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Differences in carcass traits, meat quality and chemical composition between the pigs of different *CAST* genotype

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Abstract. The study was carried out on 89 Pig Improvement Co. (PIC) pig carcasses, with the aim to investigate the differences between three *CAST* loci in carcass and meat-quality traits, as well as chemical composition of *longissimus dorsi* (LD) muscle. The differences among genotypes at *CAST*/*Hinf*I locus were significant in all carcass traits measured, where AB genotype exhibited preferable values in carcass lengths, ham length, muscle thickness, loin eye area, fat thickness and fat area. Among meat-quality traits analysed, genotypes at *CAST*/*Hinf*I locus differed in pH_{45} in SM muscle, both pH_{24} in *semimembranosus* (SM) and LD muscles, as well as luminosity; genotypes at *CAST*/*Msp*I differed in pH_{24} and EC_{24} measured at LD muscle and in red colour intensity, level of yellowness and hue angle; while genotypes at *CAST*/*Rsa*I differed in pH_{45} and EC_{45} in SM muscle, pH_{24} in LD muscle, paleness and redness, as well as in shear force and calpain activity. EF genotype at this locus exhibited the highest pH values and the lowest CIE L^*, with more pronounced red colour, but also highest shear force and lowest calpain activity values. Furthermore, significant differences in chemical composition of LD muscle were found only among genotypes at *CAST*/*Rsa*I loci, where FF genotype had the lowest intramuscular fat and the highest relative share of protein.

Additional keywords: *CAST* genotype, calpain activity, carcass composition, meat quality, pig.

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Introduction

The perception of meat quality from consumer aspect refers to sensory attributes such as colour, flavour and meat tenderness. Among these traits the consumers are complaining mainly about large variability in meat tenderness (Maltin *et al*. 2003). Despite the efforts to control and optimise conditions before, during and after the slaughter, still large variability in this trait can be found on the market. This fact suggests that it is a very complex quality attribute and is influenced by many structural and metabolic factors, mainly connective tissue concentration, final pH, muscle contraction during *rigor mortis*, and probably the most important, activity of proteolityc enzymes (calpains and cathepsins). It seems that the role of calpains is most pronounced in early *post mortem* tenderisation of meat, after which their activity is decreasing due to pH decline, giving a lead to cathepsins released from lysosomes (Cong *et al*. 1989*b*; Cong and Goll 1993).

Calpastatin is an endogenous inhibitor of calpains, proteins considered to be the main determinants of meat tenderness (Cong *et al*. 1989*a*; Cong and Goll 1993). The molecule is consisted of one L domain, coded by Exons 2–8 and four repeating domains, coded by Exons 9–14 (Stearns *et al*. 2005). Meyers and Beever (2008) found that heterozygosity in the first 60 kb of calpastatin gene was concordant with the quantitative trait loci for tenderness in their Illinois Meat Quality Pedigree population. In this block, non-coding regions appeared to be more polymorphic than coding regions, presumably due to less selection pressure on non-coding sequences. This suggests that the causative mutation resides in regulatory region (Lindholm-Perry *et al*. 2009).

Unlike calpains, which are coded by at least 14 genes (Kemp *et al*. 2010), calpastatin is coded by only one gene – *CAST*, mapped on SSC2 in Region 2q2.1–q2.4 (Ernst *et al*. 1998). Ciobanu *et al*. (2004) sequenced complete *CAST* gene and identified two single nucleotide polymorphisms (SNPs) associated with meat tenderness, namely Arg249 Lys and Ser638Arg. The influence of these polymorphisms on the quality of raw meat and dry-cured products has been investigated by several authors (Ramos *et al*. 2005; Stalder *et al*. 2005; Škrlep *et al*. 2010, 2012; Gou *et al*. 2012); however, their findings were rather inconsistent.

In the already mentioned study of Ciobanu *et al*. (2004), apart from meat tenderness identified, SNPs were also associated with cooking loss and juiciness of the pig meat. The authors stressed the possibility of significant improvements in production of pig meat by the use of these polymorphisms in marker-assisted selection. In that way, naturally tender and juicy pork could be produced avoiding the additional processing steps. Investigation of Stalder *et al*. (2005) showed that polymorphism at Ser638Arg in pigs was a significant source of variation for moisture content in cured hams and a tendency of influencing the ham yield. Although Ramos *et al*. (2005) could not detect the influence of *CAST* polymorphisms on any trait of dry-cured hams, Škrlep *et al*.

