Lysosomal proteolysis in skeletal muscles of bulls

S. J. Rosochacki[1,2], T. Sakowski[1], J. Połoszynowicz[1], E. Juszczuk-Kubiak[1], A. Kowalik-Krupa[2], J. Oprządek[1]

[1]Institute of Genetics and Animal Breeding PAS, Jastrzębiec, Poland
[2]Białystok Technical University, Białystok, Poland

ABSTRACT: The relationship between lysosomal proteolytic enzyme activities involved in skeletal muscle proteolysis of the *longissimus lumborum et thoracis* muscle (MLLT) of bulls was described. Samples from the same region were obtained *post mortem* from 7 Piemontese (P) and 54 Black-and-White bulls (B-W) about 18 months old fed *ad libitum*. The activity of cathepsin D was determined as pepstatin (cathepsin D inhibitor) sensitive activity (PSCatD) towards 1% haemoglobin. Pepstatin-insensitive acid (PIA) and leupeptin-insensitive (thiol proteinase inhibitor) acid (LIA) autolytic activities were measured in the presence of 1 mM Mg^{++}. MLLT was also analysed for RNA, DNA and protein content. The data were processed by analysis of variance and differences between sires were tested by the contrast procedure of general linear model. In the examined muscle RNA decreased by 16% in B-W compared to P, CPS by about 14% and FCS by about 39%. DNA content was higher by 64.5% in B-W compared to P bulls ($P \leq 0.01$). Some differences were found between P bulls and B-W groups of sires in the percentage of proteins ($P \leq 0.01$), CatD and PSCatD ($P \leq 0.01$), but the most pronounced differences were determined in PIA and LIA ($P \leq 0.01$), and in the percentage of inhibition by pepstatin and leupeptin ($P \leq 0.01$) in AAA. In the Black-and-White group of sires the percentage of protein and percentage of inhibition by pepstatin and leupeptin in AAA were lowered by about 10, 17 and 22%, but PSCatD, PIA and LIA were higher by about 23.7, 41 and 57.7%, respectively, compared to Piemontese bulls. The level of aspartic and thiol proteinases was lower in the muscles of B-W compared to Piemontese. The activity was much higher in B-W compared to P. These results indicate the faster turnover of proteins in the groups after Black-and-White sires and higher anabolic increase in degradation in Piemontese bulls.

Keywords: bull; skeletal muscle; protein; proteolytic activities; cathepsin D; thiol proteinases

In the last few years there was quite a dynamic increase in the exploitation of livestock (such as bulls, lambs, goats) to increase meat production and consumption. Production and utilization of bull meat probably benefit from the belief that bull meat is of better quality compared to mutton and goat. According to chemical scores calculated by Srivasan and Moorjani (1974), the beef meat protein equals 89, compared with mutton 84, goat 84; in pork this value is 90. As the skeletal muscles contain approximately 50% of the total body protein (Simon, 1989), the changes in muscle protein mass are very important. Protein deposition depends on the balance between synthesis and breakdown of protein, which determines the efficiency of protein metabolism. It was shown that muscular accretion can be regulated at the catabolic side of protein metabolism (Dayton *et al*., 1981; Attaix *et al*., 1999). However, little is known about mechanisms and regulation of the breakdown of skeletal muscle protein, so the knowledge of protein metabolism in skeletal muscles is highly desirable for interventions to increase the efficiency of protein deposition in meat production.

