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Association of calpastatin gene polymorphisms and meat quality traits in pig

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ABSTRACT

Calpastatin is associated with the rate of *post mortem* degradation of structural proteins due to the regulation of calpain activity. In the present research, the associations between polymorphisms within 6th intron of porcine *CAST* gene and several meat quality traits were analyzed. The *CAST* gene polymorphisms affected meat colour, pH, water holding-capacity (WHC) and texture parameters (toughness, firmness, cohesiveness, chewiness, and resilience) measured in *longissimus dorsi* and *semimembranosus* muscles. The analysis performed on the most numerous breeds maintained in Poland, suggested that the most interesting polymorphisms were *CAST*/*HpaII* and *CAST*/*RsaI*, which had the greatest effect on WHC regardless of the breed analyzed and had an effect on meat pH, firmness and toughness for most breeds. Interestingly, for almost all breeds, the significant effect of both mutations on intramuscular fat content (IMF) was detected. The provided data confirmed the use of *CAST* gene as a genetic marker in breeding programmes which allows performing a selection focussed on improving the quality of pork.

1. Introduction

Tenderness, meat colour and water holding-capacity are considered to be the most important factors of meat quality (Bredahl, Grunert, & Fertin, 1998; Grunert, Bredahl, & Brunsø, 2004). According to the consumers, the most desirable features of pork are its colour, external fatness and tenderness (Fortomaris et al., 2006). On the other hand, for the meat industry the most important are traits like meat and fat content in carcasses and those which determine meat quality after thermal treatment i.e. drip loss, water-holding capacity, pH and texture characteristics (Jaworska et al., 2009). These quality traits are mainly dependent on the muscle tissue structure and influence technological and culinary values of the meat.

The activity of proteolytic enzymes – calpains and their inhibitor – calpastatin, corresponds to the meat (beef, lamb, pork) flavour and texture attributes: tenderness, juiciness, cooking loss, and cooked and fresh meat colour. Calpastatin, an endogenous inhibitor specific for the calpains, is also associated with the rate of degradation of structural proteins *post mortem* by controlling the activity of calpains (Wendt, Thompson, & Goll, 2004). Furthermore, the calpastatin protein is assumed to influence the expression levels of genes encoding structural or regulatory proteins. Melody et al. (2004) showed significant differences in calpastatin activity between three porcine muscles which correlate with various levels of desmin degradation. Thus, the changes of calpastatin activity in muscle cells can be partially explained by the differences in proteolysis rate and pork quality (Huff-Lonergan & Lonergan, 2005).

To date, numerous research indicated that several QTLs related with pork quality traits were localized on SSC2 within the *CAST* gene region (Meyers & Beever, 2008; Meyers, Rodriguez-Zas, & Beever, 2007; Rohrer, Thallman, Shackelford, Wheeler, & Koohmaraie, 2006). For the first time, the *CAST* gene was proposed as a candidate gene associated with pork tenderness by Ernst, Robic, Yerle, Wang, and Rothschild (1998). In the recent years, intensive researches focussed on the identification of the functional *CAST* polymorphisms were performed. Ciobanu et al. (2004) indicated significant linkage disequilibrium between alleles of calpastatin and reported that both haplotypes and individual polymorphisms affected firmness, Instron force, and juiciness score of pork. The following studies confirmed the significant association of *CAST* SNPs with tenderness, cooking loss or favourable meat quality score (Lindholm-Perry et al., 2009; Nonneman et al., 2011). Rohrer et al. (2012) reported that *CAST* was one of the genes with the largest effects on pork quality and could be useful for breeding selection.

Due to calpastatin function, the *CAST* gene is considered to be a candidate gene responsible for meat quality in many domestic animals

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including pigs. According to the fact that the significance of *CAST* markers differed in populations and analyzed mutations (Rohrer et al., 2007), the objective of the present study was to determine the effect of *CAST* gene polymorphisms on important pork quality traits in five pig breeds maintained in Poland.

2. Material and methods

2.1. Animals

The study was performed on 554 pigs represented by four purebreds used in Polish breeding programmes (Polish Landrace, Polish Large White, Pietrain and Duroc; 146, 158, 115, 76, respectively) and one conservative breed — Puławska pigs (n = 59). The Puławska pigs are kept locally and are characterized by high meat quality parameters, especially a high level of IMF content. All gilts were maintained in the Pig Test Station (SKURTCh) of the National Research Institute of Animal Production (in Pawłowice, Mełno, Chorzelów) under the same housing and feeding conditions. The animals were fed ad libitum from 30 kg up to 100 (±2.5) kg until they were slaughtered and dissected.

2.2. Meat quality traits

Several meat quality traits such as: pH, meat colour, water holding-capacity and intramuscular fat content (IMF) were evaluated. The pH was measured using pH-Star Mathaus in the *longissimus dorsi* (at the last rib) and *semimembranosus* muscles, 45 min (pH_{45}) and 24 h (pH_{24}) after slaughter. Meat colour parameters (L^* — lightness, a^* — redness and b^* — yellowness) were estimated using reflectance spectrophotometer Minolta CR-310 on loin samples 24 h after dissection. Intramuscular fat content was measured (IMF) on thawed *longissimus dorsi* homogenates by the Soxhlet method using Soxtherm SOX 406 — Gerhardt, whereas water holding-capacity was evaluated according to the Graua–Hamma method (Hamm, 1986). Because the data concerning meat quality traits were not available for all animals, the number of pigs in each group for the genotype association analysis was different.

