen und 60 Jungbullen der Rasse Schwarzbunt mit einem Holstein-Friesiananteil von über 75 % ab einem Alter von 250 Tagen. Die Tiere waren Nachkommen von 27 Holstein-Friesianbullen aus der künstlichen Besamung. Die in Ställen gehaltenen Tiere erhielten bis zum 7. Monat Silage, Heu und Kraftfutter ad libitum. Zwischen dem 7. und 8. Monat erhielten die Tiere beider Geschlechter ad libitum Konzentratfutter. Die Blutentnahme erfolgte bei der Hungergruppe vor und nach einer 48stündigen Nüchterungszeit. Bei den Bullen ergaben sich keine Hungerauswirkungen bei den Enzymen: Alanine Aminopeptydase, Leucine Aminopeptydase, Acid Phosphatase, Lyosomal Esterase und β-Glucosidase, während dies bei den Färsen für β-Glucosidase, β-Glucoronidase und N-Acetyl-β-Glucosidase zutraf. Bezüglich des Geschlechtereinflusses zeigten die Färsen in fast allen Fällen größere Enzymaktivitäten als die Bullen.

Schlüsselwörter: Jungrinder, lysosomale Enzyme, Blutserum, Hunger, Geschlecht

Introduction

Numerous opinions confirmed by the results of many research papers point out the importance of the lysosomal complex in the cell, which groups the enzymes degrading the proteins, fats and carbohydrates. In this way the lysosomal complex has been included to a system of adaptation response of animal (HASULIK, 1992; JÓŹWIK et al., 2003 a b; WITEK and KOŁĄTAJ, 2000; WITEK et al., 1999). We already know that lysosomes occur in the protoplasm where, independently of ATP and ubiquitin, the long living proteins are degraded (BERLETT and STADTMAN, 1997). The lysosomal enzymes may also hydrolyze the simple chemical compounds emerging as a result of the catabolism, for further use in the cell (LANKOFF and KOŁĄTAJ, 2001).

In connection with these data, it seemed interesting to estimate the changes of the activity of some model lysosomal enzymes in the blood plasma of young bulls and heifers maintained in farm conditions, fed normally and starved for a short period. Fasting or restriction of food are a specific types of stress burden, which appears quite

often in domestic animals (OHSHITA et al., 1986; WITEK and KOŁĄTAJ, 1998). The aim of this study was to determine the influence of starvation and sex on the activity of some lysosomal enzymes in young cattle.

Material and methods

The study included 177 Polish Friesian cattle with an over 75% Holstein proportion - 117 heifers and 60 young bulls 250 days old. The animals were progeny of 27 AI Holstein sires. All individuals were housed in a tie stall and fed *ad libitum* silage, hay and concentrate until the 7^{th} month of age. From the 7^{th} to the 8^{th} month of age the animals of both sexes received an *ad libitum* full concentrate diet. The blood samples were taken from the neck vein before and after 48 hours starvation between 8 – 10 a.m. to test tubes containing heparin. During starvation the animals had access to water. The samples were centrifuged for 15 minutes at 20.000 g in K – 24 centrifuge. In the blood serum the following lysosomal enzymes were determined: β-glucoronidase (BGRD) - EC 3.2.1.31; β-glucosidase (BGLU) – EC 3.2.1.21; N-acetyl-β-glucosaminidase (NAG) - EC 3.2.1.30; β-galactosidase (BGAL) – EC 3.2.1.23; lysosomal lipase (LL) - EC 3.1.1.2; alanine aminopepdydase (AAP) - EC 3.4.11.2; leucine aminopeptydase (LAP) - EC 3.4.11.1; acid phosphatase (AP) – EC 3.1.3.2; lysosomal esterase (EL) – EC3.1.1.3.

The activity of BGRD, NAG, BGAL, and AP was determined according to the BARRET's method (1972), LAP - by the method of PFLEIDERER and CELLIERS (1963), AAP by the method of PFLEIDERER et at. (1964), EL and LL by the MAIN'S method (1960). The enzymatic activity was expressed in nMol/mg of protein per hour. Plasma protein was estimated according to the method of KIRSCHKE and WIEDERANDERS (1984).

The reagents used were produced by Sigma (Sigma-Aldrich Corp). The results obtained were analyzed statistically using the following model:

$$y_{ijkl} = \mu + Sire_i + Sex_j + T_k + S_l + e_{ijkl}$$

were:

y_{ijkl} = observed value;
μ = population mean;
$Sire_i$ = effect of father (i = 1,...,27);
Sex_j = effect of sex (j = 1,2);
$Treatment_k$ = effect of treatment – before and after starvation (k=1,2);
$Season_l$ = effect of season at blood sampling –1-January-March; 2-April-June; 3-July-September; 4- October-December (l=1...4,)
e_{ijkl} = random error.