There is a lot of evidence concerning remarkable sensitivity of the overall rate of protein degradation in muscle and liver to nutritional factors (Waterlow *et al*., 1978; Millward, 1980). In skeletal muscle protein breakdown several proteinase systems are thought to be involved (Etherington, 1984). The process of

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protein catabolism involves, at least partially in myofibrillar protein catabolism, the lysosomal acid proteinases, called cathepsins and the group of their inhibitors called cystatins (Calkins and Sideman, 1988). These enzymes exhibit their maximal proteolytic activities at low pH and are capable of breaking down myosin and actin and a large number of other myofibrillar proteins into relatively small fragments (Ouali, 1992; Uytterhaegen *et al*., 1994). They also participate in further breaking down degradation products of other proteolytic systems (Elgasim *et al*., 1985). In muscular tissues the main enzymes of this system are cathepsin D – aspartic proteinase, and cathepsins B, L, and H – cysteine proteinases (Goll *et al*., 1983). The activities of the different endogenous proteolytic enzymes depend on genetic and non-genetic factors (Rosochacki, 1985; Kretchmar *et al*., 1994).

Apart from the lysosomal pathway of protein degradation (Baricos *et al*., 1987), most cells have a non-lysosomal route of intracellular protein catabolism (Rechsteiner, 1987; Hershko, 1988; Jank *et al*., 2000, 2001). The calcium-dependent neutral proteolytic system, consisting of μ- and m-calpain and their natural inhibitor calpastatin, does not degrade any of the major myofibrillar proteins like myosin and actin (Goll *et al*., 1992; Koohmaraie, 1992; Johari *et al*., 1993; Thompson and Palmer, 1998), but the whole process of protein degradation is believed to be initiated by these enzymes, which removes the Z-disk of myofibrils at the beginning (Belcastro *et al*., 1996; Goll *et al*., 1998). Multicatalytic proteinase system (MCP) can also be involved in the initial step of myofibrillar degradation and thus in the metabolic disassembly of these proteins. ATP-ubiquitin (Ub)-dependent pathway (Thompson and Palmer, 1998) is responsible for the degradation of short-lived and abnormal proteins (Hershko and Ciechanover, 1992) and disassembled myofibrils that accumulate in the other proteolytic pathways.

According to some authors, the rate of protein degradation is reflected in the same way as the activity of cathepsin D in various rat (Millward *et al*., 1981) and chicken muscles (Stauber *et al*., 1977). The action of cathepsin D in the muscle towards purified contractile proteins as well as towards myofilaments and myofibrils has been examined (Schwartz and Bird, 1977; Millward, 1980). Therefore, by measuring the changes in the activity of cathepsin D one can easily follow changes in the degradation rate of proteins.

The main aim of this study was to examine the type of change in the activity of the degrading system involved in the degradation of metabolized proteins in skeletal muscles of Piemontese and Black-and-White bulls. The experiment was also designed to examine the activities of some lysosomal proteolytic enzymes in different strains of bulls and protein variables in the *longissimus lumborum et thoracis* muscle as indexes of protein turnover in muscles of 18 months old bulls. These measurements may help determine whether genetically different bulls can be used for the study of skeletal muscle protein turnover.

MATERIAL AND METHODS

Animals

All the animals were born on an Experimental Farm in Jastrzębiec. The experiment was conducted on 7 Piemontese (P) and 54 Black-and-White (crosses with Holstein-Friesian – HF) bulls (B-W group) about 18 months old. Black-and-White experimental bulls were the progeny of six different sires (1–6), but Piemontese bulls were the progeny of one sire. The animals were fed *ad libitum*. The bulls were slaughtered after 14 hours of fasting and the samples of *longissimus lumborum et thoracis* muscle (MLLT) from the same region of XIII vertebra were taken within 15 minutes after slaughter, frozen at –70°C until required. To perform this experiment, the Animal Care Use Committee of the Institute approved the use of animals in this study according to guidelines established by the World Society for the Protection of Animals.

Biochemical analysis

Proteolytic activity assay. In MLLT the following determinations were made:

– total cathepsin D (CatD) activity towards 1% haemoglobin as a substrate;
– extent of inhibition of total CatD activity by pepstatin (300 μg/estimation) to estimate the activity of pepstatin-sensitive CatD (PSCatD);
– acid autolytic activity (AAA) towards natural proteins extracted from tissue as a substrate;
– extent of inhibition of AAA by pepstatin to estimate the pepstatin-insensitive acid autolytic activity (PIA);

– extent of inhibition of AAA by leupeptin (5.1 μg per estimation) to estimate the leupeptin-insensitive acid autolytic activity (LIA).

