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Preliminary observations upon relation between the G77A polymorphism in *CATD* gene and lysosomal proteinases activity and sensory traits of meat from bulls of three breeds*

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Observations were carried out on 31 Black-and-White (BW), 16 Charolaise (CH) and 18 Simmental (S) bulls at the age of 12-15 months. Determined were: taste, aroma, tenderness and consistency of *longissimus dorsi* muscle. Fragment of the bovine *CATD* gene encoding procathepsin D was amplified and subjected to RFLP analysis with restriction nuclease *Apa*I. Only two genotypes were identified: *GG* and *GA*, the frequencies of the alleles being 0.806 and 0.194 in BW, 0.656 and 0.344 in CH and 0.639 and 0.361 in S bulls, respectively.

In the muscle, the total cathepsin D (CatD), pepstatin-sensitive cathepsin D (PSCatD), pepstatin-insensitive and leupeptin-insensitive acid autolytic activities (PIAAA and LIAAA, respectively) were determined. No interbreed differences in CatD, PSCatD and the percent of inhibition in cathepsin D were found. PIAAA and LIAAA significantly differed between breeds (P≤0.01), being higher in BW by 47.9, 46.4 and 34.6% than in CH and by 40.8, 37.5 and 22.7% than in S bulls, respectively. The percent AAA inhibition by leupeptin in BW bulls was by 14.9% higher than in CH and by 24.2% than in S bulls. In CH bulls, the inhibition of AAA by pepstatin depended on the genotype,

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being higher in *GA* than *GG* animals by 15.83% (P≤0.05). The protein percent of muscle in CH and S bulls was by 33.4 % and 36.7 % higher, respectively, than of BW bulls muscle. Highly significant differences in sensory traits of muscle were identified between BW and CH or S bulls.
The sensory traits assessed were higher in meat of CH and S than of BW bulls by 36.09 % and 35.54% in aroma, by 35.67% and 33.15% in taste, by 32.24% and 21.31% in tenderness and by 36.68% and 38.24% in consistency, respectively. These differences were identified as significant in aroma, taste, tenderness and consistency between the meat of BW and CH or S bulls (P≤0.01). Within tenderness, the difference was found significant also between CH and S bulls (P≤0.05, by 9.01%higher in CH). When the genotypes (*GA* and *GG*) were combined with estimated sensory parameters, only in meat of BW bulls significant differences were identified between genotypes in aroma, taste and consistency (P≤0.01) in favour of *GG* genotype bulls.

KEY WORDS: cattle / beef / *CATD* / gene polymorphism / proteolytic enzymes / sensory traits

The growth of animal muscles is a dynamic process [Grant and Helferich 1991] and it is a function of genetic and nutritional factors reflected mostly in the growth of muscle weight, and fibril types and diameter [Rehfeld and Bünger 1990, Fiedler *et al*. 1998, Smith *et al*. 2000]. The muscle lysosomal proteinases and calpain [Calkins and Seidman 1988, Goll *et al*. 1992, 1998] activities are highly related to the rate of muscle protein turnover. The nonlysosomal calcium-dependent route of intracellular protein degradation, consisting of μ- and m-calpain and their natural inhibitor calpastatin, do not degrade any of the major myofibrillar proteins like myosin [Küchenmeister *et al*. 2001, Küchenmeister and Kuhn 2003], actin [Thompson and Palmer 1998, Goll *et al*. 1998] and α-actinin [Doumit and Koohmaraie 1999], but they initiate this process by removing of myofibrillar Z-disk [Belcastro *et al*. 1996].

The most important *post-mortem* change with regard to muscle protein degradation is considered to be the liberation of some lysosomal enzymes, e.g. cathepsins. It was shown by Mikami *et al.* [1987] that the main enzymes of this catheptic system (mostly cathepsin D and thiol proteinases – cathepsins B, H and L) exhibit – under physiological conditions – maximum proteolytic activity at low pH and cathepsins are capable of degrading myosin, actin and α-actinin and a large number of other myofibrillar proteins into relatively small fragments. There are also evidences pointing out the remarkable sensitivity of the protein degradation rate in muscle cells (fibrils) to nutritional factors [Millward *et al*. 1980, 1981] and that rate of protein degradation varies in the same way as activity of cathepsin D in various rat [Millward *et al*. 1981] and chicken muscles [Rosochacki 1985].

