PRELIMINARY INVESTIGATIONS ON RELATIONSHIP BETWEEN POLYMORPHISM AT CAST LOCUS AND THE QUALITY OF PORK

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Preliminary communication
Prethodno priopćenje

SUMMARY

The present study was performed in order to investigate a relationship between polymorphisms on the calpastatin gene (CAST) and pig meat quality traits. The investigation was carried out on 29 gilts and barrows, crosses of Large White x German Landrace randomly selected at slaughter line. Pigs were slaughtered at 130-150 kg of live weight and blood samples were taken for genomic DNA analysis. The following indicators of meat quality and meat chemical composition were evaluated: pH and electric conductivity measured 45 minutes post mortem in m. Longissimus dorsi (LD muscle) and in M. Semimembranosus (SM muscle); electric conductivity measured at the same locations after 24h of cooling; drip loss (determined by "bag method"); colour (measured with Minolta chromameter and expressed as Hunter L, a, b values); instrumental tenderness assessed as Warner-Bratzler (WB) shear force and moisture, fat, protein and collagen content (%) determined on cooked LD muscle after 24h of thawing. The amplification products of the CAST gene were digested with HinfI restriction endonuclease and three genotypes (AA, BB and AB) were revealed. Statistical analysis showed that meat originated from pigs of AB genotype had the lowest WB shear force and the highest protein content of cooked LD muscle. As for the indicators of technological meat quality, statistically significant differences (p<0.05) were found between genotype AA and both BB and AB genotype for electric conductivity measured in LD muscle after 24h of cooling, as well as between BB and both AA and AB genotypes for drip loss.

Key-words: CAST gene, polymorphism, pig, meat quality

INTRODUCTION

Meat quality is a complex term used to describe various properties of meat. It can be defined in many ways and from different standpoints: from the aspect of pig industry or consumer. For pig industry, the most important meat properties are pH value, electric conductivity, drip loss, cooking and curing loss, chemical properties, etc., which are often referred as "technological meat quality". On the other hand, taste, colour, fat content, texture or tenderness are meat properties affecting "sensory or eating quality", thus important for consumer. Among these traits, tenderness is considered as a limiting factor of meat acceptability for a consumer (Miller et al., 2001). It is believed that the key determinant of the final meat tenderness is the proteolysis of key target proteins within the muscle fibres (Taylor et al., 1995). The investigations on all major livestock species have shown that calpain enzyme family is responsible for this peptide cleavage (Koohmaraie, 1996). The calpain system comprises of two omnipresent isoforms called μ-calpain and m-calpain, and calpastatin, a specific endogenous inhibitor specifically acting on μ- and m-calpain Ca^{2+} endonuclease.

There is evidence suggesting that calpastatin activity postmortem is highly related to meat tenderness in different species (Koohmaraie et al., 1991; Sensky et al., 1998). Studies on the randomly selected commercial pig fatteners have shown that high level of calpastatin in the first few hours *post mortem* were associated with increased prevalence of tough meat (Parr et al., 1999). Although there are at least eight calpain genes (Braun et al., 1999), it is believed that there is only one calpastatin gene.

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The investigations on ninety-seven unrelated pigs from 11 breeds genotyped for polymorphisms identified with *Hinf*I, *Msp*I and *Rsa*I enzymes, revealed chromosomal localization of pig CAST gene at pig chromosome 2; region q2.1-q2.4 (Ernst et al., 1998). Krzęcio et al. (2005) investigated an association of the CAST/*Msp*I genotype on certain meat quality traits of fatteners and found its significant effect on drip loss from *Longissimus lumbor*um muscle at 48h and 96 h *post mortem*. Moreover, Krzęcio et al. (2008) established a significant effect of CAST/*Rsa*I polymorphism on pH values at 35 min and 2, 3, 24, 48, 96 and 144h post mortem, IMP/ATP ratio, electric conductivity at 3h and 4h *post mortem*, technological yield of meat in curing and thermal processing and protein content in muscle tissue.
Ciobanu et al. (2004) identified two CAST polymorphisms affecting pork texture: Arg249Lys and Ser638Arg encompassing exon 13 and exon 28, respectively. Stalder et al. (2005) established that CAST Ser638Arg polymorphism was a significant source of variation for cured ham moisture content and has a tendency to be a significant source for yield, ham weight loss, salt content and Minolta colour change. Škrlep et al. (2009) demonstrated the effect of CAST Lys249Arg polymorphism on green ham weight and CAST Lys249Arg polymorphism on green ham colour.
The aim of the present study was to investigate the relationship between polymorphisms of porcine calpastatin gene fragment encompassing intron 6 identified with *Hinf*I restriction endonuclease and the traits important for technological and sensory quality of pig meat.