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(2010) reported the significant influence of *CAST* Lys249Arg and *CAST* Lys249Arg polymorphisms on ham weight and colour, respectively. In the study of genetic polymorphisms connected to quality traits of hams, Gou *et al*. (2012) identified Arg/Arg *CAST* genotype at both loci (249 and 638) as the most favourable for quality of Spanish dry-cured hams.

Despite the fact that introns do not translate into protein, mutations in these parts of gene can influence phenotype, if they are located in the regulatory region. Their influence is manifested through the improvement of transcription effectiveness or by their effect on mRNA stability (Le Hir *et al*. 2003). Furthermore, there is a large possibility that intronic variants can be in linkage disequilibrium (LD) with causative mutations that might not be in introns, particularly for variants near the intron and exon boundaries (Koufariotis *et al*. 2014). As earlier stated, the causative mutation seems to be located in regulatory region, which indicates that it may affect the amount of *CAST* mRNA either by regulating expression, by affecting mRNA stability or half-life, or by alternate splicing rather than the amino sequence of the protein (Lindholm-Perry *et al*. 2009). Numerous investigations in Introns 6 and 7 showed that many of the meat-quality and carcass traits were connected with the genotypes detected in intronic part of the *CAST* gene (Emnett *et al*. 2001; Koćwin-Podsiadła *et al*. 2004; Krzęcio *et al*. 2007*a*; Rybarczyk *et al*. 2010). For that reason, polymerase chain reaction–restriction fragment-length polymorphisms (PCR–RFLP) in intronic part of *CAST* gene are still considered interesting markers for improvement of pig-meat and carcass quality. The study of Ropka-Molik *et al*. (2014) showed that polymorphisms at *CAST*/*Hpa*II and *CAST*/*Rsa*I, located in Intron 6 of *CAST* gene, had the greatest effect on water holding capacity, meat pH, firmness and toughness for most breeds analysed. Furthermore, the investigation of Gandolfi *et al*. (2011) showed a significant influence of *CAST* EU137105: g.76872 G > A, SNP located in Intron 6 on the activity of autolysed calpain and drip loss; the effect of the specific *CAST* genotype on the shear force was not significant.

Therefore, the aim of the present paper is to evaluate the effect of nine genotypes identified in Intron 6 of CAST gene and meat-quality traits, as well as the chemical composition of hybrid pigs, with special emphasis on shear force and calpain activity.

Materials and methods

Animals

The study was conducted on 89 pig carcasses originating from P337xC23 PIC hybrid fatteners (45 gilts and 44 barrows). In accordance with European legislation (2008/120/EC 2008) on available floor space, the animals were kept in the collective pens with 12–15 animals/pen, resulting in an available floor space of 0.65–1.00 m^2 per pig. The animals were fed same diets and kept in the same environmental conditions with *ad libitum* access to food and water. The pigs were fed three different diets consisted of 13.58 MJ ME and 17.36 g/kg crude protein (CP) to 30 kg liveweight (LW); 13.26 MJ/ME and 16.05 g/kg CP from 30 kg to 70 kg LW; and 12.95 MJ/ME and 14.06 g/kg CP from 70 kg LW to slaughter weight. Pigs remained within the same pen the entire fattening period so as to prevent aggression resulting by mixing. LW of the animals included in the experiment was in the range of 110–150 kg. Animals were slaughtered in a commercial abattoir following electrical stunning (225–380 V, 0.5 A, 5–6 s). The carcasses were dressed according to the conventional procedure.

Carcass composition

After 24 h of cooling, the carcass length 'a' (from *os pubis* to *atlas*), length 'b' (*from os pubis* to 1st rib), ham length (from the anterior edge of the *symphysis pubis* to the hock joint), together with the ham circumference at its widest point, were measured. Back-fat and loin eye area were measured by geometric procedure according to Comberg *et al*. (1978) and expressed as the fat : loin eye ratio.

