IMPACT OF POLYMORPHISM OF THE REGULATORY SUBUNIT OF THE μ-CALPAIN ON THE PROTEOLYSIS AND TENDERNESS OF MEAT FROM YOUNG BULLS

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***Abstract* - The objective of this study was to estimate the impact of polymorphism of the regulatory subunit of the μ-calpain (*CAPN1S*) gene on the course of proteolysis and the process of bovine meat tenderisation during 10-day cold storage. The analysis was performed on *longissimus thoracis and lumborum* (LT&L) muscles collected from 76 bulls 6 to 12 months of age. Polymorphism identification of the above-mentioned gene was conducted using the PCR-RFLP technique. Its effect on the course of the proteolysis was assessed by monitoring changes in proportions of muscle proteins during 10-day process of meat ageing. Special attention was focused on changes in native titin (T1) share and products of its degradation (proteins of molecular weight (*m.w.)* of 2400 kDa and 200 kDa) and protein of 37 kDa as well as myosin heavy chains (MHC). In the case of the last proteins, their polymorphism was evaluated as well. Meat tenderness was estimated measuring the value of shear force and sensorially. The highest tenderness was ascertained for the heterozygote. Its improvement was associated with a significant decrease in proportions of proteins of approximately 37 kDa accompanied by an increase of those with 200 kDa. Muscles derived from CT genotype were characterised by the highest proportions of type 2a MHC isoform. Value differences between proportions determined for the heterozygote and CC and TT homozygotes of the *CAPN1S* gene were statistically significant.**

Index Terms: beef tenderness, *CAPN1S* gene, proteolysis, MHC

I. INTRODUCTION

The proteolysis of myofibrillar proteins plays a major role in *post mortem* (*p.m.*) meat tenderisation. It is associated with the action of two enzymatic systems: calpain and cathepsin. It is believed that the calpain system plays the main role in the *p.m.* proteolysis and the process of meat tenderisation. A special role in this regard is assigned to μ-calpain (Geesink, Kuchay, Chishti & Koohmaraie 2006; Koohmaraie & Geesink 2006).

There are many factors influencing the activity of proteolytic enzymes. Special attention is paid to genetic once (Casas, White, Wheeler, Schackelford, Koohmaraie, Riley, Chale, Jonson and Smith 2006; Costello, O'Doherty, Troy, Ernst, Kim, Stapleton, Sweeney and Mullen 2007). Page, Casas, Heaton, Cullen, Hyndman, Morris, Crawford, Wheeler, Koohmaraie, Keele and Smith (2002) described two mutations in the μ-calpain gene (*CAPN1*) which were associated with bovine meat tenderness. Transversion in exon 9 (C3709G) and transition in exon 14 (A4558G) were significantly correlated with bovine meat tenderness in Piemontese x Angus as well as Jersey x Limousine breeds measured 48 h *p.m.* Polymorphism in intron 17 of this gene was significantly associated with meat tenderness measured on days: 7, 14 and 21 *p.m.* in Brahman breed. Experiments conducted by Juszczuk-Kubiak (2006) revealed a links of the RFLP/*Fok*I polymorphism of the *CAPN1* gene (intron 14 and exon 6 – RFLP-*Hpy*CH4IV/*Age*I) with bovine meat quality, including its tenderness and *CAPN2S* (3'UTR – RFLP/*Mbo*II*)* gene. i.e. a regulatory subunit of m-calpain with the above-mentioned traits. Recently, polymorphism of a new SNP in the 3'UTR region of the bovine μ-calpain small subunit (*CAPNS1*) gene (exon 11 – RFLP/*Mbo*II) has been discovered (Juszczuk-Kubiak, Flisikowski & Wicińska 2010).

Therefore, it was considered appropriate to investigate its impact on the course of proteolysis and the process of bovine meat tenderisation.

II. MATERIALS AND METHODS

Analyses were performed on *LT&L* muscles collected from 76 bulls of four cattle breeds (Holstein-Friesian, Polish Red, Hereford and Limousine) slaughtered at the age of 6 to 12 months. The number of samples for individual genotypes with regard to *CAPN1S* gene ranged from 8 to 36.

CAPN1S gene polymorphism identification of bovine muscles was performed with the assistance of the PCR-RFLP technique employing *Mbo*II endonuclease (Juszczuk-Kubiak et al., 2010). Primers with CAPN1S-F 5'-CCTCACTGTCTGTCCCTTCC-3' and CAPN1S-R 5'-ACACAAATGTTGGGCTTGG-3' sequences amplifying 332 bp were used in the experiment. The identification of the μ-calpain gene was performed in the 3'UTR – axon 11 region.

Samples for analyses were collected 45 minutes *p.m.* and proportions of MHC isoforms in fractions of washed myofibrils were determined according to Mozdziak, Greaser & Schulz (1998). Changes in protein proportions of muscle tissue were evaluated 45 minutes, 48, 96 and 240 hours *p.m.* using SDS-PAGE (15% polyacrylamide) with addition of 8M urea (Pospiech, Szalata., van Lack, Sośnicki & Greaser 2001) in which the acrylamide to bis-acrylamide ratio was 199:1 (Fritz, Swartz & Greaser 1989). From among muscle tissue proteins, particular attention was paid to the proteins with the m.w. of approximately 3700 kDa, 2400 kDa, 200 kDa and 37 kDa.