Catheptic proteolytic system has been shown to be involved in *post-mortem* beef basic composition as related to certain sensory traits in cattle [Taylor *et al*. 1995, Harper 1999]. The measured activity of cathepsin D might be the consequence in the expression of *CATD* gene variants. These variants at *CATD locus* can be correlated with beef quality indicators, so the *CATD locus* might be considered as a candidate gene for beef tenderness.

The main aim of this study was to examine and investigate the polymorphism in the bovine *CATD* gene and its association with the activity of protein-degrading

system in skeletal muscle of bulls of three wide-spread breeds. The study has also been designed to examine the effect of identified mutation on selected quality traits of beef.

Material and methods

Animals

Used were 31 Black-and-White (Polish Fresian - BW), 16 Charolaise (CH) and 18 Simmental (S) bulls. BW bulls were kept in Experimental Farm in Institute of Genetics and Animal breeding, Jastrzębiec, and fed *ad libitum* maize silage, hay and concentrate, while during the growth performance-testing period (20 days from month 7 to 8 of age) a full concentrate diet *ad libitum*. CH and S bulls came from Czech Republic and were fed *ad libitum* with standard food with access to water and kept up to 12-15 months of life. After 24 hours of fasting, the bulls were weighted, slaughtered and the samples of *longissimus dorsi* muscle (LD) from the region of XIII vertebra were excised 15 min post-slaughter, weighted and placed on ice, then frozen at -70°C for subsequent determination of protein content and lysosomal enzymes activity. Moreover, 900 g LD samples were taken to perform the meat sensory evaluation in the Laboratory of Meat Technology in Warsaw University of Agriculture. All procedures involving animals were approved by a Local Ethics Commission (permission No. 67/2001).

Analytical

Proteinase activity measurements. In a sample of LD the following proteolytic enzyme activities were determined: lysosomal cathepsin D and PSCatD, acid autolytic activity with pepstatin (PIAAA) and leupeptin (LIAAA) as inhibitors. Pepstatin is an inhibitor of cathepsin D while leupeptin inhibits thiol proteinases. Tissue samples were homogenized in 10 vol. of cold 0.1% triton x-100 in water on ice. The samples were incubated at 45^{o}C in 500 mM formic buffer, pH=3.75, for 1 h and the reaction was stopped by the addition of trichloracetic acid. The activity of cathepsin D was determined as PSCatD towards 1% hemoglobin according to Rosochacki [1985]. PIAAA and LIAAA activities were measured in the presence of 1 mM Mg^{++} (only autolytic activity with pepstatin was measured in buffer of pH 3.25). All proteolytic activities were measured using an alkaline cooper reagent, with tyrosine as a standard. Enzyme activity was expressed as μg of tyrosine per mg of protein per hour. Protein was determined according to Lowry *et al*. [1951].

PCR-RFLP genotyping. From each animal 10 ml of venous blood was collected on K_2EDTA, for DNA isolation, according to Kanai *et al.* [1994]. The *CATD* gene was screened with primers coding of cathepsin D propeptide. Primer pair was chosen according to Higuchi *et al.* [2003] as follows: FW - 5'-CGCTGCACAAGTTCACGTCC-3', length 20 bp; REV - 5'-ATCCATGTAGTTCTTGAGCA-3', length 20 bp. Amplified

was the 155 bp long fragment. PCR analysis involved 100 ng of DNA 1.0 µl, 2.5 µl 10 x Taq-amplification buffer, 0.25 µl of each of nucleotide at final concentration of 2.5 mM/µl, 0.10 µl of primers (concentration 10 pmol/µl), 0.5 µl (1 U) Taq-polimerase (SIGMA) in a final volume with water of 25 µl. The PCR of 28 cycles were: 1 min at 94°C, 30 s. at 58°C, and 1 min at 72°C; the final extension was 5 min at 72°C. The PCR product was separated by electrophoresis in 2% agarose gel (GIBCO-BRL, England) and visualized with ethidium bromide.