2.3. Texture analysis

The meat texture parameters were determined for *longissimus dorsi* and *semimembranosus* muscles of 262 animals belonging to four breeds: Polish Landrace, Polish Large White, Duroc and Puławska pigs (73, 93, 37, and 59; respectively). All muscle samples were collected after dissection and stored at −20 °C. Texture analysis was performed at room temperature using the Texture Analyser TA-XT*plus* (Stable Micro Systems, Godalming, UK). The WBS analyses (Warner–Bratzler shear force) were performed for both raw and cooked meat. The muscle slices (3.5 cm wide, approximately 200 g) were placed in a polyethylene bag, cooked in a water bath until a core temperature reached 80 °C and chilled for 24 h at 4 °C. The 3 cores (15 mm diameter) were taken from the muscle slice parallel to the muscle fibre direction and sheared using a WB triangular blade at 4.5 mm/s on a Texture Analyser TA-XT*plus*. The peak force was recorded in N. The two cores for texture profile (TPA) determination were doubly compressed by a cylinder (SMS P/25, base diameter 50 mm) to 70% of their height at a rate of 2 mm/s with a 3 s break between the storage of compression.

Selected texture parameters (texture profile analysis; TPA) such as hardness, cohesiveness, chewiness and resilience for both muscles (raw meat) were measured from force–deformation curves. Hardness was defined as the maximum force applied to the samples during the first compression cycle and expressed in N. Cohesiveness was calculated as the ratio of the area under the second curve to the area under the first curve. Springiness (mm) was determined as a ratio of time of contact with the sample during the second compression to the first compression. Resilience was defined as the ratio of the negative force input to positive force input during the first compression while chewiness was obtained by multiplying hardness, cohesiveness and springiness (Meullenet, Lyon, Carpenter, & Lyon, 1998). The data obtained was collected and calculated by Texture Expert, version 1.20 software.

2.4. Genotyping

The genomic DNA was isolated from whole blood using the Genomic Wizard Purification Kit (Promega) following the instruction provided in the protocol. All animals analyzed were free of C1843T mutation in the *RYR1* gene. The three *CAST* polymorphisms, localized in intron 6, were genotyped by PCR-RFLP method using *Hpa*II, *Hinf*I and *Rsa*I endonucleases according to Ernst et al. (1998).

2.5. Statistical analysis

The association analyses between meat quality traits and different *CAST* genotypes were performed using GLM procedure (SASv. 8.02). The most comprehensive model was:

$$Y_{ijklm} = \mu + s_i + d_j + b_k + g_l + (b * g)_{kl} + e_{ijklm}$$

where,

Y_{ijklm}	$_{ijklm}$ observation,
μ	overall average,
s_i	the effect of pig station,
d_j	the effect of date of slaughter (for meat quality traits: pH, colour, water holding-capacity),
b_k	the fixed effect of breed,
g_l	the fixed effect of different *CAST* genotypes,
$(b^*g)_{kl}$	the interaction between *CAST* genotypes and breeds (included in the model when significant),
e_{ijklm}	the random error.

Additive and dominant effects were estimated using the REG procedure of SAS, where the additive effect was denoted as 1, 0 and −1 for genotypes *AA*/*CC*/*EE* (0), *AB*/*CD*/*EF* (1) and *BB*/*DD*/*FF* (2), respectively, and the dominance effects represented as 1, −1 and 1 for *AA*/*CC*/*EE* (0), *AB*/*CD*/*EF* (1) and *BB*/*DD*/*FF* (2), respectively.

The Hardy–Weinberg equilibrium for each genotype and breed was estimated by using Court Lab — HW calculator. The linkage disequilibrium between *CAST* polymorphisms was evaluated using PowerMarker V3.25 software.

3. Results

3.1. Genotype frequencies

Concerning three *CAST* polymorphisms, frequencies of genotypes did not differ significantly within all analyzed breeds. In each breed the most numerous were *BB CAST*/*HinfI*, *DD CAST*/*HpaII*, and *FF CAST*/*RsaI* genotypes (frequencies about 67%, 58%, 61%, respectively) while less numerous were pigs with opposite genotypes: *AA CAST*/*HinfI*, *CC CAST*/*HpaII* and *EE CAST*/*RsaI* (about 6%, 12%, and 6%, respectively). Only in Polish Large White pigs the highest number of *CC* homozygotes and the lowest *DD* genotypes compared to other breeds were observed (28% and 42%, respectively). Analyzed polymorphisms were in linkage disequilibrium, the r^2 values of *CAST*/*HinfI* and *CAST*/*HpaII*, *CAST*/*HinfI* and *CAST*/*RsaI*, and *CAST*/*HpaII* and *CAST*/*RsaI* were 0.813, 0.663, and 0.671, respectively. All populations were consistent with the Hardy–Weinberg equilibrium (Table S1).