Meat quality

Post mortem measurements of muscle pH were taken at *m. semimembranosus* (SM) and *m. longissimus dorsi* (LD) 45 min and 24 h *post mortem* with Mettler MP 120-B portable pH meter (Mettler-Toledo, Schwerzenbach, Switzerland). Drip loss was measured by a bag method according to Kauffman *et al*. (1992) after 48 h of cooling the samples at 4°C. Light reflectance scores for CIE $L^*a^*b^*$ (CIE 2007) and hue angle were obtained by Minolta CR-300 colourimeter (Konica Minolta Sensing Ltd, Singapore) calibrated against a white plate ($L^* = 93.30$; $a^* = 0.32$ and 1.8; $b^* = 0.33$) with a D65 light source and 10-degree standard observer. For the purpose of instrumental tenderness evaluation, 2.54-cm-thick chops of LD muscle were frozen for 2 weeks, defrosted for 24 h at 4°C, sealed in vacuum bags, cooked in water bath to 73°C internal temperature and cooled at 4°C overnight. Cooking loss was calculated from weights taken before and after cooking of LD chops and expressed as a percentage. Shear force was measured on at least six 1.27-mm thick cores using a TA. XT*plus* Texture Analyser (Stable Micro Systems, London, UK) fitted with a 1-mm-thick Warner–Bratzler shear attachment. The mean value of maximal strength necessary for cutting the samples was calculated using a Texture Exponent 4.0 Software (Stable Micro Systems) and presented as Warner–Bratzler Shear Force (WBSF, N).

Calpain-activity assay

Calpain activity was quantified using a Calpain Activity Assay Kit (Abcam, UK), according to the manufacturer's instructions. Frozen muscles (30 mg) were pulverised and homogenised on ice in the extraction buffer provided by the manufacturer. Homogenates were centrifuged at 10 000 *g*, 4°C for 5 min and the supernatant was collected. Protein content was determined using Bradford method (Bradford 1976) with bovine serum albumin as a standard. After quantification of protein in the supernatant, 100 µg of protein was used for the calpain-activity assay with Acetyl–Leu–Leu–Tyr-7–amino-4–trifluoromethylcoumarin (Ac–LLY–AFC), a fluorescent calpain substrate. Active calpain I and calpain inhibitor (Z–Leu–Leu–Tyr–fluoromethylketone) were used for positive and negative controls, respectively. The reaction was carried out at 37°C in the dark for 1 h and fluorescence of released free AFC was monitored using a Fluoroscan Ascent microplate reader (Labsystems, Vantaa, Finland) equipped with a 400-nm excitation filter and a 505-nm emission filter. The enzyme activity

was expressed as relative fluorescence units per milligram of protein (RFU/mg proteins) of each sample.

Chemical composition

Relative contents of water, intramuscular fat (IMF), protein and collagen in LD muscle were determined by NIR spectroscopy (AOAC 2007) using the FoodScan™ Meat analyser (Foss, Hillerød, Denmark).

DNA analysis

Total DNA was isolated from 50 mg of pig tissue using a commercially available High Pure PCR Template Kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the protocol provided by manufacturer. The genotypes at different *CAST* loci were identified with *Hinf*I, *Msp*I and *Rsa*I restriction enzymes according to the method described by Ernst *et al*. (1998). PCR was performed in a reaction mixture of 20 µL containing 50 ng genomic DNA, standard PCR buffer, 1.5 mM MgCl2, 200 µM of each dNTP, 5 pmol of each primer and 1 U *Taq* DNA polymerase (Fermentas, Vilnius, Lithuania). Cycling conditions consisted of initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C (45 s), annealing at 58°C (45 s) and extension at 72°C (1 min), with a final extension at 72°C for 6 min. PCR products were purified using High Pure PCR Clean Up Micro Kit (Roche Diagnostics GmbH) and digested with three restriction enzymes (*Hinf*I, *Msp*I, *Rsa*I) in a total volume of 15.0 µL containing 12.0 µL of PCR product, 10 × reaction buffer and 1 U of each restriction endonuclease (Fermentas). The resulting digests were incubated at 37°C for 2 h and resolved on 3% agarose gel stained with ethidium bromide.

Statistical analyses

Kolmogorov–Smirnov normality tests followed by Levene's test for equality of variances were performed for all 27 traits as a whole and within each of the genotypes separately. For the traits where Levene's test showed significance, the Kruskal–Wallis analysis of variance (ANOVA) was used to evaluate heterogeneity of the means across genotypes. Kruskal–Wallis ANOVA was followed by Mann–Whitney U test as a *post hoc* test for two-genotype comparisons. For the traits where Levene's test showed no significance, the differences between different genotypes at *CAST* loci were analysed using mixed ANCOVA (analysis of covariance), a procedure of the general linear model with genotype and sex as the main factors and batch number as a random effect. Since genotype × sex interaction was not observed, gender was excluded from further analysis. Hot carcass weight was used as a covariance when proved to be significant for a trait. The differences between genotypes were determined by Fisher's l.s.d. test, where $P < 0.05$ was classified as significant difference and $P < 0.1$ as a tendency. All data were analysed using Statistica 10 (StatSoft Inc., Tulsa, OK, USA).