Meat tenderness measurements were conducted using an Instron type 1140 apparatus with a Warner-Bratzler attachment (Grześ, Pospiech, Rosochacki, Łyczyński, Mikołajczak & Iwańska 2007). Additionally, sensory tenderness assessment according to linear scale (Adamik 1997, Baryłko-Pikielna & Matuszewska 2009) was carried out where the score of 10 points corresponded to very tender and 1 point – very tough meat. Tenderness analyses, both instrumental and sensory, were conducted on days: 2, 4 and 10 of the meat cold storage ageing.

Statistical calculations were based on the analysis of variance, whereas the significance of differences was calculated using Tukey test (*Statistica* v. 8.0 software).

III. RESULTS AND DISCUSSION

Depending on genotypes, the determined proportions of native titin (T1 of 3700 kDa) in the muscle tissue of the experimental cattle ranged from 2.53 to 3.21% (Tab. 1) and were relatively low in comparison with data reported in other experiments (Kołczak, Pospiech, Palka & Łącki 2003; Huff-Lonergan, Mitsuhashi, Beekman, Parrish, Olson Jr. & Robson 1996) where these proportions fluctuated from 7 to 10%. At the same time, a relatively large share (from 3 to 5%) occurred for the 2400 kDa band which was very likely the product of titin degradation referred to as T2. This could indicate that the process of titin degradation in the meat of the examined bulls was relatively well advanced and its total proportion determined on the basis of the entire band comprising titin T1 and T2 ranged from 8 to 9%.

The highest share of 2400 kDa protein was observed in homozygotes but differences between homozygotes and heterozygote amounted only to 0.22-0.23% and were not statistically significant ($P \geq 0.05$) (Tab. 1). The proportion of 200 kDa protein corresponding to MHC and titin degradation products (Taylor, Geesing, Thompson, Koohmaraie & Goll 1995, Iwanowska, Iwańska, Grześ, Mikołajczak, Pospiech, Rosochacki & Łyczyński 2010) increased during the cold storage period from the initial 14% to the final level of over 16% (Tab. 1). Statistically significant differences were determined for the heterozygote and homozygote CC. As evident from experiments conducted by Sawdy, Kaiser, St-Pierre & Wick (2004) and Morzel, Terlouw, Chambon, Micel & Piccard (2008), MHC undergo *p.m.* degradation the effect of which can also be the process of meat tenderisation. The observed increased proportion of the 200 kDa band at simultaneous possibility of MHC degradation allows to assume that this increase could have been associated with the advancing titin degradation since products of its transformation are also observed in the band with the same m.w.

In the course of *p.m.* cold storage, changes were recorded in proportions of 37 kDa protein which, with respect to m.w., corresponds to troponin T (Ho, Stromer & Robson 1994). This protein may correspond also to the glyceraldehyde 3-phosphate dehydrogenase - GAPDH (Okumura, Yamada & Nishimura 2003, Okayama, Fukumoto, Nakagawa, Yamanoue & Nishikawa 1992). Beginning with the 45 min. until the 240 h after slaughter, the share of this protein decreased from 7.03% to 3.70% (Tab. 1). The process of degradation of this protein was similar for the heterozygote and homozygotes, although its course was most dynamic (decline in proportions of about 50%) for the TT homozygote and the slowest for the heterozygote. Among the analysed genotypes of the *CAPN1S* gene, statistically significant differences were observed in proportions of this protein between the analysed terms of ageing. In the case of the TT homozygote, these differences concerned extreme values, whereas for the heterozygote and CC homozygote, differences were recorded between three terms of cold storage. The lowest level of the 37 kDa protein following 240 h storage was observed for CC homozygote and differences between its proportions for this genotype and the heterozygote were statistically significant (Tab. 1). This appears to indicate similar relationships regarding changes of this protein to those observed in the case of

GAPDH in pork and beef meat (Okumura et al., 2003; Okayama et al. 1992). The above remark does not rule out the participation in this process of troponin T whose degradation is associated with increased meat tenderness.

When analysing the course of changes taking place in muscle tissue *p.m.*, which are associated with proteolysis and exert influence on its tenderness, attention is frequently focused on the character of muscle fibre metabolism (Xiong 1994, Huff-Lonergan et al., 1996) emphasising that the course of proteolysis is usually faster in muscles with the majority of type 2a fibres in comparison with the red fibres. In the discussed experiment, the highest (38.08%) proportion of the type 2a MHC was determined for the heterozygote of the *CAPN1S* gene and differences between values determined for the heterozygote and homozygotes were statistically significant (Tab. 2). Statistically significant differences also occurred for proportions of the type 1 MHC between heterozygote and homozygotes (Tab. 2). The observed similar course of muscle protein degradation changes recorded for the compared genotypes accompanied by significant differences in MHC polymorphism may indicate that muscle fibre metabolism affected by *CAPN1S* was a decisive factor in the meat tenderisation process.