Amplified DNA samples were subjected to RFLP analysis of a total volume 10 µl reaction solution including 1.0 µl of specific NEBuffer, with 0.5 µl (2.5 U) of *Apa*I restriction enzyme (BIOLABS, New England, USA) and 8.5 µl of PCR product solution, at 25°C for *Apa*I for 3 hours. The obtained products were separated by electrophoresis in 4% agarose (NuSive GTG agarose:2 SIGMA agarose) in 1 x TBE buffer (0.09 M Tris-boric acid, 0.002 M EDTA) with 0.5 µg/ml ethidium bromide, visualized under UV light, and scanned in an FX Molecular Imager apparatus (Bio-Rad, Hercules, CA, USA).

Meat sensory evaluation. Associations between *CATD*/*Apa*I gene polymorphism and meat sensory parameters were determined fort LD samples from a total of 65 bulls. Sensory panel traits were analyzed at 72 h *post-mortem* and included: taste, aroma, tenderness and consistency. The sensory evaluations were carried out according to a 5-point scale as described by Baryłko-Pikielnia *et al*. [1964] and prescription given in the Polish Standard PN-IS06564. About 150 g sample was kept for 72 h at 4°C and next in 1% table salt solution for consequence four days, fried for 8 minutes on each side at 180°C.

Statistical. The data were analysed by one-way analysis of variance. The t-test was used to identify significant intergroup differences. To check the statistical differences between percent of inhibition, the square root transformation was done, and analysis of variance was performed.

Results and discussion

The first time the cDNA sequence of bovine procathepsin D was shown for the first time by Higuchi *et al*. [2003]. A 1580 bp-long sequence was found to consist of 1160 bp of translated regions and 420 bp of 3'UTR fragment of the gene. In this sequences, the mentioned authors identified 9 SNP, of which 7 were silent mutations and two others changed the amino acid sequence of protein. G/A substitution in coding region of procathepsin D recognized by *Apa*I endonuclease changed glycine for serine, but the other transversion – G/C – which was recognized by *Sma*I restriction endonuclease, changed the glycine for alanine and was found characteristic only for Japanese Shorthorn and Japanese Black cattle [Higuchi *et al*. 2003] appearing with the very low frequency (in only two out of 126 bulls analysed).

Using RFLP method the polymorphism in position 77 of the *CATD* gene was studied in 31 BW, 16 CH and 18 S bulls. Figure 1 shows only two genotypes shown after digestion with *Apa*I endonuclease – *GG* and *GA*. Similarly as to Japanese authors, homozygotic *AA* animals were not fund out in any of the bulls examined. The frequencies of the *G* and *A* alleles were: 0.806 and 0.194 in BW, 0.656 and 0.344 in CH and 0.639 and 0.361 in S bulls (Tab. 1).