Results

The differences among investigated genotypes in carcass traits are presented in Table 1. AB genotype had the highest carcass length ('a' and 'b'), ham length, muscle thickness and loin eye area. This genotype also had the lowest fat thickness and fat area. However, this genotype did not differ from AA genotype in

Table 1. The differences among genotypes at *CAST/Hinf*I, *CAST/Msp*I and *CAST/Rsa*I loci and carcass traits of investigated pigs expressed as least square means (LSM) and mean square errors (MSE)

Length a, length from *os pubis* to *atlas*; length b, length from *os pubis* to 1st rib; ham length, length from the anterior edge of the *symphysis pubis* to the hock joint; MSE, mean square error. Within rows, mean values without a common lower-case differ significantly ($P < 0.05$); within rows, mean values without a common upper-case letter show a different trend ($P < 0.1$)

Genotype	CAST/*Hinf*I					CAST/*Msp*I					CAST/*Rsa*I				
	AA	AB	BB	MSE	P	CC	CD	DD	MSE	P	EE	EF	FF	MSE	P
N	13	61	15			45	17	27			26	15	48		
Length a (cm)	92.00b	95.11a	94.80a	0.86	0.121	94.82	93.76	94.78	0.78	0.394	95.31	94.27	94.33	0.80	0.494
Length b (cm)	110.46b	112.44a	111.53ab	0.87	0.205	112.36	112.12	111.33	0.78	0.142	112.31	112.60	111.65	0.80	0.779
Ham length (cm)	34.85ab	35.34a	34.53b	0.31	0.043	35.36A	34.71B	35.04AB	0.27	0.141	35.19	34.73	35.23	0.28	0.143
Ham circumference (cm)	72.77b	74.75a	74.73a	0.86	0.552	74.64	74.41	74.19	0.77	0.138	74.81a	75.53a	73.94b	0.78	0.241
Loin eye area (cm^2)	50.65b	55.48a	53.67ab	1.54	0.159	54.92	53.46	54.37	1.39	0.657	55.16	53.75	54.32	1.43	0.638
Fat area (cm^2)	18.31c	20.82b	22.92a	1.26	0.262	20.04b	20.75ab	22.13a	1.12	0.103	22.41a	21.29ab	19.80b	1.14	0.103
Fat : meat ratio at LD cut	0.36b	0.38b	0.43a	0.02	0.189	0.37b	0.39ab	0.41a	0.02	0.151	0.41a	0.40ab	0.37b	0.02	0.573

meat : fat ratio at the LD muscle cut. Three obtained genotypes at *CAST*/*Msp*I locus differed in ham length, fat area and loin eye area, where the lowest fat thickness was found in CD genotype, while the lowest fat area and fat : meat ratio at LD cut was found in CC genotype. As for the *CAST*/*Rsa*I locus, significant differences among genotypes were found in ham circumference, fat area and fat : meat ratio at LD cut. EE genotype had the highest ham circumference, with the highest fat : muscle ratio.

Among 16 investigated meat-quality traits, *CAST*/*Hinf*I locus influenced both pH_{45} and pH_{24} in SM muscle and ultimate pH values in LD muscle. Significant differences among genotypes at this locus were also found for hue angle ($h°$) and level of paleness (Table 2). The most favourable pH values measured 24 h *post mortem* in LD muscle were observed in BB genotype and for SM muscle in AB genotype. As for the level of paleness, the most favourable values were noticed in BB genotype. Furthermore, the same genotype exhibited the lowest $h°$ values, indicating that meat from this genotype was redder with less MMb (AMSA 2012).

Genotypes obtained by the use of *Msp*I restriction enzyme differed in pH_{24} and EC_{24} values measured in LD muscle and in almost all Minolta colour scores (a^*, b^* and $h°$), where CD genotype had meat with most pronounced red colour.

