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Calpastatin (*CAST)* gene polymorphism and selected meat quality traits in pigs

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The investigation was carried out on a group of 245 pigs (114 *NN* and 131 *Nn* as regards *RYR1*) belonging to different breeds. Determined was meat pH, colour, water holding capacity, drip loss during storage and sensory assessment of raw meat consistency. Several significant relations were proved between the examined meat traits and polymorphic forms of the calpastatin gene. Polymorphism at *locus CAST*/*Hinf*I was related to pH_1 value (P<0.05), colour saturation and colour lightness of meat (P<0.05). Polymorphism of the calpastatin gene identified with endonucleases *Msp*I and *Rsa*I has influenced other meat traits connected with water binding capacity and consistency of raw meat. It was found that at *locus CAST*/*Msp*I animals of *AA* genotype had firmer and springier meat (P<0.05) than those of *AB* and *BB* genotypes. At *CAST*/*Rsa*I *locus* meat of pigs of *BB* genotype appeared firmer and springier than that of *AB* (P<0.01) or *AA* genotype pigs (P<0.05). Significantly lower drip loss during meat storage was observed in pigs of *AA* genotype at *locus CAST*/*Msp*I, and of *BB* genotype at *locus CAST*/*Rsa*I when compared to pigs of remaining genotypes (P<0.05).

KEY WORDS: calpastatin / gene polymorphism / meat / pigs

The major parameters of quality, culinary value and technological value of meat include colour and water holding capacity. Meat colour is largely dependent on the structure of muscle tissue that modifies the depth of light beam penetration, its reflection or diffusion, as shown mainly in the differences in colour lightness measurements. Water holding capacity describes a highly complex mechanism of muscle protein hydration and determines a number of properties related to drip loss and consistency of meat [Hamm 1960].

Advances made in the biochemistry of muscle tissue have clarified many mechanisms of meat quality formation on the one hand, and discovered new research areas at the biochemistry and molecular genetics level on the other [Warner *et al*. 1997, Sensky *et al*. 1999, Koćwin-Podsiadła and Kurył 2003, Koćwin-Podsiadła *et al*. 2003, Pospiech *et al*. 2001, 2003]. A significant role in these studies is played by the calpain system whose *in vivo* activity in the animal muscle is transferred to the period of *post mortem* changes and even to the time when meat matures and tenderizes [Lonergan *et al*. 1995, Goll *et al*. 1998, Geesink and Koohmaraie 1999, Kristensen *et al*. 2002, Szalata *et al*. 2002]. The system contains two Ca^{2+}-dependent proteolytic enzymes, μ-calpain and m-calpain, while calpastatin serves as the inhibitor of both calpains. The calpain system was also shown to play a significant role during animal growth by regulating synthesis and degradation of muscle proteins, and by shaping the proteolytic potential of meat and its tenderization *post mortem* [Kristensen *et al*. 2002].

The objective of this study was to show the effect of polymorphism of the calpastatin gene identified with *Hinf*I, *Msp*I and *Rsa*I endonucleases on meat quality traits in pig with the *NN* and *Nn* genotype with regard to *RYR1*.

Material and methods

Used were 245 pigs, including 47 Polish Landrace, 25 Złotnicka Spotted, 19 crossbreds (75% Pietrain × 25% Złotnicka Spotted), 115 crossbreds derived from the Torhyb programme of high-lean fattening pig production, and 39 Stamboek fatteners, with 1:1 gilts to castrated males ratio. There were 114 animals of the *NN* and 131 animals of the *Nn RYR1* genotype.

Genotypes at the *locus RYR1* were identified according to Fujii *et al*. [1991], and those at the *CAST* locus according to Ernst *et al*. [1998] using *Hinf*I, *Msp*I and *Rsa*I endonucleases.

The pH_1 was determined 45 min. *post mortem* in the *longissimus lumborum* (LD) muscle of the left carcass-side between the 4th and 5th lumbar vertebra. Measurements of pH_1 were made with a portable glass spearhead electrode pH-meter, and ultimate pH 48 hours *post mortem* (pH_u) was measured in aqueous solution of the muscle tissue.

On the next day after slaughter, LD samples were taken during dissection for laboratory analyses (the first 3 lumbar vertebrae). Sensory evaluation of meat was performed on a fresh cut, which was analysed for colour, exudation and consistency [Clausen and Thomsen, 1956] Ground meat colour was measured using Spekol 11 spectrophotometer with a reflectance attachment and colour parameters were calculated from the regression equations derived by Różyczka *et al*. [1968]. Water holding capacity (WHC) was determined with the filter paper method according to Grau and Hamm [1956] and expressed as per cent of loose water in meat. Area of the compressed meat sample was assumed as a measurement of meat plasticity [Grajewska *et al*. 1998]. Drip loss was measured on meat slices according to Honikel [1987].