MATERIAL AND METHODS

Animals
The study was performed on 29 carcasses of gilts and barrows, crosses of Large White x German Landrace. The animals were randomly selected at a slaughter line in one slaughterhouse in eastern Croatia. They were slaughtered at 130-150 kg of live weight, when blood samples for DNA analysis were taken.

Meat quality evaluation
Value of pH (pH_{45}) and electric conductivity (EC_{45}) were measured on *Longissimus dorsi* (LD) muscle and on the *Semimembranosus* muscle (*SM*) 45 minutes after slaughter. The electrical conductivity (EC_{24}) was measured in the same locations after 24h of cooling of the carcasses at temperature of 4°C. The measurements of pH value and electric conductivity were performed with digital pH-meter (Mettler MP 120-B) and LF Star device (Matthäus, Germany), respectively. Drip loss was determined on *LD* muscle by bag method according to Honikel (1987). Cooking loss was established on *LD* chops used for shear force determination, and expressed in percent of sample weight prior to cooking. The colour of *LD* muscle was measured 24h after cooling of the carcasses (15 minutes of blooming) using a Minolta chromameter (Model CR-300, Minolta Camera Co. Ltd., Osaka Japan) and presented as Hunter L, a, b measurements. For tenderness measurements, 2.54 cm thick chops of *LD* muscle were sealed in plastic bags and frozen at -20°C. Prior to measurements the samples were defrosted at 4°C for 24h, cooked in a water bath until internal temperature reached 73°C and cooled at 4°C over night. Shear force was measured on at least four subsamples of each chop taken with a core cylinder. The subsamples were analysed with a TA.XT*plus* Texture Analyser fitted with a 1 mm thick Warner-Bratzler (WB) shear attachment. The mean value of maximal strength necessary for cutting of the sample was calculated with a Texture Exponent 4.0 Software (Stable Micro Systems Ltd., UK).

Chemical composition of cooked meat
The content of fat, moisture, protein and collagen were determined on cooked sample of *LD* muscle with FoodScan Lab NIT analyser (Foss, Denmark).

PCR-RFLP analyses
Genomic DNA was isolated from the blood by standard extraction protocol with phenol-chloroform-isoamil (25:24:21) alcohol (Ausubel et al., 2000). Polymerase chain reaction (PCR) was carried out in a total volume of 20 µl containing 100 ng genomic DNA, 1 x PCR buffer, 200 µM dNTP, 1 mM $MgCl_2$, 1 U Taq DNA polymerase (Fermentas, Litva) and 5 pmol of each oligonucleotide. Primer

sequences were: 5‘-GCGTGCTCATAAAGAAAAAGC-3‘; and 5‘-TGCAGATACACCCAGTAACAG -3‘.
PCR was performed according to the following protocol: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C (45 sec), annealing at 58°C (45 sec), extension at 72°C (1 min), with final extension step at 72°C for 6 minutes. Restriction digestion of PCR products was carried out in a total volume of 15.0 µl containing 12.0 µl of PCR product, 10 x reaction buffer and 1 U *Hinf*I restriction endonuclease (Promega, USA). Reactions were incubated at 37°C for 2h and resolved on 3% agarose gel.

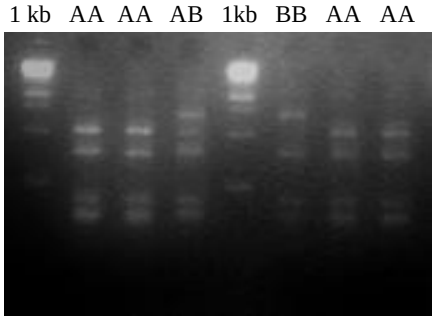

Figure 1. Profile of CAST gene digested by HinfI endonuclease. There are five fragments (174, 200, 372, 503 and 646 bp) detected. Genotype AB has all five fragments; genotype AA lacks the longest fragment, while in genotype BB the 503 bp fragments did not exist. M: 1 kb DNA step ladder (Promega, USA)
Slika 1. Genotipovi na CAST genu dobiveni restrikcijom sa HinfI endonukleazom. Detektirano je pet fragmenata (174, 200, 372, 503 i 646 bp). Genotip AB sadrži svih pet fragmenata; genotipu AA nedostaje najduži fragment, dok genotipu BB nedostaje fragment od 503 bp. M: 1 kb DNA step ladder (Promega, USA)

Statistical analysis
The obtained data were subjected to one-way analysis of variance using GLM procedure where gene polymorphisms were included in the linear model as categorical predictor variable and the measured traits as dependant variables. Means, which were significantly different, were identified using Fisher’s LSD test (STATISTICA 7.1 for Windows Software, 1984-2006).