Genotypes at *CAST*/*Rsa*I locus differed in pH and EC values measured 45 min *post mortem* in SM muscle, pH_{24} values of SM muscle, level of paleness and redness, as well as shear force and calpain activity. EF genotype had highest pH values in SM muscle and lowest CIE L^*, with more pronounced red colour, but also highest shear force and lowest calpain-activity values.

The comparison between investigated genotypes for chemical composition of LD muscle is shown in Table 3. The only locus that influenced investigated traits was *CAST*/*Rsa*I; FF genotype had the lowest IMF content and highest protein content.

Discussion

It is known that calpain–calpastatin system plays a significant role in the muscle growth and development. This system is associated with apoptosis and myogenesis (Pandurangan and Hwang 2012), where calpain activity is required for myoblast fusion, cell proliferation and growth (Goll *et al*. 1998; Cruzen 2013). Increased skeletal muscle development may be a result of a reduced rate of protein degradation in muscle, which is connected with reduced activity of calpain mainly due to increased calpastatin activity (Goll *et al*. 1998).

The genotypes at *CAST*/*Hinf*I locus significantly differed in all investigated carcass traits. Genotype AB exhibited the most favourable values for all traits, which indicates its potential use as a marker for improved carcass traits in marker-assisted selection. Contrary to results of the present study, Kurył *et al*. (2003) and Rybarczyk *et al*. (2010) did not determine significant influence of *CAST*/*Hinf*I locus on any of the carcass traits measured. However, the investigations of Koćwin-Podsiadła *et al*. (2004) showed a significant influence of this locus on back-fat thickness and weights of the back-fat with bones, ham, loin, shoulder and ham muscle tissue in fatteners free of *RYR1* allele. The authors concluded that BB genotype at *Hinf*I and *Msp*I loci can be used in selection on ham weight and AA genotype at the same loci can be used for selection on loin weight.

Table 2. Least square means (LSM) and mean square errors (SME) for relationships among genotypes at *CAST*/*Hinf*I, *CAST*/*SMp*I and *CAST*/*Rsa*I loci and meat quality of investigated pigs

SM, *musculus semimembranosus*; LD, *musculus longissimus dorsi*; EC, electric conductivity, CIE $L^*a^*b^*$, colour coordinates; h°, hue angle; SME, mean square error. Within rows, mean values without a common lower-case letter differ significantly ($P < 0.05$); within rows, mean values without a common upperc-case letter show a different trend ($P < 0.1$)

Genotype	CAST/*Hinf*I					CAST/*SMp*I					CAST/*Rsa*I				
	AA	AB	BB	SME	P	CC	CD	DD	SME	P	EE	EF	FF	SME	P
N	13	61	15			45	17	27			26	15	48		
pH_{45} SM	6.36A	6.33AB	6.20B	0.06	0.184	6.33	6.33	6.27	0.05	0.968	6.20b	6.46a	6.32b	0.05	0.010
pH_{45} LD	6.18	6.26	6.23	0.06	0.502	6.23	6.22	6.27	0.06	0.162	6.20	6.28	6.25	0.06	0.736
EC_{45} SM	4.82	5.49	5.35	0.32	0.495	5.24	5.81	5.30	0.29	0.373	5.80a	5.69ab	5.03b	0.29	0.126
EC_{45} LD	4.04	4.59	5.01	0.30	0.316	4.52	4.30	4.86	0.27	0.404	4.78	4.57	4.47	0.27	0.843
pH_{24} SM	5.58B	5.68A	5.63AB	0.04	0.314	5.64	5.65	5.64	0.04	0.188	5.65ab	5.78a	5.62b	0.04	0.027
pH_{24} LD	5.58b	5.65ab	5.68a	0.03	0.406	5.64AB	5.73A	5.63B	0.03	0.958	5.65	5.69	5.63	0.03	0.528
EC_{24} SM	7.95	8.34	8.37	0.40	0.751	8.32	8.72	7.97	0.35	0.413	8.16	7.89	8.48	0.36	0.512
EC_{24} LD	4.98	4.97	4.97	0.41	0.953	4.73B	5.69A	4.91AB	0.35	0.173	5.29	4.97	4.79	0.37	0.359
CIE L^*	51.70a	50.82ab	49.40b	0.73	0.332	51.12	50.79	49.95	0.65	0.630	50.36ab	49.46b	51.28a	0.65	0.307
CIE a^*	7.21	7.40	7.58	0.27	0.923	7.34AB	7.83A	7.24B	0.23	0.153	7.49AB	7.78A	7.24B	0.24	0.491
CIE b^*	3.33	3.26	2.98	0.19	0.300	3.30a	3.53a	2.90b	0.16	0.027	3.16	3.19	3.27	0.17	0.618
$h°$	24.80A	23.60AB	21.20B	1.11	0.157	24.09A	24.29A	21.59B	0.70	0.095	22.56	22.17	24.19	1.01	0.278
Drip loss (%)	5.92	5.61	5.22	0.66	0.930	5.16	6.14	5.95	0.57	0.280	5.97	4.98	5.57	0.59	0.396
Cooking loss (%)	33.68	33.47	32.46	0.44	0.238	33.29	33.49	33.30	0.39	0.926	33.21	33.01	33.49	0.40	0.886
Shear force (N)	48.27	48.01	45.19	1.58	0.270	47.68	48.92	46.56	1.40	0.5340	46.03B	51.17A	47.28A	1.40	0.074
Calpain activity (RFU/mg)	9.24	8.77	8.59	0.36	0.747	8.81	9.10	8.63	0.32	0.677	9.54a	8.33b	9.30ab	0.31	0.011

