Animal Science Papers and Reports vol. 34 (2016) no. 1, 61-72
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Effect of calpastatin gene (*CAST*) polymorphism on pork quality

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(Accepted November 3, 2015)

The aim of the study was to assess the effect of calpastatin gene (*CAST/RsaI*) polymorphism on technological and sensory quality of pork, the range of glycolytic and proteolytic *post mortem* changes and slaughter value. The up-till-now study results on the correlation between *CAST/RsaI* polymorphism and pork quality are inconsistent and the effect of the CAST gene polymorphism on meat quality is not fully explained.The research was carried out on 65 hybrid pigs of the PenArLan breeding company. Fatteners of the DD genotype at *locus CAST/RsaI* had higher meat content in carcass, higher ultimate pH and cooking yield, lower glycolytic potential and protein content as compared to CD and CC genotypes. This has been reflected in higher sensory tenderness. Results concerning the proteolysis level of myofibrillar proteins indicated that the DD genotype was intermediate between CC and CD. In conclusion, fatteners of the DD genotype are more suitable for meat production and pork quality than the other *CAST/RsaI* genotypes.

KEY WORDS: pork quality / calpastatin gene/ glycolysis/ proteolysis

Pork has major economic importance in all European countries, Asia and North America. Nowadays, consumers pay more attention to the quality of meat they purchase. This aspect is particularly important in case of the types of meat used for culinary purposes.

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Majority of traits associated with the carcass and meat quality belongs to the quantitative traits. The value of these traits is dependent on the interaction of genetic and environmental factors. Most of environmental factors such as maintaining, nutrition, pre-slaughter handling and slaughter technology itself are quite well examined. They are often precisely controlled or specified in the regulations focused on the animal's welfare.

Nowadays, mainly the genetic factors are studied, especially their effect on meat quality. As a result of fast development of methods of molecular biology, it has become possible to know the structure, location and function of several genes that are responsible for shaping the individual quantitative traits associated with meat quality and musculature. A detailed knowledge of a few first major genes affecting carcass and meat quality ($RYR1^T$, RN^-) was the basis for further exploration. Other genes, called candidate genes, are currently studied and their effect on meat quality is evaluated. Among the tested candidate genes the calpastatin gene can be distinguished [Ernst *et al*. 1998, Ciobanu *et al.* 2004]. Studies have shown the occurrence of calpastatin gene polymorphism and then the relationship between the proteolytic system of calpastatine-calpain and the meat quality in some breeds of pigs [Ciobanu *et al.* 2004]. The effect of this gene on several traits (slaughter value, technological and sensory meat quality and proteolysis of muscle proteins) was observed [Koćwin-Podsiadła *et al.* 2003, Krzęcio *et al*. 2007, Kurył *et al*. 2003, Sieczkowska *et al*. 2010]. However, the results of these studies are varied and the effect of the CAST gene polymorphism on meat quality is not fully explained.

For the above reasons the aim of the present study was to determine the effect of calpastatin gene polymorphism on carcass quality, sensory and technological qualities of pork used for processing and cooking purposes.

Material and methods

Animals

The study was carried out on 65 gilts from hybrid pig lines of the PenArLan Polska company. The animals originated from crossbreeding of Nad'ma gilts with P76 boars. The Nad'ma pig line was created from crossbreeding Redone (maternal line created from the following breeds: Large White x Landrace x Pietrain x Hampshire x Meishan x Jian Xing) and Galia (maternal line created with Large White x Landrace). The P76 line was created from the Laconie (built from Large White, Hampshire and Pietrain breeds in equal proportions) and the Penshire (built from Hampshire, Duroc and Large White breeds in the following proportion: 50%, 35% and 15%) synthetic line.