The PSCatD/PIA ratios should identify the pathway possibly predominating in the MLLT protein catabolism – CatD against cysteine proteinases. Proteolytic activities were determined according to Rosochacki (1985). Standard samples of tissues (approximately 1 g of MLLT) were homogenized in cold 0.1% Triton X100 (Serva, Germany) in water (0°C) and used for enzymatic activity measurements. All enzymatic activities were determined in 500 mM of formic buffer (POCH, Poland), pH 3.75 (only for the PIA pH was 3.25) at 45°C during 1 hour. After incubations 3% trichloroacetic acid (POCH, Poland) was added (to remove proteins) and in the obtained supernatant the proteolysis products were measured with Folin reagent (Lowry *et al*., 1951), using tyrosine (between 2 and 40 μg/ml) as a standard. Haemoglobin, pepstatin and leupeptin were obtained from Sigma, USA. Both PIA and LIA were measured in the presence of 1 mM Mg^{++} (POCH, Poland). The activity of proteinases was defined as μg of tyrosine released per mg protein. Pepstatin is an inhibitor of cathepsin D while leupeptin inhibits thiol proteinases.

DNA, RNA and protein assay. MLLT were assayed for DNA, RNA and protein content. The procedure of Munro and Fleck (1966) was used for determining muscle and liver DNA, RNA concentrations in approximately 100 mg of tissue. The protein was determined in an alkaline solution of homogenates dissolved in 3% trichloroacetic acid with Folin-Lowry (1951) reagent (Merck, Germany), against albumin (POCH, Poland) as a standard. The same method was used to determine the residual protein in the RNA solution, and thus to obtain the necessary correction for the RNA estimation.

Capacity for protein synthesis (CPS) and functional cell size (FCS) were expressed as 10^3RNA/protein and protein/10^3DNA ratios, respectively.

Statistics

The data were processed by analysis of variance (SAS/STAT guide for personal computers, SAS Institute Inc., Cary, NC) according to the following model:

$$y_{ij\,k} = \mu + (GO)_{ij} + e_{ijk}$$

where: y_{ijk} = observation
$(GO)_{ij}$ = random effect of i-th sire within j-th breed
e_{ijk} = random error

Differences in mean values ± SE between and within groups of sires were tested by the contrast procedure of general linear model. To check the statistical differences between percentages of inhibition, firstly the square root transformation was done, and then analysis of variance was performed. Differences were considered to be significant at $P \leq 0.05$ (single asterisk or small letters) and $P \leq 0.01$ (double asterisk or capital letters), where asterisks describe differences between Piemontese and Black-and-White groups, and letters describe differences between the particular Black-and-White sires.

RESULTS

The content of protein in MLLT in Piemontese and B-W bulls is shown in Table 1, and was higher by about 10% in Piemontese compared to B-W bulls ($P \leq 0.01$).

The enzymatic activities in MLLT of bulls are shown in Table 1. Cathepsin D activity increased to 42.5 in B-W group compared to 34.8 μg of tyrosine/mg of protein in Piemontese bulls ($P \leq 0.01$). The AAA activity increased in B-W bulls from 20.4 to 22.4 μg of tyrosine/mg of protein in P sires. The most marked increase in LIA occurred in B-W group of bulls compared to P bulls by 57.7% ($P \leq 0.01$) with parallel increase in PSCatD (by 23.66%; $P \leq 0.01$) – both reflecting the activity of cathepsin D. It is supported by the activity of PIA, but with pepstatin as an inhibitor of aspartic cathepsins (rise by 40.9%; $P \leq 0.01$), reflecting thiol proteinases. The percentage of inhibition by pepstatin and leupeptin in AAA was almost the same in B-W bulls, being around 50%, and was lower than in P sires (average 63.5%).