Table 3. Least square means (LSM) and mean square errors (MSE) for relationships among genotypes at *CAST*/*Hinf*I, *CAST*/*Msp*I and *CAST*/*Rsa*I loci and chemical composition of *longissimus dorsi* muscle

IMF, intramuscular fat content; MSE, mean square error. Within rows, mean values without a common lower-case letter differ significantly ($P < 0.05$); within rows, mean values without a common upper-case letter show a different trend ($P < 0.1$)

Parameter	CAST/*Hinf*I					CAST/*Msp*I					CAST/*Rsa*I				
	AA	AB	BB	MSE	P	CC	CD	DD	MSE	P	EE	EF	FF	MSE	P
N	13	61	15			45	17	27			26	15	48		
IMF (%)	2.69	3.00	2.81	0.16	0.254	2.69	3.00	2.81	0.16	0.409	2.74ab	3.07a	2.64b	0.16	0.210
Moisture (%)	73.17	73.09	73.02	0.16	0.567	73.17	73.09	73.02	0.16	0.736	73.39A	72.93B	73.11AB	0.16	0.181
Protein (%)	23.75	23.65	23.80	0.11	0.462	23.75	23.65	23.80	0.11	0.889	23.77AB	23.51B	23.81A	0.11	0.725
Collagen (%)	1.00	0.98	0.95	0.04	0.901	1.00	0.98	0.95	0.04	0.892	0.91b	0.97ab	1.02a	0.04	0.068

Ham length and circumference represent important traits due to the possibility of selecting the best carcasses for further processing into valuable dry-cured products, such as ham or prosciutto. In the present study, CC genotype at *Msp*I locus exhibited the highest ham length and EE genotype at *Rsa*I locus the highest ham circumference. The lowest fat thickness was found in CD genotype. Krzęcio *et al*. (2007*a*) reported a significant effect of *CAST*/*Msp*I locus on the ham thickness at the last rib, as well as the weights of LD muscle, ham and shoulder. In their investigation, DD genotype had higher belly weight, back weight and fat thickness than did other investigated genotypes. In addition, Kuryłł *et al*. (2003) determined the lowest fat thickness and the highest muscle area in DD genotype. In contrast to results of the present study, Kuryłł *et al*. (2003) also reported the lowest back-fat thickness in EE genotype.