The pigs were produced and kept under identical environmental conditions and were fed a standard diet. Pigs originating from the herds were included in the program of elimination of disadvantageous genes' ($RYR1^T$ and RN^-). All animals were transported to the meat plants in the same transport conditions. The fatteners were slaughtered at about 110 kg of live weight in accordance with the legally binding

procedures, including automatic electric stunning and exsanguination in a horizontal position. After slaughter, about 1 kg of *Longissimus dorsi* (LD) muscle (at the last rib) was taken to evaluate meat quality.

Carcass and meat quality traits

Slaughter value of the fatteners was determined on a carcass (directly on the technological line in the meat plant) using a CGM apparatus (Sydel Corporation, France). The back fat thickness and *longissimus* thickness were measured at the last rib *ca*. 7 cm from the carcass mid-line. On the basis of this measurement meat percentage in a carcass was estimated with the equation of Borzuta [1998].

The pH value was measured at 1, 24 and 48 h after slaughter with the WTW 330i pH-meter (Germany). Meat colour was measured in CIE L*a*b* system with the CR310 Minolta Chroma Meter (Japan) 48 h *post mortem*. As a standard a white tile (L* = 93.90, a* = 0.32, b* = 0.33) and D^{65} light source were used. The aperture was 8 mm, and illuminant D65 and a 10° standard observer were used. The 2 cm-thick loin chops were cut and bloomed for 1 hour at 4°C with no surface covering before color measurement. The natural drip loss (DL) was determined according to the Prange *et al.* [1977] methods as modified by Honikel [1987]. The meat cooking yield was determined on 500 g meat samples. The cooking process was conducted in a salt solution (0.8% NaCl) to reach 72°C inside the meat. The cooking yield was the weight of the cooked meat sample as a percentage of the raw sample. The contents of protein and fat in the muscle were determined according to the ISO Standard, respectively by means of the Kjeldahl's [AOAC, 1974] and Soxhlet's [ISO 1444:2000] methods.

Glycolytic potential was measured at 24 h *post mortem* in the *Longissimus dorsi* muscle. Muscle tissue (1 g) was homogenized with 10 ml of 0.5 M perchloric acid. Aliquots of homogenate (0.5 ml) were taken for enzymatic determination of glycogen, glucose and glucose-6-phosphate according to Dalrymple and Hamm [1973] method. Lactic acid was determined in the supernatant of homogenate centrifuged for 15 min [Bergmeyer, 1974]. Glycolytic potential (GP) was calculated according to the following formula adapted from Monin and Sellier [1985]:

$$PG = 2\ ([glycogen] + [glucose\text{-}6\text{-}phosphate] + [glucose]) + lactate$$

The sensory quality of cooked meat were evaluated on the basis of flavor, color intensity, color homogeneity, fat perception, tenderness and juiciness of cooked meat according to the sensory QDA method [ISO 13299.2:1998] with an unstructured, linear graphical scale of 100 mm, converted to numerical values (0-10 conventional units c.u.). The meat samples were cut into portions (cubes) of approximately equal size and weight (ca. 25 g), and the samples were then placed in plastic, odorless, and disposable boxes covered with lids. All samples were separately coded for the assessment using three digit codes and were presented in random order to avoid the carryover effect. The assessment was performed by a formally trained panel of 10 people (3–8 years of sensory evaluation practices).

Proteolysis degree of myofibrillar proteins

Composition analysis of the myofibrillar proteins contained in muscular tissue was performed with the SDS-PAGE method [Bollag and Edelstein 1991] using the STANDARD system (Kucharczyk TE, Poland). Proteins were separated on a 12 % separation gel and 5 % stacking gel. Myofibrillar proteins were extracted from 20 mg of muscle, homogenized with 800 µl of a Tris–HCl buffer (pH 6.8) containing 0.375 M 2-mercaptoethanol, 3 % SDS, 8 M urea, and 2 M thiourea. Muscle protein concentration was determined as total nitrogen using the AOAC method [1974]. The concentration of soluble protein from the drip loss was determined using the Biuret Method [Lowry *et al.* 1951]. Myofibrillar proteins from the drip loss were dissolved 1/1 (v/v) in Tris–HCl sample buffer (pH 6.8; composition: 0.375 M 2-mercaptoethanol, 3 % SDS, 8 M urea, 2 M thiourea, and 0.05 % bromophenol blue) so that the final sample concentration was 2.5 µg/µl. The mixture was then heated for 3 min at 95°C, and 10 µl of the mixture was then placed in gel. The gels were first run for approximately 0.5 h at 70 V followed by 1 h at 150 V. Gels were stained with Coomassie Brilliant Blue R250 by 0.5 h. They were destained according to the Weber and Osborn [1969] recommendation. The protein mass standards of Fermentas International Inc., Canada, Burlington were used. Image analysis and quantification were performed using the GelScan v. 1.45 software (Kucharczyk TE, Poland).