Table 2 shows the composition of bull's MLLT. The DNA concentration was 237.2 in P and 390.2 μg/g of tissue (on average) in B-W bulls, being higher by 64.5%. Protein/10^3DNA ratio (mg/mg) called FCS was decreased by 39% in B-W compared to P bulls. RNA/g of muscle was higher in P bulls by about 16% compared to B-W, being 303.5 μg/g of tissue (on average) in P and 254.8 μg/g of tissue (on average) in B-W. CPS (10^3RNA/protein) was higher in P bulls by about 14% compared to B-W bulls. RNA/DNA

Table 1. Enzymatic activity in MLLT of bulls (in μg of tyrosine per mg of protein) and inhibition (in %) of cathepsin D by pepstatin and acid autolytic activity by pepstatin and leupeptin (mean ± SD)

Group	*n*	% of protein	Cat D	Cat D + PEP	PSCatD	% of inhib.	AAA	PIA	% of inhib.	LIA	% of inhib.	PSCatD/ PIA
Piemontese (P)	6	19.47 ± 2.34	34.80 ± 4.42	7.62 ± 1.55	27.18 ± 3.07	78.22 ± 2.16	20.38 ± 3.94	7.62 ± 1.52	62.43 ± 4.67	7.12 ± 1.15	64.81 ± 3.85	3.57
1-VII, B-W	7	19.18 ± 1.01	46.47 ± 4.00	8.72 ± 0.89	37.79 ± 3.82	81.20 ± 2.22	18.88 ± 1.88	10.12 ± 0.97	46.34 ± 2.82	10.38 ± 1.43	45.47 ± 5.31	3.73
2-VIII, B-W	8	18.66 ± 2.11	37.87 ± 1.99	8.26 ± 1.04	29.55 ± 1.69	78.04 ± 2.44	17.25 ± 1.17	9.15 ± 1.62	46.70 ± 9.87	9.05 ± 0.68	47.47 ± 2.01	3.23
3-IX, B-W	9	15.58 ± 1.25	40.77 ± 4.65	8.43 ± 1.47	32.01 ± 3.33	79.44 ± 1.48	26.41 ± 1.99	11.42 ± 2.17	56.82 ± 7.80	13.53 ± 1.61	48.89 ± 3.26	2.80
4-I A, B-W	10	17.75 ± 1.36	46.69 ± 3.96	9.12 ± 1.35	36.88 ± 3.90	80.04 ± 2.90	25.97 ± 2.60	12.46 ± 1.36	53.26 ± 4.71	12.35 ± 1.89	53.23 ± 3.41	2.96
5-XII, B-W	10	17.58 ± 1.95	39.15 ± 3.37	8.39 ± 1.87	30.74 ± 2.98	78.51 ± 4.27	20.24 ± 3.22	9.52 ± 1.98	53.17 ± 4.69	10.00 ± 1.54	50.34 ± 4.68	3.23
6-I B, B-W	9	17.74 ± 1.67	44.42 ± 3.45	9.41 ± 1.85	35.12 ± 3.00	78.48 ± 3.45	24.33 ± 3.00	11.72 ± 1.20	52.71 ± 5.00	11.56 ± 1.78	53.88 ± 4.66	2.91
Mean 1–6, B-W	53	17.67 ± 1.89	42.51 ± 4.93	8.72 ± 1.48	33.61 ± 4.32	79.23 ± 3.04	22.42 ± 4.25	10.74 ± 1.99	51.80 ± 7.01	11.23 ± 2.13	50.26 ± 4.76	3.17
% of Piemontese		90.08	122.16	114.44	123.66	101.29	110.01	140.94	82.97	157.72	77.55	88.69
P vs. B-W statistical differences		*	**	–	**	–	**	**	**	**	**	
Statistical differences between B-W groups	A	3–1,2, 4,6	2–1,6 3–1,4,6	5–1,4,6	2–1,4,6 3–1,4,6 5–1,4,6		1–4,6 2–4,6 3–1,2 5–1,3,6	1–4 2–3,6 5–4,6	3–1,2	2–4,6 3–1,2,6 5–3,4	1–4,6 2–4,6	
	a	3–5				1–2		3–5	1–4,6 2–4 5–1,2	1–4 5–6	1–5 3–4,6	