Meat quality is characterised by traits that are the consequence of different metabolic processes in muscle caused by conditions related to the *ante mortem* handling of the animal, slaughter and carcass manipulation. Muscle acidity, i.e. pH value and its drop rate, represent the most important meat-quality trait due to its influence on the many related properties such as, for example, protein denaturation during meat aging, drip loss, colour and losses during thermal processing. The influence of calpastatin on pH values can be explained through its ability of calcium channel regulation (Lee *et al*. 1992) and the fact that phosphorylase responsible for glycogenolysis is also a calpain substrate (Lametsch *et al*. 2002). In this way, glycogen in *post mortem* muscle degrades at different rates, depending on the activity of calpain system regulated by the activity of calpastatin (Boehm *et al*. 1998). It is interesting to notice that all three investigated *CAST* loci influenced pH values measured in SM and LD muscle (Table 2), which is supported by other authors who have reported results similar to ours (Koćwin-Podsiadła *et al*. 2003; Kapelański *et al*. 2004; Krzęcio *et al*. 2004, 2007*a*, 2007*b*; Ngu *et al*. 2012). The electrical conductivity measured 24 h *post mortem* is often used as the predictor of drip loss in pork muscle; an increase in EC_{24} values is related to a decrease in water-holding capacity (Lee *et al*. 2000; Łyczyński *et al*. 2009; Czyzak-Runowska *et al*. 2010). From the results shown in Table 2, it can be noticed that CD genotype at *CAST*/*Msp*I locus exhibited the highest ($P < 0.1$) EC_{24} values measured in LD muscles and the highest drip loss. Opposite to the results of the present study, Krzęcio *et al*. (2004, 2008) reported a significant influence of *Rsa*I mutation on EC values, water-holding capacity and drip loss, where EE genotype showed favourable values for these traits.

Significant differences in CIE L^* values were observed for *CAST*/*Hinf*I and *CAST*/*Rsa*I loci, while genotypes at *Msp*I locus differed in CIE a^*, CIE b^* values and hue angle. In concordance with our results Kapelański *et al*. (2004) and Ropka-Molik *et al*. (2014) reported that meat from animals of the AA genotype at *CAST*/*Hinf*I locus was characterised with shorter colour-dominant waves than that from animals of AB and BB genotypes, which indicates paler meat. On the other hand, Rybarczyk *et al*. (2010) found a significant influence of *CAST*/*Hinf*I locus on CIE b^* and CIE a^*, where BB genotype exhibited higher values of both CIE a^* and CIE b^* than did AB genotype, while Ngu *et al*. (2012) reported a significant influence of *Msp*I mutation on the meat redness, where the reddest meat was observed in DD genotype. However, Krzęcio *et al*. (2008) did

not determine the effect of *CAST*/*Rsa*I on colour-reflectance scores.

Muscle is an ATP- and Ca^{2+}-sensitive organ. With slaughter process and consequent decrease of ATP, an increase of Ca^{2+} in muscle cell can be observed. The calpain system activates at the time when pH begins to drop and Ca^{2+} ions begin to leak out of the endoplasmic reticulum. Binding of Ca^{2+} ions to calpain cause molecule alterations that enable it to become active, but also allows interaction of calpastatin with enzyme (Moldoveanu *et al*. 2008). The amount of calcium required to allow half-maximal binding of calpastatin to calpains is lower than that required for half-maximal activity of the unautolysed and autolysed forms of calpain (Huff Lonergan *et al*. 2010). By further decrease of pH values, calpastatin is degraded due to the increase of calpain activity. The rate of its degradation depends on the rate of meat proteolysis and tenderisation (Lonergan *et al*. 2001), but the exact factors that regulate calpastatin degradation by calpains are still unknown (Huff Lonergan *et al*. 2010). In the present study, a relationship between calpain activity and shear force at *CAST*/*Rsa*I locus was noticed, indicating its potential role in calpain activity regulation and meat tenderisation. Using the different methodology of tenderness evaluation, Kapelański *et al*. (2004) also found the most tender meat in EE genotype. These results suggest the possibility of using EE genotype at *CAST*/*Rsa*I locus as a marker for increased tenderness in marker-assisted selection.

The differences in all investigated chemical parameters of LD muscle were found only among genotypes obtained by *Rsa*I restriction enzyme. Contrary to our results, Krzęcio *et al*. (2008) reported highest protein share and lowest IMF in EE genotype, while Cheng (2004) found a significant influence of *Msp*I mutation on IMF in different breeds and suggested selection of the DD genotype at *CAST*/*Msp*I for the improvement of its mean values.

Conclusions

Our study indicated an effect of *CAST*/*Hinf*I locus on economically important carcass traits in PIC hybrid pigs, suggesting its potential use as genetic marker for improvement of these traits. Furthermore, we found significant ($P < 0.05$) and suggestive ($P < 0.10$) differences among investigated *CAST* loci in most meat-quality traits. Most importantly, a relationship between shear force and calpain activity was noticed, where EE genotype at *CAST*/*Rsa*I locus exhibited the highest calpain-activity and the lowest shear-force values. This result suggests a potential role of this intronic mutation in calpain-activity regulation and meat tenderisation of PIC fatteners.

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