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An association between genotype at the *CAST locus* (calpastatin) and meat quality traits in porkers free of *RYR1^T* allele*

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The study aimed at determining whether parametres describing the quality of pork and its basic composition are significantly related to the genotype at *locus CAST* (calpastatin). Calpastatin is a calpain inhibitor and the proteolytic system calpain/calpastatin plays an important role in the growth and development of muscles, as well as in the processes of *post mortem* degradation of muscle proteins. The investigations covered 397 porkers free of the *RYR1^T* gene, of which 91 were purebred Landrace and the remaining were crossbreds: 65 Landrace × Yorkshire, 129 Landrace × Duroc, 83 [(Landrace × Yorkshire) × Duroc] and 29 [(Landrace × Yorkshire) × (Duroc × Pietrain)]. The results obtained indicate that the value of a series of parametres characterizing the quality of pork is significantly dependent on the animal's genotype at *locus CAST*. Selecting animals for a favourable *CAST* genotype would render it possible to control not only the quality of fresh meat (tenderness),

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but also lower the losses occurring during storage (natural drip) and processing (curing and thermal processing – the TY indicator).

KEY WORDS: calpastatin gene / candidate gene / gene polymorphism / pig / pork

Genes *RYR1* and *RN* are described as major genes for meat quality traits in pigs [Sellier i Monin 1994]. Despite this however, among animals of the same breed and the same *RYR1* genotype one may observe considerable differences as regards certain pork quality traits. Therefore, it is necessary to identify other genes affecting the phenotypic differentiation of animals as regards those traits. Projects for mapping of the pig genome rendered it possible, among other things, to locate QTLs for meat quality traits [Bidanel and Rothschild 2002], on all chromosomes, what confirms the hypothesis that quantitative traits, and such are some meat quality traits, are polygenic. Selection for increased muscling, conducted over many years, has led to a dramatic lowering in meat quality, which is determined by a series of metabolic processes taking place in the muscle tissue, both pre- and post-slaughter. The *post mortem* rate of degradation of the muscle structural proteins is a factor determining meat tenderness, water holding capacity, drip loss, conductivity and colour. Those traits decide to a considerable degree about the technological value of meat [Bertram *et al*. 2000]. In the *post mortem* proteolysis of muscle proteins the calpain system and its inhibitor calpastatin, play a significant role and is decisive in relation to numerous meat quality traits, including tenderness, water holding capacity and water drip [Melody *et al*. 2004].

The present study aimed at evaluating how significant is the effect of the porker's genotype at the *CAST locus* on meat quality traits in animals free of allele $RYR1^T$.

Material and methods

Animals

The studies were conducted on 397 porkers (211 castrated males and 186 gilts) free of the $RYR1^T$ allele, of which 91 were purebred Landrace and the remaining were crossbreds: 65 Landrace × Yorkshire, 129 Landrace × Duroc, 83 [(Landrace × Yorkshire) × Duroc] and 29 [(Landrace × Yorkshire) × (Duroc × Pietrain)]. The animals came from the Pedigree Centre Jagodne, owned by SOKOŁÓW S.A. Meat Processing Plant. The parental material (except for the Pietrain breed) of the porkers analysed originated from Denmark. The maintenance and nutrition (Cargill complete diet offered according to age) conditions during rearing and fattening were uniform for all animals.

The animals were slaughtered during the autumn-winter season, 2-4 hours after transport (300 km). Slaughter was preceded by electric stunning (Dutch line INARCO) and bleeding in horizontal position, in accordance with the technique in force applied at the SOKOŁÓW S.A. Meat Processing Plant.

Methods

The quality of fresh and cooled meat was evaluated after slaughter on the *Longissimus lumborum* (LL) muscle on the basis of the parametres given below.