RESULTS AND DISCUSSION

Table 1 shows results of LSD test between investigated pig genotypes for traits of technological meat quality. From the presented results, an influence ($p<0.05$) of polymorphism at the CAST locus on ultimate electric conductivity (EC_{24}) and drip loss can be observed. According to Hoffmann (1994), meat of desirable technological quality traits (Red, Firm, Non-exudative) is characterised by EC_{24} values less than 7.0 mS/cm^2 and pH_{45} values greater than 6.0. It can be observed that all three genotypes meet these criteria. The low values of electric conductivity indicate a lower amount of free water in muscle structure and consequently lower values of drip loss. As it can be seen from the Table 1, out of the three investigated genotypes, the pigs of AA genotype had significantly lower EC_{24} and drip loss values. This is supported by the investigation of Kurył at al. (2004), who concluded that the presence of allele A in heterozygotes (AB) at CAST/*Hinf*I locus is related to a significant lowering of WHC ($P\leq0.001$) among fatteners with genetically conditioned stress resistance.
In the research of Koćwin-Podsiadła et al. (2003) and Krzęcio et al. (2004), a highly significant ($p<0.001$) influence of polymorphism at CAST/*Hinf*I locus on pH values at different times *post mortem* was found. Krzęcio et al. (2004) found that the lowest pH_{96} in pigs of BB genotype and assumed that this may be the result of a lower activity of the calpastatin variant that is encoded by allele B. Although we could not establish a statistically significant influence of pig genotype on pH_{45} values, the obtained values are similar to the results of Koćwin-Podsiadła at al. (2003), where the lowest pH_{45} was for meat originated from pigs of AB genotype (6.13), followed by BB genotype (6.19) and AA genotype (6.40).

Table 1. LS Means and standard errors (in brackets) for indicators of technological meat quality of investigated pigs
Tablica 1. Procijenjene srednje vrijednosti i standardne greške (u zagradama) tehnoloških svojstava kvalitete mesa istraživanih svinja

Trait / *Svojstvo*	Genotype / *Genotip*		
	AA (N=11)	AB (N=10)	BB (N=8)
*pH_{45} *SM*	6.12 (0.10)	5.98 (0.09)	5.99 (0.09)
**pH_{45} *LD*	6.45 (0.12)	6.20 (0.10)	6.39 (0.10)
***EC_{45} *SM* (mS/cm^2)	7.50 (0.86)	5.22 (0.77)	6.19 (0.73)
EC_{24} *SM* (mS/cm^2)	7.81 (0,78)	9.43 (0,69)	8.14 (0.66)
EC_{45} *LD* (mS/cm^2)	3.25 (0.75)	4.85 (0.67)	3.65 (0.64)
EC_{24} *LD* (mS/cm^2)	3.06^a (0.74)	5.53^b (0.66)	5.05^b (0.63)
Drip loss (%) / *otpuštanje mesnog soka (%)*	3.48^a (0.44)	4.24^a (0,40)	4.93^b (0.38)
Cooking loss (%) / *kalo kuhanja (%)*	31.13 (0.54)	32.14 (0.48)	31.66 (0.46)

^{a,b}Different letters within a column indicate significant differences at the 5% level by Fisher's LSD test
*M. Semimembranosus pH.; ** M. Longissimus dorsi pH; ***electric conductivity / *električna provodljivost*

Table 2 presents meat sensory quality indicators (colour and WB shear force) for investigated pig genotypes. Kapelansky et al. (2004) reported that CAST/Hinf*I* locus influenced significantly on meat colour, i.e. pigs of AA genotype had lower *a* and higher L values, indicating lighter and less red meat. Although we could not establish such a relationship, it can be observed that our results corroborate with their findings. Of the four investigated traits related to sensory meat quality, the statistically significant influence ($p<0.05$) of CAST/*Hinf*I genotype was found only for WB shear force values. From the Table 2 it can be seen that meat originated from pigs of AB genotype had the lowest values of WB shear force, i.e. their meat was the most tender. This is supported by findings of Krzęcio et al. (2004), who also determined that pigs of AB genotype had the most tender meat.