Genotyping and PCR-RFLP analysis

Genomic DNA was extracted from the blood sample using a QIAamp DNA Blood Mini kit (Qiagen). Genotypes CAST/*RsaI* were identified by the PCR/RFLP method according to Ernst *et al.* [1998].

Statistical analyses

Data were analyzed using Statistica 10.0 software [StatSoft, Inc., 2012]. Population genetic equilibrium was estimated by Chi^2 test. A one-way analysis of variance of the effect of genotype on the traits was performed. The significance of differences between means was identified with the Tukey's test. The effects of genotypes for each trait were reported as the means ± standard deviation (SD).

Results and discussion

Three genotypes were identified at *locus* CAST/*RsaI* (CC, CD, and DD – Table 1). In the present study, a significant effect of the CAST/*RsaI* polymorphism on the technological quality of meat has been confirmed. In the present study, as in the study of Koćwin-Podsiadła *et al.* [2009], this gene polymorphism had a positive effect both on slaughter value and meat quality. The association between the polymorphism of the CAST gene and the values of main pork quality traits was shown by Koćwin-Podsiadła *et al.* [2003], Ciobanu *et al.* [2004], Kapelański *et al.* [2004], Krzęcio *et al.* [2007], Gandolfi *et al.* [2011] and McBryan [2010], while the research of Rybarczyk

Table 1. Allele and genotype frequencies at the CAST/*RsaI locus*

Trait	Genotype		
	CC	CD	DD
No. of pigs	14	21	30
Allele frequencies	C=0.38 D=0.62		
Genotype frequencies	0.21	0.33	0.46

et al. [2010] and Hamill *et al.* [2012] did not confirm this relationship. Nevertheless, in the studies of Rybarczyk *et al.* [2010], the values of carcass, meat quality or meat basic chemical composition were compared only between one type of homozygotes DD and heterozygotes CD, which could make it difficult to prove the "genotype - trait quality" relationship.

Fatteners with the DD genotype at *locus CAST/RsaI* had higher meat content in the carcass. Meatiness of these fatteners was higher by approximately 2% compared to the CC and CD genotypes, which did not differ significantly from each other (Tab. 2). The expected impact of the calpastatin gene polymorphism on the slaughter value may be due to the effect exerted upon the protein turnover after slaughter [Goll *et al.* 1998]. As it was reported by Goll *et al.* [1998], the calpain-calpastatin proteolytic system plays an important role in the normal growth of skeletal muscle during the postnatal period and significantly shapes the number of muscle fibers. The increased skeletal muscle growth may be the result of the reduced protein degradation due to the inhibition of calpains by calpastatin [Hopkins and Geesink 2009].

Table 2. Characteristics of slaughter value of fatteners with different CAST/*RsaI* genotypes

Trait	Genotype		
	CC	CD	DD
Hot carcass weight (kg)	88.10±5.38	88.38±9.26	88.11±7.54
Longissimus dorsi muscle thickness (mm)	54.78±4.85	55.28±6.61	59.36±5.58
Backfat thickness (mm)	13.77±4.37	14.74±3.00	13.28±3.03
Meat in carcass (%)	55.78^{a}±2.29	55.83^{a}±2.94	57.84^{b}±2.00

^{ab}Within row means bearing different superscripts differ significantly at P≤0.05.