Abbreviations: Cat D – cathepsin D; PSCatD – pepstatin sensitive cathepsin D (measured towards 1% haemoglobin); AAA – acid autolytic activity; PIA – pepstatin insensitive acid autolytic activity; LIA – leupeptin insensitive acid autolytic activity; PEP – pepstatin; B-W – Black-and-White bulls; 1–6 – six different Black-and-White groups of bulls

Statistical differences between P vs. B-W and within B-W groups at: ** and A = $P \leq 0.01$; * and a = $P \leq 0.05$, respectively

Table 2. Composition of MLLT (mean ± SD)

Group	*n*	μg RNA/g tissue	10^3RNA/protein (CPS)	μg DNA/g tissue	Protein/10^3DNA (FCS)	RNA/DNA
Piemontese, P	7	303.5 ± 18.08	1.126 ± 0.068	237.2 ± 28.7	1151.4 ± 140.9	1.292 ± 0.136
1-VII, B-W	8	248.0 ± 8.9	0.932 ± 0.047	386.1 ± 75.3	712.4 ± 138.5	0.669 ± 0.159
2-VIII, B-W	8	252.7 ± 41.9	1.031 ± 0.154	396.5 ± 37.5	639.1 ± 105.9	0.639 ± 0.105
3-IX, B-W	9	251.0 ± 20.8	0.945 ± 0.120	407.2 ± 39.0	664.8 ± 72.0	0.623 ± 0.062
4-I A, B-W	10	230.6 ± 5.1	0.875 ± 0.050	466.6 ± 16.9	567.3 ± 44.7	0.490 ± 0.014
5-XII, B-W	10	278.2 ± 17.4	1.074 ± 0.072	353.3 ± 52.3	789.7 ± 167.2	0.812 ± 0.135
6-I B, B-W	9	267.2 ± 16.9	0.954 ± 0.060	327.5 ± 19.6	857.7 ± 39.5	0.807 ± 0.085
Mean 1–6 B-W	54	254.8 ± 25.53	0.968 ± 0110	390.2 ± 61.8	705.3 ± 141.2	0.673 ± 0.152
% of Piemontese		83.95	85.96	164.50	61.25	52.08
P vs. B-W statistical differences		**	**	**	**	**
Statistical differences between B-W groups	A	6–4 3–4; 5–1,3,4	2–4 5–1,3,4,6	1–4,6 2–4,6; 3–4,6 5–3,4; 4-6	1–4,6; 2–6 3–6; 4–6; 5–2,4	1–4; 2–4,6 3–4,6; 4–6 5–1,2,4
	a	2–4,5	2–3	2–5	3–5	1–6

CPS – capacity for protein synthesis; FCS – functional cell size

Statistical differences between P vs. B-W and within B-W groups at the level of: ** and A = $P \leq 0.01$; a = $P \leq 0.05$

ratio was much higher (by 47%) in Piemontese bulls compared to W-B.

DISCUSSION

Piemontese and Black-and-White breeds come from *Bos taurus primigenius*. The selection over the last hundred years changed these breeds from dual-purpose cattle to meat breed and milk breed, respectively. The group of Black-and-White bulls we used has HF genes and is of dairy type. Piemontese bulls, recognized as having hypertrophic muscle mutation, are also characterized by lower intramuscular fat content in the MLLT, compared to the other breeds. This breed is characterized by thin skeleton and low fat content compared to B-W bulls.