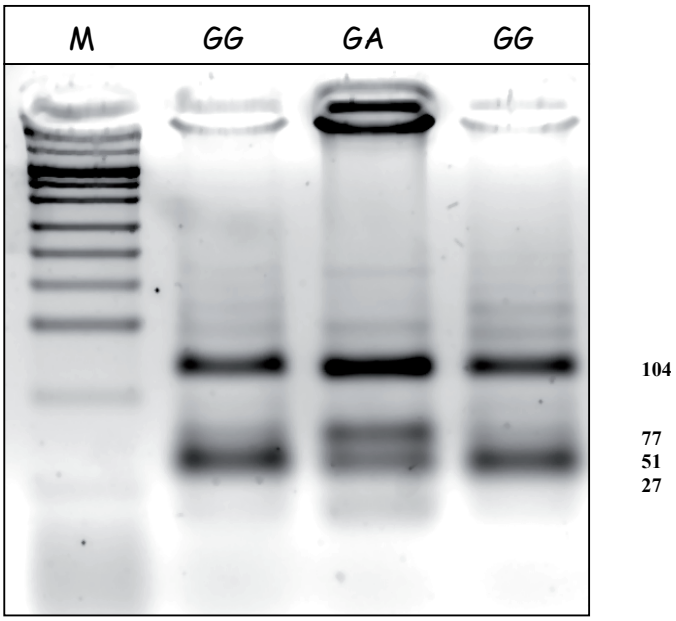

Fig.1. Agarose gel (4%) showing the polimorphism G/A at position 77 in the region coding procathepsin D in bovine *CATD* gene after digestion of the PCR product with endonuclease *Apa*I. M –DNA marker size (1444-80 bp); homozygote *GG* – restriction fragments: 104 bp, 51 bp and 27 bp; heterozygote *GA* – fragments of: 104 bp, 77 bp, 51 bp and 27 bp.

Table 1. Frequencies of genotypes at *Apa*I sites in G77A mutation in procathepsin D coding region of *CATD* gene

Genotype/allele	Black-and-White (n=31)		Charolaise (n=16)		Simmental (n=18)	
	observed	expected	observed	expected	observed	expected
GG	0.613	0.650	0.313	0.431	0.278	0.408
GA	0.387	0.312	0.688	0.451	0.722	0.461
AA	0.000	0.037	0.000	0.118	0.000	0.131
Frequencies of alleles						
G	0.806		0.656		0.639	
A	0.194		0.344		0.361	

The analysis was performed of the association between G77A polymorphism, recognized by restriction endonuclease *Apa*I at *CATD locus* of cattle (*GenBank* AB055312) localized in the coding region of procathepsin D, and the activity of cathepsin D and meat quality traits.

The activity of cathepsin D and autolysis measured in the LD muscle of BW, CH and S bulls is shown in Tabele 2. In the muscle of BW bulls the mean activities of CatD, PSCatD, AAA, PIAAA and LIAAA were the highest, as compared with CH and S bulls. Statistical analysis showed the effect of the breed on the activity of AAA, PIAAA and LIAAA being lower by 47.94% and 40.77% in AAA, by 46.41% and 37.45% in PIAAA and by 34.61% and 22.71% in LIAAA in CH and S than in BW bulls, respectively. The higher inhibition of AAA by leupeptin (CatD measured with the natural proteins as a substrate), was found out also in BW bulls, being lower in CH and S animals by 14.86% and 24.17%, respectively.

Earlier, Rosochacki *et al*. [2004, 2005] showed some interbreed differences in the measured activity of cathepsin. In their study the activity of thiol proteinases and cathepsin D were higher in muscles of BW than in Piedmontese or Angus, Hereford, Charolaise, Limousine and Simmental bulls. Piedmontese bulls are characterized by the double muscling (hypertrophic) phenotype controlled by a single dominant-recessive gene, like in the Belgian Blue breed. McPherron and Lee [1997] revealed the absence of mutations present in Belgian Blue or Piedmontese bulls (11-nucleotide deletion in the exon 3 of myostatin gene) in some others 15 breeds, including Charolaise, Simmental, Angus, Hereford and Limousine. The phenotype of the mutation in Piedmontese bulls is related to the missense mutation in exon 3 of myostatin gene, but in Limousines – to the multiple gene complex with additive effects. The results presented here and in the earlier authors' reports [Rosochacki *et al*. 2004, 2005] show the highest activity of CatD and PSCatD in meat of BW and also (among the examined breeds) in the muscles of Angus and Simmental bulls [Rosochacki *et al*. 2005]. Chambaz *et al.* [2003] reported that the Angus bulls showed a growth rate similar to Simmental and Charolaise, while Limousine grew more slowly. Based on the ratio PSCatD/PIAAA we could conclude, that the proteolysis of muscle proteins in CH and S bulls was mostly influenced by cathepsin D (ratio in CH and S was 3.97 and 3.73, but in BW – 2.73). It is in accordance with the earlier authors' results [Rosochacki *et al*. 2004, 2005]. The mentioned differences in the activity of lysosomal degrading system between BW and CH and S bulls (this and earlier studies) reflects the differences in the protein turnover and even more – in overall protein metabolism in the skeletal muscle. These results support the authors' earlier conclusions, that in LD of meat-type bulls an anabolic decrease in degradation occurred.