Based on the results obtained, meat quality was graded following the principles given by Grajewska *et al*. [1984].

Statistical calculations, analysis of variance and significance of differences between particular genotypes *CAST/Hinf*I, *CAST/Msp*I, *CAST/Rsa*I in respect of studied meat traits, were made using STATISTICA 5.5 PL software [2000].

Results and discussion

The effect of *RYR1* gene polymorphism on the analysed meat quality traits and the interactions between *RYR1* and *CAST* genotypes are discussed in detail in another paper. Here it should only be mentioned that in the material analysed, significant ($P<0.05$) interactions between *RYR1* and *CAST*/*Msp*I and *CAST*/*Rsa*I genotypes concerned only the chemical composition of meat (dry matter, protein or fat).

The number of animals representing different genotypes at the locus *CAST*/*Hinf*I, *CAST*/*Msp*I and *CAST*/*Rsa*I, with regard to the number of pigs with the *NN* and *Nn* genotype, are given in Table 1. The smallest group were animals with the *AA* genotype at the *CAST*/*Hinf*I *locus* (9 out of 245), just as were *AA* homozygous at *CAST*/*Msp*I (14 out of 237) and *BB* homozygous at *CAST*/*Rsa*I (13 out of 229 pigs). The above groups of *CAST* homozygotes simultaneously were almost exclusively *NN* homozygotes in relation to *RYR1*. As a rule, the meat of these few animals differed from the other genotype groups in a significantly higher value of pH_1 ($P<0.05$). A similar result concerning the effect of polymorphism at *locus CAST*/*Hinf*I on muscle pH45 was reported by Koćwin-Podsiadła *et al*. [2003]. It must be stressed that means of this trait for the meat of all genotype subgroups ranged within the limits described for high quality meat by Grajewska *et al*. [1984].

Sensory evaluation of raw meat, performed by a panel of judges, concerned its colour, exudation and consistency. The analysis of colour and exudation showed no differences between the genotype subgroups at the *locus CAST*. Meat consistency, which characterizes its firmness and springiness, was differentiated to a small extent by the genotype with regard to *CAST*/*Msp*I and *CAST*/*Rsa*I. In the case of *CAST* polymorphism identified by *Msp*I endonuclease, the differences concerned the *AA* genotype in relation to *AB* and *BB* genotypes ($P<0.01$). The meat of *AB* and *BB* showed lower firmness and springiness than that of *AA* niamals. Similar to the polymorphism of *CAST* identified with *Rsa*I endonuclease, the meat of *BB* pigs was significantly more firm than that of *AB* ($P<0.01$) and *AA* animals ($P<0.05$).

If we assume that tenderness of cooked meat is mediated by the same process of enzymatic proteolysis that is responsible for the consistency of raw meat, it can be predicted that tenderness of the meat of pigs with the *AB* and *BB* genotype at the *CAST*/*Msp*I *locus* will be greater than in the pigs with the *AA* genotype. Just as for *CAST*/*Rsa*I, the most desirable tenderness of meat would be shown by the pigs with the *AB* genotype at the *CAST*/*Rsa*I *locus*.

The results of objective assessment of meat colour, water holding capacity, plastic-

Table 1. Number of pigs, mean values and standard deviations of meat quality traits in calpastatin genotypes CAST/HinfI, CAST/MspI and CAST/RsaI

Number	CAST/HinfI			CAST/MspI			CAST/RsaI		
	AA	AB	BB	AA	AB	BB	AA	AB	BB
	9 [illegible]	91 [illegible]	[illegible]	[illegible]	101 [illegible]	[illegible]	[illegible]	[illegible]	[illegible]
pH_1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
pH_{24}	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Colour score	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] score	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Consistency score	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

a,b — within rows means bearing different superscripts differ significantly: small letters – P≤0.05; capitals – P≤0.01

Table 2. Meat colour, water holding capacity and drip loss as influenced by calpastatin polymorphisms of CAST/HinfI, CAST/MspI and CAST/RsaI

Item	CAST/HinfI			CAST/MspI			CAST/RsaI		
	AA	AB	BB	AA	AB	BB	AA	AB	BB
Dominant wavelength (nm)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Colour saturation (%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Colour lightness (%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
WHC, loose water (%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Plasticity (cm^2)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Drip loss (%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

a,b — within rows means bearing different superscripts differ significantly: small letters – P≤0.05; capitals – P≤0.01

ity and drip loss from 24 to 72 h *post mortem* are given in Table 2. With reference to the genotypes at the *locus CAST*/*Hinf*I, *AA* animals compared to the other genotypes were characterized by the colour with a shorter dominant wavelength (582.9 *vs* 583.9 and 584.4 nm), *i.e*. less red colour shifted towards the spectrum of yellow colour ($P<0.05$) and by brighter, less desirable colour (*AA vs AB* and *BB*, $P<0.05$).