The aim of this study was to compare some proteolytic enzymes and protein variables in the *longissimus lumborum et thoracis* muscle between two different breeds of bulls. There were some statistically significant differences between the progeny of six sires of B-W bulls as it can be seen in Tables 1 and 2. It should be emphasized that the investigated variables were always different between both groups of bulls.

Lysosomal proteinases that are active at acid pH play an important role in the metabolism of proteins. Changes in the skeletal muscle lysosomal proteinases should be accompanied by changes in the protein synthesis and degradation and the latter by changes in proteinase activity (Millward *et al.*, 1981). The changes in the rate of degradation in the muscle of these breeds can occur either in the capacity or activity of the degradation system or in the sensitivity of the substrate; the latter can hardly be measured. Measuring the amount and activity of the degradation system (capacity for degradation) is much easier and some changes were observed in the concentration of proteinases in muscle (Rosochacki and Millward, 1979; Millward *et al.*, 1980, 1981; Rosochacki, 1985; Jank *et al.*, 2000, 2001). The activity of the degradation system is usually much higher in the liver than in MLLT (Rosochacki and

Piekarzewska, 1988; Rosochacki, 1989). This may be due to the structure of the cytoplasm, where in the muscle about 70% of cytoplasmic proteins are tightly bound within the myofibrils. Assuming that the degradation system is a part of the sarcoplasmic reticulum in the intermyofibrillar space, there is a separation of the substrate and degrading system, and factors which affected the binding of individual proteins to the myofibril would control this separation. On the contrary, in the liver there is no obvious separation although there is a lysosomal membrane between the substrate and the degrading system (Millward *et al*., 1981).

Cathepsin D activity was 22% higher in B-W group compared to Piemontese ($P \leq 0.01$). This is supported by the most marked increase (in B-W group of bulls) in LIA (by 57.7%; $P \leq 0.01$) with parallel increase in PSCatD (by 23.66%; $P \leq 0.01$) – both reflecting the activity of cathepsin D, substantially differentiating the degradation apparatus in MLLT of these strains (Table 1). It is also supported by the activity of PIA, which measures the acid autolytic activity towards natural substrate – homogenate, but with pepstatin as an inhibitor of aspartic cathepsins (rise by 40.9%; $P \leq 0.01$), reflecting thiol proteinases. It can be seen that the ratio PSCatD to PIA activity is higher in Piemontese bulls than in B-W (Table 1). So enzymatic degradation in Piemontese MLLT was more cathepsin D dependent than in B-W. It means that proteolytic enzymes are more active in B-W bulls (less total protein in the muscle), which is in agreement as far as these two breeds are concerned.

An increase in the proteolytic activity of enzymes, e.g. cathepsin, could result from changes in cathepsin-cystatin interactions rather than from the gene expression. It was shown by Hasselgren (1999) that an increased amount of mRNA was not directly correlated with the increase in protein breakdown rate. Therefore it is not possible to explain muscle degradation only by an increase or decrease in mRNA concentration. The level of proteolytic enzymes reflected by the inhibition by pepstatin and by leupeptin in AAA was lowered by about 20% (in average) in B-W group compared to Piemontese. So, the level of aspartic and thiol enzymes was lower in the muscle of B-W bulls compared to Piemontese, but their activity was much higher. It means that proteolytic enzymes are more active in B-W bulls (less total protein in the muscle). In the present experiment, the inhibition by leupeptin in MLLT was about 50% in B-W group, much higher than in chickens where the inhibition by leupeptin was 26% and 17% in breast and leg muscle, respectively (Rosochacki *et al*., 1986). This inhibition in isolated anterior *latissimus dorsi* (ALD) and posterior *latissimus dorsi* (PLD) chicken muscles was about 20.9% in control and 16.5% in hypertrophic muscles (Rosochacki, 1985). This suggests that the extent of enzymatic response to the catabolism of protein in bulls differs from chickens. It also supports the hypothesis that in chicken muscles some other proteolytic systems degrade myofibrillar proteins. On the contrary, in the bulls' muscles, aspartic and thiol proteinases play the major role in myofibrillar proteolysis.