In the earlier authors' study [Juszczuk-Kubiak 2006] performed on 157 BW and 48 beef-type bulls, G/A mutation in *CATD locus* influenced the activity of cathepsin D (CatD), PSCatD both in BW and beef-type animals, however in the opposite direction (higher activities in BW bulls of genotype *GG* and lower in beef-type bulls of the same genotype). The highest cathepsin D activity was found in the muscles of beef-

Table 2. Enzymatic activity in LD muscle of bulls of different breeds (µg of tyrosine per mg of protein per hour) and inhibition (%) of cathepsin D by pepstatin, and acid autolytic activity by pepstatin and leupeptin (means±SE)

Breed		Protein (%)	Cat D	Cat D + pepstatin	PSCatD	Inhib. of CatD by pepstatin	AAA	PIAAA	Inhib. of AAA by pepstatin	LIAAA	Inhib. of AAA by leupeptin
BW	mean	16.46	40.17	10.13	30.03	74.74	26.17	11.01	57.25	15.13	41.66
	SE	1.81	4.90	1.80	3.98	3.52	4.76	2.11	7.23	3.00	8.40
CH	mean	21.9	39.81	9.98	29.85	74.84	17.69	7.52	56.55	11.24	36.27
	SE	0.956	4.29	1.12	3.63	2.33	2.71	1.90	8.75	1.85	7.00
S	mean	22.50	39.98	10.26	29.79	74.45	18.59	8.01	56.97	12.33	33.55
	SE	1.50	4.59	1.35	3.76	2.68	2.58	1.95	8.44	1.68	6.76
Significant differences		BW-CH, SIM**					BW-CH, S**	BW-CH, S**		BW-CH, S**	BW-CH* BW-S**

BW – Black-and-White; CH – Charolaise; S – Simmental; CatD – activity of cathepsin D; PSCatD – pepstatin-sensitive cathepsin D (measured towards 1% hemoglobin); AAA – acid autolytic activity; PIAAA – pepstatin-insensitive acid autolytic activity; LIAAA – leupeptin-insensitive acid autolytic activity.
*, **Difference between breeds significant at P≤0.05 and 0.01, respectively.

Table 3. Least squares means (LSM) and their standard errors (SE) for sensory-evaluated traits of meat of BW, CH and S bulls scored according to 5 points scale

Breed		Aroma	Taste	Tenderness	Consistency
BW	LSM	3.63	3.56	3.66	3.19
	SE	0.33	035	0.31	0.49
CH	LSM	4.94	4.83	4.84	4.36
	SE	0.26	0.22	0.28	0.34
S	LSM	4.92	4.74	4.44	4.41
	SE	0.38	0.48	0.73	0.51
		BW-CH, S**	BW-CH, S**	BW-CH, S** CH-SIM*	BW-CH, S**

**Differences between breeds significant at P≤0.01.
*Differences between breeds significant at P≤0.05.

Table 4. Leeast squares means (LSM) and their standard errors (SE) for sensory-evaluated traits of meat from BW bulls (scored according to 5 points scale) and for inhibition of AAA by pepstatin in CH bulls across the *GA* and *GG* genotypes

Breed	Trait		Genotype *GA*	Genotype *GG*	Significant difference (t-test)
BW	Aroma	LSM	3.48	3.80	**P≤0.01
		SE	0.38	0.10	
	Taste	LSM	3.40	3.76	**P≤0.01
		SE	0.28	0.15	
	Tenderness	LSM	3.58	3.76	ns
		SE	0.25	0.17	
	Consistency	LSM	2.99	3.43	**P≤0.01
		SE	0.38	0.26	
CH	Inhib. of AAA by pepstatin	LSM	60.70	52.40	*P≤0.05
		SE	5.41	5.01	

type bulls with genotype *GG*, being higher in CatD and PSCatD by 9.1% and 11.2% respectively, as compared to bulls of a genotype *GA* (P<0.05). Similar influence of *G* allele in the region coding *CATD* gene was found out for the per cent inhibition of CatD by pepstatin, which showed approximate quantity of the enzyme. That inhibition was by 7.0% higher in muscle of *GG* bulls (P<0.05). The protein content measured with the aid of Folin reagent was higher by 6.4% in the muscles of heterozygotic bulls at *CATD locus* [Juszczuk-Kubiak 2006].