Animals with genotype DD had higher ultimate pH values measured at 24 and 48 h after slaughter. It was probably the result of the lowest glycolytic potential and lower level of lactic acid observed in this group of animals. The lowest protein concentration and a higher cooking yield were also recorded. It should be noted that there were no significant differences between the DD and CC genotypes in relation to the content of lactic acid and cooking yield. Results, analogous to those obtained in our studies, and concerning the effects of the CAST/*RsaI* polymorphism on the

ultimate pH, cooking yield and the protein content, were obtained by Krzęcio *et al.* [2008]. In turn Kapelański *et al.* [2004] observed the influence of the CAST/*RsaI* polymorphism on water holding capacity during storage and consistency of raw meat. A significantly lower value of the glycolytic potential, reduced protein content and a higher fat content (though not statistically significant) were found in pigs with the DD genotype. Similarly in the study of Larzul *et al.* [1998] in pigs selected for lower muscular glycolytic potential, an increased fat deposition was observed.

The present results showed higher tenderness of cooked meat in fatteners with genotype DD, but did not differ significantly from the CC genotype. Tenderness quality was probably the result of higher values of: the ultimate pH, fat content, and cooking yield (Tab. 3 and 4). A significant effect of ultimate pH, intramuscular fat and cooking yield on tenderness, juiciness and overall quality were found in Fortin *et al.* [2005], Ngapo *et al.* [2007] and Czarniecka-Skubina *et al.* [2010]. The determination

Table 3. Meat quality traits of pigs with different CAST/*RsaI* genotypes

Trait	Genotype		
	CC	CD	DD
pH_1	6.43±0.34	6.36±0.28	6.40±0.21
pH_{24}	5.51^{a}±0.12	5.53^{a}±0.07	5.62^{b}±0.12
pH_{48}	5.48^{a}±0.06	5.46^{a}±0.05	5.58^{b}±0.14
Glikogen residual (µmol/g)	11.88±8.32	9.59±6.40	6.98±6.18
Lactate in meat (µmol/g)	97.89^{ab}±10.95	102.57^{a}±9.11	92.36^{b}±11.85
Glycolytic potential (µmol/g)	121.68^{a}±22.58	121.76^{a}±16.73	106.34^{b}±20.26
Intramuscular fat (%)	1.27±0.74	1.58±0.86	1.96±1.46
Protein content (%)	23.11^{a}±0.28	23.07^{a}±0.15	22.45^{b}±0.54
Colour coordinates: L*	54.47±1.94	54.36±2.31	54.32±2.83
a*	16.22±1.09	16.33±1.00	15.75±1.11
b*	6.03±1.42	6.58±1.65	6.35±1.77
Drip loss (%)	4.73±1.94	3.78±2.88	3.20±1.75
Cooking yield (%)	70.88^{ab}±1.61	69.79^{b}±2.06	72.50^{a}±2.51

^{ab}Within row means bearing different superscripts differ significantly at P≤0.05.

Table 4. Sensory quality of cooked meat of fatteners of different CAST/*RsaI* genotypes

Sensory attributes (0-10 c.u.)	Genotype		
	CC	CD	DD
Odour intensity	8.02±0.30	7.88±0.36	8.11±0.42
Ton of colour	8.39±0.43	8.32±0.37	8.52±0.28
Colour homogenity	8.08±0.45	8.19±0.49	8.45±0.20
Tenderness	6.36^{ab}±0.72	5.36^{b}±1.77	7.04^{a}±0.91
Juiciness	4.45±1.26	4.64±1.18	5.14±1.78
Fat perceptability	2.40±0.37	2.15±0.31	2.41±0.33
Meat flavour	6.39±0.91	6.98±0.91	6.87±0.66
Overall quality	6.05±0.65	5.79±1.03	6.53±0.90

^{ab}Within row means bearing different superscripts differ significantly at P≤0.05.

coefficient obtained by Czarniecka-Skubina *et al.* [2010] indicated that the level of IMF and pH_{24} *post mortem* determined the overall sensory quality of meat in 59 %. Simultaneously, these studies have shown that in meat with higher pH_{24} (above 5.50), increase of the intramuscular fat content resulted in the improvement of the sensory quality (tenderness, juiciness, meat flavour and overall quality) after cooking, whereas this was not the case in the group of pH_{24} <5.5.