The maximum capacity for protein synthesis – CPS – (measured as 10^3RNA/protein ratio) was found in Piemontese animals, being 1.126 for 18 months (about 540 days) old bulls and only 0.968 in B-W, supporting the higher content of protein in the Piemontese muscles (Tables 1 and 2); the measured RNA is mostly of ribosomal origin. In our experiment with much younger calves, the values in the *semidentinosus* muscle were 6.84 for 0–34 days old calves, 6.00 for 66–115 days old and 4.8 for 190 days old ones (Rosochacki *et al*., 1990). The value in a mouse leg muscle was about 7.13 (Rosochacki and Piekarzewska, 1988) or 10.9 (Rosochacki, 1989). In isolated ALD – slow, red and PLD – fast, white muscles of adult chickens these values were 9.4 and 5.0, respectively (Rosochacki, 1985). The CPS in MLLT of 6-month-old Duroc pigs was 1.96, but in Pietrain – 1.60 (Rosochacki *et al*., 2000). Our results show that the capacity for protein synthesis is quite small in muscles of 18 months old bulls compared to muscles of younger and other animals; this value decreases during the time of animals growth. As it can be seen from Table 2, the capacity for protein synthesis is lower by about 24% in B-W sires compared to Piemontese bulls. It can be concluded that the decrease in CPS was mostly attributed to a fall in rRNA transcription in B-W sires. This is in agreement with the concentration of RNA, and from the present study it appears that Piemontese bulls accumulate protein in the cells faster than B-W sires.

Because of the multinucleated state of the myotube in muscle, total DNA is not an acceptable measure of myotube number. Rather, total DNA/mg tissue allows comparisons of nuclei content between populations. Protein/10^3DNA ratio, called "functional cell size" – FCS, is a very good factor to estimate the cell size. This ratio differed very much between animals. Muscles constitute 42–47% of the ruminant body weight, and due to their growth, the

total protein content of the animal also increases, while the percentage of protein does not change during the experiment. FCS is the imaginary volume of cytoplasm controlled by a single nucleus (Cheek *et al*., 1971). 60–80% of muscle DNA resides inside the muscle fibrils and it must be assumed that any changes in DNA content occurred proportionally inside and outside of muscle fibrils. Within a given muscle there are different proportions between individual fibril types and this may affect its size and metabolism. Our results demonstrate quite marked changes between groups of bulls (by 64%), being much higher in B-W groups (390 on average) than in Piemontese (237), indicating the higher FSC in B-W bulls. This is also supported by the RNA/DNA ratio – the concentration of ribosomes per nucleus – that was much higher in Piemontese than in B-W sires (1.292 versus 0.673). In another experiment with mice (Rosochacki, 1989) the changes in protein/10^3DNA ratio were at about 60% affected by different nutritional conditions (different amount of protein, fat and carbohydrates in the diet). In our experiment with the *semitendinosus* muscle of calves, the protein/10^3DNA ratio increased during the time of experiment 2.3 fold in hypertrophic muscles and 2.6 fold in others, being in six months old calves about 787 and 755, respectively, but in 15 weeks old ones 522 and 452, respectively (Rosochacki *et al*., 1990). The lower DNA concentration and higher protein/DNA ratio in P bulls compared to B-W suggests that Piemontese bulls have higher muscle (as well as physiological) maturity.

The rise in the activity of proteolytic enzymes – mostly CatD (by 22%), PSCatD (by 24%), PIA (by 41%) and LIA (by 57.7%) – the level of statistical differences being as high as 0.0001 – in the LDM of B-W bulls indicates a higher protein breakdown in the tissue compared to Piemontese.