Results

The analysis of variance (Table 1) showed that the enzyme activity is generally influenced by physiological state (starvation), sex, sire, and least by season of blood sampling. Activities of LAP, AP, EL, LL and NAG are influenced by sire. This suggest, that they are genetically determined. AAP, EL, LL, BGLU and NAG are influenced by sex. Season influenced the activity of LAP, AP, and BGAL. The starvation influenced, statistically confirmed, on the activity of all the studied enzymes. The means ($\bar{x}$) and standard deviation (Sd) are presented in Tables 2 and 3. A great variation for all the analysed enzymes activities can be observed. The

coefficients of variance for enzyme activity in blood serum sampled before starvation were higher for EL, LL, BGRD and BGLU (bulls) and AAP, BGRD, BGLU (heifers) than after starvation. BGLU and BGRD in both sexes and AAP in heifers showed the highest variation coefficient (55% and more). A great difference of variances between the sexes was observed for AAP (22.22% and 79.94%). The data in Table 4 show that the activity of some studied lysosomal enzymes was influenced by sex (before starvation); for example, activity of NAG , AAP, EL, LL, BGLU and NAG. Almost in the all cases the activities of lysosomal enzymes were higher in heifers (except AP, BGRD, NAG). The starvation did not influence on AAP, LAP, AP, LL, BGLU activity in bulls and BGRD, BGAL, BGLU and NAG in heifers.

Table 1
Effect of different factors on the activity of studies enzymes (Einfluss einzelner Faktoren auf die untersuchten Enzymaktivitäten)

No	Enzyme		Sire	Sex	Treatment	Season
1.	Alanine aminopeptydase	AAP	N.S	**	**	N.S
2.	Leucine aminopeptydase	LAP	**	N.S	*	**
3.	Acid phosphatase	AP	*	N.S	**	**
4.	Lysosomal esterase	EL	**	**	**	N.S
5.	Lysosomal lipase	LL	*	**	**	N.S
6.	β-glucoronidase	BGRD	N.S	N.S	**	N.S
7.	β-galactosidase	BGAL	N.S	N.S	*	**
8.	β-glucosidase	BGLU	N.S	**	*	N.S
9.	N-acetyl-β-glucosaminidase	NAG	**	*	*	N.S

* - statistically significant p≤ 0,05; ** - statistically significant p≤ 0,01

Table 2
The activity ($\bar{x}$ ± Sd) of estimated enzymes in blood serum of bulls (Enzymaktivitäten im Blutserum der Bullen)

Enzyme	Before starvation					After starvation				
	$\bar{x}$	± Sd	V	from	to	$\bar{x}$	± Sd	V	from	to
Alanine aminopeptidase AAP	5.31	1.18	22.22	3.22	8.43	4.47	1.40	31.32	1.91	9.05
Leucine aminopeptidase LAP	4.79	1.14	23.95	2.97	9.21	4.54	1.31	28.85	1.89	8.91
Acid phosphatase AP	2.31	0.68	29.44	0.84	3.97	2.36	0.92	38.98	0.73	5.53
Lysosomal esterase EL	2.25	0.88	39.11	0.02	4.10	3.82	1.01	26.44	2.19	7.35
Lysosomal lipase LL	0.22	0.11	50.00	0.06	0.49	0.20	0.05	25.00	0.12	0.34
β-glucoronidase BGRD	0.20	0.11	55.00	0.05	0.69	0.15	0.06	40.00	0.08	0.51
β-galactosidase BGAL	0.41	0.13	31.70	0.18	0.76	0.48	0.21	43.75	0.17	0.99
β-glucosidase BGLU	0.11	0.08	72.72	0.03	0.36	0.14	0.08	57.14	0.03	1.79
N-acetyl-β-glucosaminidase NAG	26.74	11.43	42.74	5.88	53.45	34.98	15.08	43.11	9.01	64.43

Table 3
The activity ($\bar{x}$, ± Sd) of estimated enzymes in blood serum of heifers (Enzymaktivitäten im Blutserum der Färsen)