Table 2. LS Means and standard errors (in brackets) for LD muscle colour and WB shear force - indicators of sensory meat quality of investigated pigs
Tablica 2. Procijenjene srednje vrijednosti i standardne greške (u zagradama) boje MLD-a i otpornosti na presijecanje – indikatora senzorske kvalitete mesa istraživanih svinja

Trait / *Svojstvo*	Genotype / *Genotip*		
	AA (N=11)	AB (N=10)	BB (N=8)
Minolta Hunter L	46.38 (1.12)	49.05 (1.01)	47.58 (0.96)
Minolta Hunter a	6.40 (0.73)	7.27 (0.66)	7.22 (0.62)
Minolta Hunter b	4.47 (0.52)	5.39 (0.46)	4.68 (0.44)
WB shear force (N) / *otpornost na presijecanje (N)*	38.66^{ab} (0.92)	37.86^a (0.82)	41.10^b (0.78)

^{a,b}Different letters within a column indicate significant differences at the 5% level by Fisher's LSD test

Statistical comparison of pig genotypes showed that pigs of AB genotype had significantly higher LD muscle protein content ($p<0.05$) compared to the pigs of AA genotype, whereas there was no statistically significant differences between BB and AB genotype for this trait. It should be mentioned here that chemical composition of the meat in the present study was performed on the cooked samples. In the investigations on the influence of CAST and RYR1 genes on meat quality parameters of four-breed fatteners, Kurył et al. (2004) have found that BB fatteners had significantly higher water content in *LD* muscle than pigs of the AB and AA genotype. Consequently, the inverse tendency was confirmed for dry matter content. However, their analysis was performed on the samples taken from fresh pig meat.

Table 3. LS Means and standard errors (in brackets) for chemical composition of cooked LD muscle of investigated pigs

Tablica 3. Procijenjene srednje vrijednosti i standardne greške (u zagradama) kemijskog sastava kuhanog MLD-a istraživanih svinja

Component / *Komponenta*	Genotype / Genotip		
	AA (N=11)	AB (N=10)	BB (N=8)
Moisture (%) / *Vlaga (%)*	56.92 (0.80)	57.11 (0.71)	57.96 (0.68)
Fat (%) / *Mast (%)*	10.45 (1.14)	8.60 (1.02)	8.43 (0.97)
Protein (%) / *Protein (%)*	34.07[a] (0.59)	35.90[b] (0.53)	35.33[ab] (0.51)
Collagen (%) / *Kolagen (%)*	2.51 (0.14)	2.49 (0.12)	2.45 (0.12)

[a,b]Different letters within a column indicate significant differences at the 5% level by Fisher's LSD test

CONCLUSION

From the results of the presented research it can be observed that polymorphism at CAST locus was related to certain properties of meat quality, namely on electric conductivity at 24h *post mortem*, drip loss, WB shear force and protein content of cooked LD muscle. Since this is only a preliminary investigation conducted in order to evaluate whether polymorphism at the CAST/*Hinf*I locus can influence pig meat quality, it can be assumed that from the results presented in the paper, further research on this candidate gene is needed.

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PRELIMINARNO ISTRAŽIVANJE ODNOSA POLIMORFIZAMA NA CAST GENU I KVALITETE SVINJSKOG MESA

SAŽETAK

Istraživanje je provedeno na 29 križanaca velikog jorkšira i njemačkog landrasa nasumično odabranih na liniji klanja u cilju utvrđivanja veze između polimorfizama na kalpastatin genu (CAST) i svojstava kvalitete mesa. Svinje su zaklane pri 130-150 kg tjelesne mase, kada su izuzeti uzorci krvi za izolaciju genomske DNK. Na liniji klanja izmjerena su slijedeća svojstva kvalitete mesa: početna pH vrijednost i početna vrijednost električne provodljivosti (mjereni na musculus Longissimus dorsi i musculus semimembranosus 45 minuta nakon klanja); završne vrijednosti električne provodljivosti (izmjerene na istim mjestima 24 h nakon hlađenja polovica); otpuštanje mesnog soka ("metodom vrećice"); boja (izmjerena Minolta kolorimetrom i izražena kao Hunter L, a i b vrijednosti); tvrdoća/nježnost mesa te udio vlage, masti, proteina i kolagena (na skuhanom odsječku MLD). Umnoženi produkti CAST gena podvrgnuti su restrikciji HinfI endonukleazom te su utvrđena tri genotipa (AA, AB i BB). Statističkom analizom utvrđeno je da su svinje AB genotipa imale najpoželjnije vrijednosti tvrdoće/nježnosti mesa te najviši udio proteina u MLD-u, dok su za tehnološka svojstva kvalitete mesa statistički značajne razlike utvrđene između genotipa AA i BB te AB za završne vrijednosti električne provodljivosti u MLD-u, kao i između BB te AA i AB za vrijednosti otpuštanja mesnog soka .

Ključne riječi: CAST gen, polimorfizam, svinje, svojstva kvalitete mesa

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