The effects of the myofibrillar proteins proteolysis degree seem to confirm the above mentioned relationship. In the pigs of genotype DD the higher proteolysis degree of muscle proteins was not recorded. Conversely, the current results, presented in table 5, showed that the proteolysis degree of actin in the LD muscle of fatteners of the DD genotype was lowest but did not differ statistically from pigs with genotype CD. What regards the proteins of 31 and 30 kDa weight, considered as markers of the degree of proteolysis, they slightly modified the results. A relatively high amount of the polypeptides was found in the CC pigs. However, it did not differ significantly from the one found in pigs of the DD genotype. The observed values for genotype CD did not differ either from the DD. Significant differences were only found between CC and CD, indicating a higher proteolysis degree of proteins in pigs with CC. No significant differences between genotypes with regard to other proteins of higher molecular weight were observed (Tab. 5). The observed differences in the degree of proteolysis were due to differences in the inhibition of calpains by calpastatin depending on genotype at CAST/*RsaI*. These results are consistent with the data related to slaughter value as in those pigs the highest meatiness was recorded (Tab. 2).

Table 5. Quantification of muscle proteins from the *Longissimus dorsi* in pigs of different CAST/*RsaI* genotypes

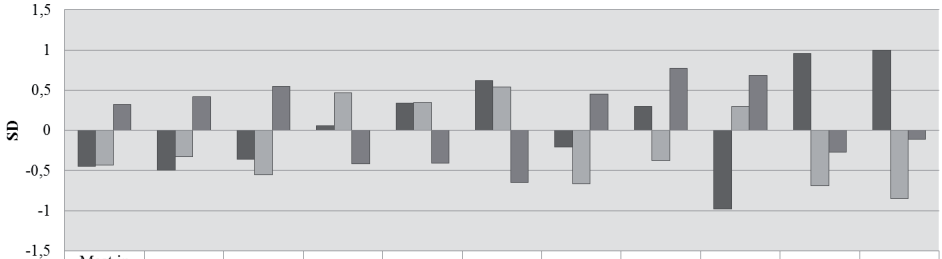
Trait	Genotype		
	CC	CD	DD
Myosin	27.30±4.08	32.07±1.91	30.83±0.02
115 kDa	1.40±0.26	1.47±0.29	1.40±0.46
100 kDa	0.97±0.25	1.27±0.21	1.00±0.10
α-actinin	4.90±0.26	5.27±0.55	4.77±0.85
μ-calpain	0.63±0.06	0.87±0.21	0.83±0.42
65 kDa	1.63±0.94	0.97±0.98	1.43±0.15
60 kDa	3.77±1.01	2.33±0.92	2.47±0.65
55 kDa	1.13±0.38	0.87±0.11	0.77±0.30
50 kDa	5.63±1.38	4.37±1.00	4.63±0.38
Actin	14.33^{a}±2.29	16.77^{ab}±0.61	17.50^{b}±0.72
Troponin T	6.10±0.36	6.27±1.15	6.10±0.70
Tropomyosin	8.37±1.15	6.93±0.68	8.37±0.55
31 kDa	7.60^{a}±1.75	4.83^{b}±1.12	5.53^{ab}±0.85
30 kDa	2.37^{a}±0.46	1.37^{b}±0.42	1.77^{ab}±0.11
29 kDa	2.80±0.56	2.23±0.06	2.30±0.35

^{ab}Within row means bearing different superscripts differ significantly at P≤0.05.