In this work, the mutation G77A in *CATD* gene influenced only the per cent of inhibition by pepstatin in AAA (concerning the thiol proteinases in the muscle), and it was higher in heterozygotic CH animals by 15.83% (P<0.05; Tab. 4).

For statistical analysis of polimorphism association in the region coding procathepsin sequence in *CATD* gene the data concerning the sensory meat evaluation were used. In the analysis of polymorphism in procathepsin D gene (by RFLP/*Apa*I genotypes *GG* and *GA*) and meat sensory traits, 31 BW and 16 CH and 18 S beef-type bulls were used. The means for all traits (aroma, taste, tenderness and consistency) scored, by a panel of five experienced people are presented in Table 3.

All measured meat sensory trait values appeared much lower in BW than in CH and S bulls. The sensory traits were higher in meat of CH and S bulls than in meat of BW: by 36.09% and 35.54% for aroma; by 35.67% and 33.15% for taste, by 32.24% and 21.31% for tenderness and by 36.68% and 38.24% for consistency, respectively ($P<0.01$). Within tenderness, significant differences were also identified between CH and S bulls in favour of the former by 9.01% ($P<0.05$). The genetic differences in measured traits must be related to genetic differences in early *post-mortem* structural changes in the myofibrillar (connective tissue, fiber type, marbling) and /or to early *post-mortem* proteolytic activity.

The differences between estimated genotypes *GG* and *GA* in *CATD locus* (RFLP/*Apa*I mutation) but only in BW bulls were also noticed in taste, aroma and consistency (Tab. 4), being higher in genotype *GG* ($P<0.01$); the meat of *GG* animals had higher values by 9.20% in aroma, 9.30% in taste and by 14.72% in meat consistency ($P<0.01$) – Table 4 – being in accordance with Juszczuk-Kubiak [2006] data, but not statistically confirmed. Juszczuk-Kubiak [2006] showed the effect of genotype on meat consistency, but in the beef-type bulls, being the best in *GG* animals ($P<0.05$).

Juszczuk-Kubiak [2006] showed on 76 BW and 71 beef-type bulls, that meat of the former with *GA* variant of the *CATD* gene contained more fat (%) and was more tender (lower SF_{48}) than that of bulls with *GG* genotype, what could be related to the localization of fat in the muscle fibrils [Jurczak 2000]. Allele *G* was also related to the higher water holding capacity 48 h *post-mortem*.

In the present study, for the first time, the associations were shown between RFLP/*Apa*I of the bovine *CATD* gene and the activity of cathepsins and several meat quality traits in a group of Polish Black-and-White, Charolaise and Simmental cattle. As the investigation was performed on a total of 65 animals only, the findings presented might not be numerous enough to allow the proper definite conclusions concerning the influence of the mutation on activity of some lysosomal proteinases in different cattle genotypes and on meat quality traits.

REFERENCES

1. BARYŁKO-PIEKIELNA N., KOSSAKOWSKI T., BALDWIN Z., 1964 – Wybór optymalnej metody przygotowania mięsa wołowego i wieprzowego do oceny sensorycznej. ***Roczniki Instytutu Przemysłu Mięsnego*** 1, 132-139.
2. BELCASTRO A.N., ALBISSER T.A., LITTEJOHN B., 1996 – Role of calcium-activated neutral protease (calpain) with diet and exercise. ***Canadian Journal of Applied Physiology*** 21, 328-346.