1. Acidity (pH) measured directly in the LL muscle 35 min, 2 h, 3 h, 24 h, 48 h, 96 h and 144 h *post mortem*, using a Master pH-meter (DRAMINSKI). Moreover, 45 min post-slaughter the pH was measured in a water homogenate of muscle tissue according to the Polish Standard PH-77/A-82058, using a CP-311 pH-meter (ELMERTON) with a combined glass electrode type OSH-10-OO.
2. Electric conductivity (EC) measured on a LF-Star conductometer (MATTHAUS) 35 min, 2 h, 3 h and 24 h post-slaughter.
3. Colour lightness (L*) of the muscle tissue, determined with a MINOLTA CR310 apparatus, 24 h post-slaughter.
4. The ATP breakdown rate, expressed by indicator R_1 = IMP/ATP, determined 45 min post-slaughter according to Honikel and Fischer [1997].
5. Water holding capacity (WHC), determined 24 h post-slaughter according to Grau and Hamm [1952] as modified by Pohja and Ninivaara [1957].
6. Natural drip, determined 48 h, 96 h and 144 h post-slaughter according to Prange *et al*. [1977].
7. Meat yield during the curing and thermal processing (72°C), expressed by the TY indicator, determined according to Naveau *et al*. [1985] with own modification.
8. Tenderness, measured instrumentally with an INSTRON apparatus with a Warner-Bratzler's countershaft and expressed in Newtons/cm^2 (N/cm^2).

LL samples obtained 45 min post-slaughter were analysed for the glycolytic potential as well as for glycogen and lactic acid content. The content of glycogen, glucose and glucose-6-phosphate was determined by the enzymatic method according to Dalrymple and Hamm [1973], while the level of lactic acid – according to Bergmeyer [1974].

The glycolytic potential of muscles, measured in μM of lactic acid per 1 g of muscle tissue, was calculated according to the formula proposed by Monin and Sellier [1985].

Moreover, in LL samples the content of dry matter was determined according to the Polish Standard PN-73/A82110. Total protein was determined with the Kjeldahl method and intramuscular fat with the method of Soxhlet (Polish Standards PN-75/A04018 and PN-73/A82111, respectively).

Genomic DNA was isolated from blood leucocytes after Kawasaki [1990] and *CAST* genotypes were determined with PCR/RFLP technique according to Ernst *et al*. [1998].

The effect of *CAST* genotypes on the basic composition and quality of meat was calculated on the basis of a two-factor analysis of variance in an non-orthogonal arrangement [Ruszczyc 1981]. The means obtained were compared by the NIR test [Oktaba 1980].

Results and discussion

The effect of genotype *CAST*/*Hinf*I on meat quality traits

Out of the 28 traits measured, genotype *CAST*/*Hinf*I was shown to affect the value of seven, related to meat quality and composition: pH measured directly in the LL muscle at various times post-slaughter, R_1, characterizing the *post mortem* ATP breakdown rate in the muscle, natural drip determined 96 h and tenderness measured 48 h post-slaughter (Tab. 1). The highest pH values in the second and third hour *post mortem*, with a simultaneously lowest R_1 value and smallest natural drip, were recorded for porkers with genotype *AB*. With the passing time of post-slaughter, the highest pH was recorded for porkers with genotype *AA*, although significant differences between genotype *AA* and *BB* were observed only 96 hours *post mortem*, what is of importance for culinary meat. One should emphasise that within the group of animals of genotype *AB* the most favourable changes were observed in relation to the tenderness of meat stored 48 to 144 h – the cutting force decreased from 60.5 to 38.99 N/cm^2, thus reaching a value significantly different from the two remaining *CAST*/*Hinf*I genotypes, *i.e*. *AA* and *BB*. This indicates a correct, slow progress of pork maturation in animals with genotype *AB*, favourable for meat quality.

The effect of genotype *CAST*/*Msp*I on meat quality traits

The pH values, measured directly in the LL between hour 24 and 144 post-slaughter were significantly related to the genotype of porkers at *locus CAST*/*Msp*I (Tab. 1). Animals of genotype *AA* demonstrated significantly highest values for those parametres. Moreover, their LL presented the least level of total protein, lower by 0.24 per cent points (pp) from that recorded for animals of genotype *BB* ($P \leq 0.05$).