As stated by many authors, the 30 kDa polypeptide is a degradation product of troponin T (TnT) [Kołczak *et al.* 2003, Nowak 2005, Hopkins and Gessink 2009, Huff-Lonergen *et al.* 2010]. According to Huff-Lonergen *et al.* [2010], degradation of TnT may simply be an indicator of the overall *post-mortem* proteolysis that occurs faster at lower pH levels. The amount of 30 kDa polipeptide partly corresponds to the amount of non-degraded troponin-T (Tab. 5). Similarly to the results reported by Żelechowska *et al.* [2012], higher proteolysis degree of muscle proteins in the meat with a low pH was observed. These authors reported that the 31 and 30 kDa polypeptides were significantly more abundant in the meat of low ultimate pH.

The results presented in Table 5 are in accordance with the results illustrated in Table 3. Pigs of the genotype CC had meat of the highest degree of acidification (Tab. 3) and advanced level of myofibrillar proteolysis. Moreover, homozygotes DD showed average values between genotypes CC and CD (Tab. 5). The highest content of polypeptides, which are markers of the degree of proteolysis (31 and 30 kDa), was observed (especially in animals of the CC genotype). These results were confirmed by the sensory quality of the meat after heat treatment, as it was characterized by a higher degree of tenderness from the porkers of genotype DD and was similar to homozygotes CC. The lowest values were recorded for the heterozygotes. These results partially confirmed the relationship that was observed by Josell *et al.* [2003] and Miller *et al.* [2000]. They showed that meat with low pH is characterized by higher enzymatic activity, which increases the aging rate, myofibrillar fragmentation and higher tenderness and juiciness.

There is a significant effect of the D allele in the homozygous state, which resulted in decreasing (lactic acid and protein contents) or increasing (pH_{24} and pH_{48}, the cooking yield and tenderness) the values of traits (Fig. 1). As for most of the traits, it can be stated that the D allele is recessive to the C allele. The mean values for heterozygotes did not differ significantly from the CC homozygotes for the following



	Meat in carcass (%)	pH24	pH48	Lactate (µmol/g)	PG (µmol/g)	Protein (%)	Cooking yield (%)	Tenderness (0-10 c.u.)	Actin (%)	31 kDa	30 kDa
CC	-0,45	-0,5	-0,36	0,06	0,34	0,62	-0,21	0,3	-0,98	0,96	1
CD	-0,43	-0,33	-0,55	0,47	0,35	0,54	-0,67	-0,38	0,3	-0,69	-0,85
DD	0,32	0,42	0,55	-0,42	-0,41	-0,65	0,45	0,77	0,68	-0,27	-0,11

Fig. 1. CAST/*RsaI* genotype effects as expressed in standard deviation units in relation to the overall mean.

traits: meat content in carcass, pH_{24}, pH_{48}, glycolytic potential and content of protein. Totally different positions of the heterozygotes in regard to both homozygotes (CC, DD) was observed for the lactic acid content, cooking yield and tenderness. It was shown that the average values of heterozygous pigs in terms of actin were between the values of both homozygotes at *locus* CAST/*RsaI* (Tab. 5).

The results of the study showed significant effect of the polymorphism of calpastatin gene CAST/*RsaI* on some traits related to slaughter value, technological and sensory quality of pork and the range of glycolytic and proteolytic changes occurring post mortem in muscle.

Fatteners of the DD genotype at *locus CAST*/*RsaI* had higher meat content in carcass and ultimate pH value, cooking yield, more tender meat and lower glycolytic potential and protein content as compared to CD and CC genotypes. The effect of CAST/*RsaI* polymorphism revealed also a lower proteolysis degree of muscle proteins in pigs of genotype CD compared to the CC animals. Results concerning the proteolysis level of myofibrillar proteins indicated that the DD genotype was intermediate between CC and CD. In conclusion, fatteners of the DD genotype are more suitable for meat production and pork quality than the other *CAST*/*RsaI* genotypes.

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