Enzyme	Before starvation					After starvation				
	$\bar{x}$	± Sd	V	from	to	$\bar{x}$	± Sd	V	from	to
Alanine aminopeptidase AAP	7.48	5.98	79.94	3.14	9.28	5.46	1.18	21.61	2.57	9.92
Leucine aminopeptidase LAP	5.03	0.93	18.48	2.82	7.74	4.64	0.99	21.33	2.59	7.90
Acid phosphatase AP	2.18	0.63	28.89	1.05	5.52	2.68	0.91	33.95	1.10	5.44
Lysosomal esterase EL	4.12	0.71	17.23	2.06	5.95	3.70	0.77	20.81	2.30	5.59
Lysosomal lipase LL	0.33	0.06	18.18	0.17	0.49	0.27	0.05	18.51	0.12	0.39
β-glucoronidase BGRD	0.18	0.12	66.66	0.05	1.33	0.21	0.10	47.61	0.05	0.56
β-galactosidase BGAL	0.48	0.13	27.08	0.20	0.78	0.49	0.16	32.65	0.12	0.85
β-glucosidase BGLU	0.21	0.12	57.14	0.03	0.80	0.18	0.08	44.44	0.04	0.43
N-acetyl-β-glucosaminidase NAG	24.84	11.79	47.46	5.13	99.0	25.57	12.5	48.88	6.12	84.39

Table 4
Analysis of variance for activity of estimated lysosomal enzymes (Varianzanalyse für die lysosomalen Enzymaktivitäten)

Enzyme	sex	before starvation		after starvation		
		LSM	Se	LSM	Se	
Alanine aminopeptidase AAP	bulls	4.74^{A}	0.53	4.51^{A}	0.51	N.S
	heifers	6.79^{A}	0.42	4.69^{A}	0.44	**
Leucine aminopeptidase LAP	bulls	4.56	0.14	4.82	0.14	N.S
	heifers	5.02	0.11	4.55	0.12	**
Acid phosphatase AP	bulls	2.45	0.10	2.39	0.10	N.S
	heifers	2.02	0.08	2.54	0.09	**
Lysosomal esterase EL	bulls	3.91^{A}	0.11	2.27^{A}	0.10	**
	heifers	4.07^{A}	0.08	3.70^{A}	0.09	**
Lysosomal lipase LL	bulls	0.20^{A}	0.01	0.22^{A}	0.01	N.S
	heifers	0.34^{A}	0.01	0.27^{A}	0.01	**
β-glucoronidase BGRD	bulls	0.15	0.01	0.20	0.01	*
	heifers	0.18	0.01	0.20	0.01	N.S
β-galactosidase BGAL	bulls	0.49	0.02	0.41	0.02	**
	heifers	0.44	0.02	0.44	0.02	N.S
β-glucosidase BGLU	bulls	0.12^{A}	0.02	0.10^{A}	0.02	N.S
	heifers	0.23^{A}	0.01	0.22^{A}	0.02	N.S
N-acetyl-β-glucosaminidase	bulls	35.4^{a}	1.78	26.2	1.65	**
NAG	heifers	26.6^{a}	1.36	26.7	1.44	N.S.

*- statistically significant p≤ 0,05; **- statistically significant p≤ 0,01
a – between sexes p≤0,05; A – between sexes p ≤0,01

Discussion

The results indicated a great variation of the studied enzyme activities. Our earlier studies revealed that the activity of the degradation system of the lysosomal compartment is a significant factor maintaining the animal cell in the state of dynamic homeostasis, as well as an important indicator of stress reactivity. Observations conducted on pigs (KOŁĄTAJ et al., 1996), cattle (KOŁĄTAJ et al., 1998), sheep (KOŁĄTAJ et al., 2002) as also on mice (JÓŹWIK et al., 2003 a b; LANKOFF and

KOŁĄTAJ, 2001; WITEK and KOŁĄTAJ, 1998, 2001), rabbits (KONECKA et al., 2002; WITEK and KOŁĄTAJ, 2000) and quail (WITEK et al., 2000) confirmed this hypothesis. The data obtained from them indicate that the stress factors, employed in these studies, caused the significant changes in the reactivity of the animal cell lysosomal system. Starvation had a distinct effect respectively on some studied lysosomal enzyme activity only. The interpretation of this phenomenon is, as an adaptional system in cattle, difficult to explain.

It is worth emphasise that starvation is a factor changing the homeostasis of animal (CORWIN, 2000; OHSHITA et al., 1986). This stress factor was observed regarding to the lysosomal hydrolases, too (JÓŹWIK et al., 2003 a; WITEK and KOŁĄTAJ, 1998).

The number of studies regarding homeostasis and its disorders in cattle increases, as evoked by different level of nutrition (KIJORA et al., 2002; RICHARDT et al., 2002). Numerous indications of that adaptation has been published but we has failed to find such data regarding the lysosomal degradation enzymes in the blood serum in cattle. It seems that this reactivity may be very interesting in these animals from physiological point of view. There are no bibliographical references about the variability of lysosomal enzyme activities in the cattle.

We think, reactivity of the lysosomal enzymes can be used as indicator of adaptation ability in the cattle too. Because there is a small number of publications on these enzyme activities in cattle the further studies on this area are necessary.

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