The effect of genotype *CAST*/*Rsa*I on meat quality traits

The values of 12 out of the 28 evaluated meat quality and composition traits were affected by the genotype at *locus CAST*/*Rsa*I (Tab. 1). The highest pH, measured directly in the LL muscle 2 and 3 hours *post mortem* was observed in animals of genotype *AB*, while between hour 24 and 144 of meat storage the highest pH values were recorded for *BB* animals. The lowest R_1, *i.e*. the slowest rate of ATP breakdown in the LL was observed in heterozygotes, similarly as in the case of *locus CAST*/*Hinf*I. The highest conductivity in the hour 3 and 24 *post mortem* was demonstrated by the meat from porkers of genotype *BB*. The lowest yield during meat curing and thermal processing (indicator TY) was demonstrated in animals with genotype *AA* and it was lower by 1.1 pp from that observed for heterozygotes ($P \leq 0.05$). In turn, the total protein content of meat from animals with genotype *AA* proved the highest and by 0.42 pp higher from that noted for animals of genotype *BB* ($P \leq 0.05$).

Meat quality is characterized be several traits that are the effect not only of different metabolic processes occurring in the muscle, both pre- and post-slaughter, but also of conditions related to the slaughter procedure and subsequent carcass treatment. One of the most important parametres of meat quality is the pH value and the rate at which it decreases, subsequently affecting the degree of protein denaturation during meat matu-

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ration, the water binding capacity, colour and losses during thermal processing [Sellier i Monin 1994]. For this reason it is interesting to note the significant relation appearing between the *CAST* genotype and meat pH, measured at different times *post mortem*, and in the case of the mutation identified by enzyme *Rsa*I, during the whole period analysed, *i.e*. from minute 35 to hour 144 post-slaughter.

It is known that the activity of calpastatin as a calpain inhibitor depends on the muscle pH (decreases with the decrease of pH) and determines not only the rate of proteolysis but also its range [Geesing and Koohmaraie 1999]. The results obtained in the present study indicate that selecting animals for a given *CAST* genotype could significantly affect the *post mortem* pH levels, one of the most important parametres of meat quality and technological value. For instance, when analysing the effect of genotype *CAST*/*Hinf*I on the pH_{96}, the lowest value of this indicator was observed for porkers of genotype *BB*. Simultaneously, meat from *BB* animals, compared with those of remaining *CAST* genotypes demonstrated the highest natural drip, what could indicate the highest level of proteolysis. This in turn may be the result of a lower activity of the calpastatin variant that is encoded by allele *B*.

From the technological point of view it is also of interest that the meat from animals of genotype *AA* at *locus CAST*/*Rsa*I showed the lowest yield after cure with a simultaneous highest content of total protein.

It is known that the content of total protein has a favourable effect on the yield after cure and the production of boiled products. The results presented indicate that the genotype of animals at *locus CAST* may modify this relation.

The results of the studies presented here indicate that a series of parametres characterizing the quality of pork and its basic composition is significantly dependent on the animal's genotype at *locus CAST*. This leads to a conclusion that by selecting animals for a given *CAST* genotype it is possible not only to

regulate the quality of fresh meat (tenderness) but also to lower the losses occurring during storage (drip loss) and processing (cure and thermal processing – indicator TY).

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Wpływ polimorfizmu genu kalpastatyny (*CAST*) na cechy jakości mięsa tuczników wolnych od genu *RYR1^T*

Streszczenie

Celem badań było określenie, czy wartość parametrów charakteryzujących jakość mięsa wieprzowego i jego skład podstawowy jest istotnie zależna od genotypu tuczników względem *locus CAST* (kalpastatyny). Kalpastatyna jest inhibitorem kalpain, a system proteolityczny kalpainy/kalpastatyna pełni znaczącą rolę we wzroście i rozwoju mięśni, a także w procesach degradacji białek mięśniowych *post mortem*. Badaniami objęto 397 tuczników następujących ras i mieszańców: Landrace, Landrace × Yorkshire, Landrace × Duroc, [(Landrace × Yorkshire) × Duroc] i [(Landrace × Yorkshire) × (Duroc × Pietrain)], odpowiednio 91, 65, 129, 83 i 29 zwierząt, wolnych od genu *RYR1^T*. Wyniki prezentowanych badań wskazują, że wartość szeregu parametrów charakteryzujących jakość wieprzowiny jest istotnie zależna od genotypu zwierząt względem *locus CAST*. Selekcjonując zwierzęta o odpowiednim genotypie *CAST* można by kształtować nie tylko jakość świeżego mięsa (kruchość), ale również obniżać straty zachodzące podczas przechowywania mięsa (wyciek naturalny) oraz jego przetwarzania (peklowania i obróbki termicznej) – wskaźnik TY).