3. CALKINS C.R., SEIDEMAN S.C., 1988 – Relationships among calcium dependent protease, cathepsin B and H, meat tenderness and the response of muscle to ageing. ***Journal of Animal Sciences*** 66, 1186-1193.
4. CHAMBAZ A., SCHEEDER M.R.I., KREUZER M., DUFEY P.A., 2003 – Meat quality of Angus, Simmental, Charolaise and Limousine steers compared at the same intramuscular fat content. ***Meat Sciences*** 63, 491-500.
5. DOUMIT M.E., KOOHMARAIE M., 1999 – Immunoblot analysis of calpastatin degradation: evidence for cleavage by calpain in postmortem muscle. ***Journal of Animal Science****s* 77, 1467-1473.
6. FIEDLER I., REHFELD C.H., ALBRECHT E., HENNING M., 1998 – Histophysiological features of skeletal muscle and adrenal glands in wild-type and domestic pigs during growth. ***Archiv für Tierzucht,*** *Dummerstorf* 41, 489-495.
7. GOLL D.E., THOMPSON V.F., TAYLOR R.G., CHRISTIANSEN J.A., 1992 – Role of the calpain system in muscle growth. ***Biochimie*** 74, 225-237.
8. GOLL D.E., THOMPSON V.F., TAYLOR R.G., OUALI A., 1998 – The calpain system and skeletal muscle growth. ***Canadian Journal of Animal Sciences*** 78, 503-512.
9. GRANT A.L., HELFERICH W.C., 1991 – An overview of growth. In: "Growth regulation in farm animals. Advances in Meat Research" (Edited by PEARSON, A.M.; DUSTON, T.R.), Elsevier, London, New York, (Vol. 7), 1-16.
10. HARPER G.S., 1999 – Trends in skeletal muscle biology and the understanding of toughness in beef. ***Australian Journal of Agricultural Research*** 50, 1105-1129.
11. HIGUCHI M., MIYASHITA Y., NAGAMINE A., WATANABE A., AWATA T., 2003 – The complementary DNA sequence and polymorphism of bovine procathepsin D (*CATD*). ***Journal of Animal Breeding and Genetics*** 120, 322-330.
12. JURCZAK M., 2000 – Ocena jakości mięsa. Skrypt dla studentów SGGW, Warszawa (Meat Quality Evaluation). In Polish.
13. JUSZCZUK-KUBIAK E., 2006 – Identyfikacja markerów molekularnych powiązanych z proteolizą białka w mięśniach i jakością mięsa. Thesis. IGiHZ, Jastrzębiec.
14. KANAI N., FURII T., SAITO K., TOKOYAMA T., 1994 – Rapid and simple method for preparation of genomic DNA from easy obtainable clotted blood. ***Journal of Clinical Pathology*** 47, 1043-1044.
15. KÜCHENMEISTER U., KUHN G., 2003 – Regulation of intercellular Ca^{2+} concentration and meat quality in pigs. ***Archiv für Tierzucht,*** Dummerstorf 46, 445-454.
16. KÜCHENMEISTER U., LANGHAMMER M., RENNE U., NÜRNBERG G., ENDER K., 2001 – Effects of exercise on sarcoplasmic reticulum Ca^{2+} transport in muscle of mice lines long-term selected for different performance traits. ***Archiv für Tierzucht,*** *Dummerstorf* 44, 441-450.
17. LOWRY O. H., ROSEBROUGH N.J., FARR A.L., RANDALL R.J., 1951 – Protein measurement with Folin phenol reagent. ***Journal of Biological Chemistry*** 193, 265-275.
18. MCPHERRON A.C., LEE S.J., 1997 – Double muscling in cattle due to mutations in the myostatin gene. ***Proceedings of the National Academy of Sciences USA*** 94, 12457-12461.
19. MIKAMI M., WHITTING A.H., TAYLOR M.J., MACIEWICZ R.A., ETHERINGTON D.J, 1987 – Degradation of myofibrils from rabbit, chicken and beef by cathepsin L and lysosomal lysates. ***Meat Sciences*** 21, 81-97.
20. MILLWARD D.J., BATES P.C., BROWN J.G., ROSOCHACKI S.J., RENNIE M.J., 1980 – Protein degradation and the regulation of protein balance in muscle. In: Protein degradation in health and disease. CIBA Foundation 75, Excerpta Medica, Elsevier/North Holland, Amsterdam, 307-329.
21. MILLWARD D.J., BATES P.C., ROSOCHACKI S.J., 1981 – The extent and nature of protein degradation in the tissues during development. ***Reproduction, Nutrition and Development*** 21, 265-277.