The effect of *CAST* polymorphism also concerned a beneficial change in one of the colour parametres, *i.e*. the dominant wavelength, as well as a shift towards the longer wavelengths in pigs with the *BB* genotype at the *CAST*/*Msp*I *locus* and in *AA* and *AB* pigs at *locus CAST*/*Rsa*I ($P<0.05$). Simultaneously however, the meat of pigs from these genotype groups was characterized by greater drip loss during storage. The *CAST*/*Msp*I polymorphism was expressed by greater drip loss in the meat of *AB* and *BB* as compared with *AA* (3.48 and 3.66 *vs* 2.19%; $P<0.05$). The effect of polymorphism at the *locus CAST*/*Rsa*I also concerned the differences in drip loss between *AA* genotype groups in relation to *AB* and *BB* (3.75 *vs* 3.33 and 3.38%, $P<0.05$).

It is assumed that drip loss has its source in intracellular water, which is lost by muscle proteins *post mortem* as a result of myofibril contraction additionally induced by a rapid fall of pH and the presence of free Ca^{2+} ions during *rigor mortis* [Honikel *et al*. 1986, Offer and Cousins 1992]. The calpain system active in the muscle, as well as its proteolytic potential both can influence the early phase of the muscle protein degradation and the fragmentation of myofibrils [Kristensen and Purslow 2001 Kristensen *et al*. 2002]. The differences shown in the meat quality traits, concerning the consistency of raw meat and the extent of drip loss between pig genotypes at the *CAST*/*Msp*I and *CAST*/*Rsa*I *loci* suggest a major role of the calpastatin gene in the formation of meat quality traits.

Because the analysed population of pigs was highly differentiated in terms of breeds and slaughter value, estimated was the frequency of PSE and DFD meat faults. The results are shown in Table 3. In the smallest groups of homozygotes, *i.e*. *AA* at the *CAST*/*Hinf*I

and *CAST*/*Msp*I *loci* and *BB* at the *locus CAST*/*Rsa*I, all animals showed the meat of normal quality. In the whole population of 245 pigs, PSE meat was shown in only one animal. Meat with certain signs of PSE or DFD fault, classified as partly PSE or partly DFD, accounted for 11 to 15% in individual genotype groups.

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Polimorfizm genu kalpastatyny *(CAST)* a wybrane cechy jakości mięsa świń

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

Badania przeprowadzono na 245 świniach (*RYR1* 114 *NN* i 131 *Nn*) różnych ras i ich mieszańców. Oznaczono podstawowe cechy jakości mięsa: pH_1, barwę, wodochłonność, utratę soku podczas składowania oraz sensorycznie ocenianą konsystencję mięsa surowego. Wykazano szereg istotnych zależności między badanymi cechami a polimorficznymi formami genu kalpastatyny. Polimorfizm w *locus CAST*/*Hinf*I wiązał się istotnie z pH_1 ($P<0,05$), jasnością i nasyceniem barwy mięsa ($P<0,05$), podczas gdy identyfikowany endonukleazami *Msp*I i *Rsa*I oddziaływał na inne cechy mięsa związane ze zdolnością utrzymywania soku w czasie składowania oraz na konsystencję mięsa surowego. Wykazano, że w *locus CAST*/*Msp*I świnie o genotypie *AA* w porównaniu z genotypem *AB* i *BB* wyróżniały się większą twardością i sprężystością mięsa ($P<0,05$). W podobny sposób odbiegały zwierzęta o genotypie *BB* od osobników *AB* ($P<0,01$) i *AA* ($P<0,05$) w *locus CAST*/*Rsa*I. Istotnie mniejszą utratą soku podczas składowania mięsa ($P<0,05$) charakteryzowały się świnie o genotypie *AA* w *locus CAST*/*Msp*I oraz świnie o genotypie *BB* w *locus CAST*/*Rsa* I wobec zwierząt pozostałych genotypów.