CONCLUSION

All mentioned activities were always higher in B-W, although there were some statistical differences between B-W groups. FCS was significantly lower in MLLT of B-W bulls ($P \leq 0.01$) suggesting a higher rate of protein breakdown than of protein synthesis. The higher FCS in MLLT of Piemontese bulls suggests a higher anabolic increase in degradation compared to B-W ones. So the muscles of B-W and Piemontese bulls respond differently to the proteolytic enzymes. Therefore this study indicates the need for further, more precise investigations on protein metabolism in the muscle. Additionally, the proteinases were assayed *in vitro*, so that their potential capacity was measured, which does not necessarily represent their physiological activity. However, in particular the changes in proteolytic variables may prove useful for understanding the mechanisms of muscle growth. It appears that genetically different lines of cattle can be useful model animals for studying the skeletal muscle protein turnover. These results indicate the faster turnover of proteins in the group of Black-and-White compared to Piemontese bulls.

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ABSTRAKT

Lysosomální proteolýza v kosterních svalech býků

Byla studována závislost mezi aktivitami lyzosomálních proteolytických enzymů působících v průběhu proteolýzy v kosterním svalu longissimus lumborum et thoracis (MLLT) u býků. Vzorky svalu byly získány od sedmi býků piemontského plemene (P) a 54 býků černostrakatého plemene (B-W) po jejich porážce ve věku 18 měsíců; býci byli vykrmováni *ad libitum*. Aktivita catepsinu D byla stanovena jako aktivita CatD citlivého na pepstatin (inhibitor catepsinu D) (PCSatD) vůči 1% hemoglobinu. Kyselé autolytické aktivity necitlivé na pepstatin (PIA) a leupeptin (inhibitor thiol proteináz) (LIA) byly zjišťovány v přítomnosti 1 mM Mg++. Ve svalu MLLT byl rovněž analyzován obsah RNK, DNK a bílkovin. Získané výsledky byly zpracovány pomocí analýzy rozptylu a rozdíly mezi plemeníky a testovány srovnávací metodou obecného lineárního modelu. Ve sledovaných svalech došlo u plemene B-W ke snížení hladiny RNK o 16 % ve srovnání s býky plemene P, zatímco hodnoty CPS a FCS klesly o 14 % resp. 39 %. Ve srovnání s býky plemene P měli býci plemene B-W obsah DNK vyšší o 64,5 % ($P \leq 0,01$). Určité rozdíly mezi býky plemene P a skupinami po plemenících B-W existovaly v procentuálním zastoupení bílkovin ($P \leq 0,01$), CatD a PSCatD ($P \leq 0,01$), ale nejvýraznější rozdíly byly zjištěny u PIA a LIA ($P \leq 0,01$) a v procentuální inhibici AAA pepstatinem a leupeptinem ($P \leq 0,01$). U skupin po plemenících černostrakatého skotu došlo ke snížení procentuálního zastoupení bílkovin a procentuální inhibice AAA pepstatinem a leupeptinem o 10, 17 a 22 %, ale ve srovnání s býky piemontského plemene hodnoty PSCatD, PIA a LIA byly vyšší asi o 23,7, 41 a 57,7 %. Hladiny aspartových a thiolových proteináz byly nižší ve svalech býků B-W než u piemontského plemene. Jejich aktivita byla mnohem vyšší u B-W než u P. Tyto výsledky ukazují na rychlejší obrat bílkovin u skupin po plemenících černostrakatého plemene a vyšší anabolický nárůst degradace u býků piemontského plemene.

Klíčová slova: býk; kosterní svalstvo; bílkoviny; proteolytické aktivity; catepsin D; thiol proteinázy

Corresponding Author

Prof. Dr. hab. Stanisław Józef Rosochacki, Polish Academy of Sciences, Institute of Genetics and Animal Breeding, Jastrzębiec, 05-552 Wólka Kosowska, Poland
Tel. +44 22 756 17 11, fax +44 22 756 16 99, e-mail: s.rosochacki@ ighz.pl