22. REHFELD C.H., BÜNGER L., 1990 – Auswirkungen einer Langzeitselektion von Labormäusen auf Merkmale des Muskelwachstums und der Muskelstruktur. ***Archiv für Tierzucht,*** *Dummerstorf* 33, 507-516.
23. ROSOCHACKI S.J., 1985 – Changes in cathepsin D, proteolytic activity and RNA and DNA content in the anterior and posterior *lattissimus dorsi* muscle of the adult fowl as affected by the induced hypertrophy. ***Archiv für Geflügelkunde*** 49, 81-90.
24. ROSOCHACKI S.J., SAKOWSKI T., POŁOSZYNOWICZ J., JUSZCZUK-KUBIAK E., KOWALUK-KRUPA A., OPRZĄDEK J., 2004 – Lysosomal proteolysis in the skeletal muscle of bulls. ***Czech Journal of Animal Science*** 49, 348-356.
25. ROSOCHACKI S.J., SAKOWSKI T., JUSZCZUK-KUBIAK E., BUTAREWICZ A., POŁOSZYNOWICZ J., 2005 – Cathepsin involvement in muscle proteolysis in meat-type bulls. ***Czech Journal of Animal Science*** 50, 422-429.
26. SMITH T.P.L., CASAS E., REXROAD 3rd C.E., KAPPES S.M., KEELE J.W., 2000 – Bovine CAPN1 maps to a region of BTA29 containing a quantitative trait locus for meat tenderness. ***Journal of Animal Sciences*** 78, 2589-2594.
27. TAYLOR R.G.H., GEESINK V.F., THOMPSON M., KOOHMARAIRE M., GOOL D.E., 1995 – Is Z-disk degradation responsible for postmortem tenderization? ***Journal of Animal Sciences*** 73, 1351-1367.
28. THOMPSON M.G., PALMER R.M., 1998 – Signaling pathways regulating protein turnover in skeletal muscle. ***Cell Signaling*** 10, 1-11.

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Związek między polimorfizmem G77A w genie *CATD* a aktywnością niektórych lisosomalnych proteinaz w mięśniach szkieletowych i jakością sensoryczną mięsa buhajów trzech ras

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

Badano związek między polimorfizmem G77A w genie *CATD* a aktywnością enzymów proteolitycznych i jakością sensoryczna mięśnia najdłuższego grzbietu 31 buhajkówh rasy cb (BW), 16 rasy charolaise (CH) i 18 rasy simental (S). Wykazano wysoko istotne różnice w kwaśnej aktywności autolitycznej (AAA), AAA nieczułej na pepstatynę (PIAAA) i AAA nieczułej na leupeptynę (LIAAA) między buhajami rasy fryzyjskiej (aktywności wyższe) a zwierzętami ras charolaise i simental (aktywności niższe). Mięso buhajków rasy BW różniło się istotnie od mięsa buhajków ras charolaise i simental pod względem smaku, aromatu, tekstury i konsystencji (niższe wskaźniki tych cech charakteryzowały mięso buhajków BW). Stwierdzono, że genotyp (*GA* lub *GG)* nie wiązał się ani z aktywnością badanych enzymów proteolitycznych w mięsie buhajków wszystkich trzech ras, ani z wartościami cech mięsa buhajków ras CH i S. Mięso zwierząt rasy BW o genotypie *GG* okazało się wartościowsze pod względem aromatu, smaku, konsystencji i kruchości niż mięso buhajków o genotypie *GA*.