Meat tenderness was evaluated at three terms (48, 96 and 240 h). Together with the progressing of proteolysis a gradual drop in the shear force value was observed. After 48 h of ageing, the highest tenderness was determined for the CC homozygote, while already from the 4. day, the lowest shear force values were determined for heterozygote. On the last day of investigations, statistically significant differences were observed between the tenderness determined for the CC homozygote and heterozygote. Meat from the latter genotype was characterised by the highest tenderness (Tab. 3). Costello et al. (2007) investigating the relationship of the calpain I and II gene polymorphism with meat tenderness also determined its highest value for heterozygote.

Sensory assessment of meat tenderness reflected the results of the shear force value measurements. The highest scores were given to the meat that was ageing for 10 days, though they were not the highest in absolute terms (Tab. 3). On the 10· day of ageing, the highest scores were assigned to heterozygote (CT) but differences between it and the remaining genotypes of the *CAPN1S* gene were not statistically significant (Tab. 3).

When analysing shear force values and scores allotted to meat samples by sensory evaluation, statistically significant differences were observed between them taking into consideration cold storage time. These differences were observed when analysing all samples, irrespective of their genotype in relation to the *CAPN1S* gene and when its impact was taken into consideration.

IV. CONCLUSION

The highest tenderness of meat was determined for the heterozygote of the *CAPN1S* gene.

Its improvement was associated with a significant decrease in the proportions of 37 kDa proteins and increase of those with 200 kDa. The recorded drop in proportions of the first group of proteins could have been associated with troponin T and GAPDH degradation, whereas the increase in the second band – with titin degradation and appearance of products of its degradation.

Heterozygote of the *CAPN1S* gene was characterised by the highest share of the type 2a MHC isoform and the lowest of the type 1.

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Table 1. Share of selected proteins in bovine *LT&L* muscle depending on genotype of μ-calpain (*CAPN1S*) and storage time in chilled room (%)

Genotypes MboII	N	Molecular weight of proteins (kDa) and storage time of meat (45', 48, 96 and 240h)															
		3700				2400				200				approx. 37			
		45	48	96	240	45	48	96	240	45	48	96	240	45	48	96	240
Total	56	3,1	3,3	3,1	3,0	3,4	3,9	4,1	4,5	$14,0^{B}$	$15,5^{A}$	$15,5^{A}$	$16,1^{A}$	$7,0^{D}$	$5,9^{C}$	$5,3^{B}$	$3,7^{A}$
CT (1)	25	3,2	3,7	3,5	2,5	3,2	3,6	3,9	4,4	$13,7^{B}$	$15,9^{A}$	$15,9^{A}$	$16,0^{A}$	$7,3^{C}$	$5,8^{A}$	$5,3^{A}$	$4,1^{Bb}$
CC (2)	26	3,2	3,1	2,8	3,0	3,7	4,0	4,2	4,6	$14,2^{A}$	$15,2^{AB}$	$15,2^{AB}$	$16,1^{B}$	$6,7^{A}$	$6,0^{A}$	$5,2^{C}$	$3,4^{aB}$
TT (3)	8	2,5	2,6	3,3	4,2	3,5	4,7	5,0	4,6	14,1	15,0	15,3	16,2	$7,3^{A}$	$5,9^{A}$	$5,3^{AB}$	$3,6^{abB}$

$^{a, b, c}$ – mean values marked with different small letters differ in columns (P≤0,05); $^{A, B,C}$ – mean values marked with different capital letters differ in rows (P≤0,05); N – number of animals in the group

Table 2. Share of MHC isoforms in *LT&L* muscle depending on μ-calpain genotype (*CAPN1S*)

Genotypes MboII	N	MHC 2a	MHC 1
Total	76	34,20	36,21
CT (1)	29	$38,08^{b}$	$30,19^{b}$
CC (2)	36	$31,07^{a}$	$40,20^{a}$
TT (3)	11	$34,20^{a}$	$39,01^{a}$

$^{a, b, c}$ – explanations – see table 1

Table 3. Values of shear force (N/cm^2) and results of sensory evaluation of *LT&L* muscle tenderness (scores) depending on μ-calpain genotype (*CAPN1S*)

Genotypes MboII	N	Shear force value of meat (N/cm^2)			N	Sensory evaluation of meat (scores)		
		48h	96h	240h		48h	96h	240h
Total	69	$148,6^{C}$	$133,9^{B}$	$108,2^{A}$	71	$4,2^{A}$	$4,9^{B}$	$5,7^{C}$
CT (1)	27	$146,0^{A}$	$132,0^{A}$	$99,1^{aB}$	27	$4,2^{A}$	$4,7^{A}$	$6,1^{B}$
CC (2)	31	$153,7^{C}$	$135,5^{B}$	$117,0^{bA}$	33	$4,1^{A}$	$4,8^{AB}$	$5,5^{B}$
TT (3)	11	$140,8^{A}$	$134,2^{A}$	$105,4^{abB}$	11	4,7	5,6	5,6

$^{a, b, c, A, B,C}$ – explanations – see table 1