The effect of calpastatin polymorphism and its interaction with RYR1 genotypes on carcass and meat quality of crossbred pigs

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The aim of the study was to establish the relationship between a calpastatin gene (CAST) polymorphism, the ryanodine receptor gene (RYR1) polymorphism and carcass/meat quality traits in crossbred pigs. No significant differences in the analyzed pigs were found between genotypes CC and CT at the locus RYR1 and CD and DD at the locus CAST/MspI in terms of carcass and meat quality. However, a significant association of the CAST/ApaLI polymorphism with carcass quality and meat marbling were observed. The carcasses of AB pigs had significantly higher carcass percentage of lean meat, thinner backfat and thicker muscle, as well as lower meat marbling, as compared with the BB pigs. Furthermore, interactions CAST/MspI × RYR1 and CAST/ApaLI × RYR1 were found significant in relation to all the studied carcass traits. The results presented here imply that the CAST gene recognized with ApaLI may be considered as important in terms of the way it affects porcine carcass quality traits. Moreover, the research has revealed a relationship between CAST and RYR1 genotypes as regards formation of carcass traits in pigs. Follow-up studies, however, should be carried out on larger populations representing all possible CAST genotypes.

Key-words: pigs, CAST gene, RYR1 gene, carcass quality, meat quality
Introduction

Intensive efforts on improvement of swine percentage of lean meat with use of Pietrain pigs resulted in a number of issues, especially those linked to a high frequency of the stress syndrome gene \((RYR1^T)\) observed in this breed, which is associated with occurrence of pale, soft, and exudative (PSE) meat (Fiedler et al. 2001). Moreover, research shows, despite belonging to very different breeds, pigs of the same genotype for the \(RYR1\) gene not only do exhibit a considerable variability in carcass lean content, but also provide meat of varying quality. This may be an effect of other genes that possibly affect both carcass traits and meat quality, modifying the effect of the \(RYR1\) (Koćwin-Podsiadła and Kurył 2003).

Calpastatin \((CAST)\) is the endogenous inhibitor involved in regulation of calpain activity in muscle cells. Activity of calpains and calpastatin relies on appropriate concentration of calcium ions in the cell (Murachi 1989). Moreover, calpastatin activity is strongly correlated with muscle growth rate, proteolytic processes, and immediate post mortem changes in the muscle (Goll et al. 1998), which affects many quality traits of the meat (Koćwin-Podsiadła et al. 2003; Melody et al. 2004).

The calpastatin gene has been mapped near the centromere of SSC 2 in the region q2.1-q2.4. The calpastatin molecule consists of domain L, coded by exons 2 to 8, and four repetitive domains, each of which is coded by exons 9-14 (Stearns et al. 2005). Polymorphisms in the calpastatin gene \((CAST)\), identified in the intron with 3 restriction enzymes \(HinfI\) and \(Hpy188I\), was found significant association between calpastatin gene \((CAST/HinfI)\) and meat quality traits (Rybarczyk et al. 2010).

The aim of the study was to find a possible relationship between variants of the calpastatin gene \((CAST/MspI\) and \(CAST/ApaLI)\), the ryanodine receptor gene \((RYR1)\) and carcass/meat quality traits in crossbred pigs sired by Pietrain boars.

Material and methods

Material

The experiment was carried out on 125 pigs (76 gilts and 49 barrows) from a pig farm located in Mecklenburg-Vorpommern (Germany). The study comprised offspring from crossing German Landrace × German Large White and also Leicoma × German Large White sows with Pietrain boars, kept under similar environmental conditions and fed with a balanced feed-mix ad libitum. All subjects destined for the experiment were conveyed in one group to the “Agryf” Meat Plant in Szczecin (Poland) in the evening after 4 hours transportation from a distance of 250 km, and slaughtered on the next day in the morning.

Slaughter value

After animal CO₂ stunning during the slaughter, blood was collected for identification of \(CAST\) and
RYR1 genotypes. Subsequently, carcass percentage of lean meat was measured, hot carcass weight, as well as the thickness of the longissimus dorsi (LD) muscle and back fat between the 3rd and 4th rib, 7 cm from the line of carcass partition into the left-hand side of the carcass half, by means of an optic-needle CGM apparatus (Sydel, France). Mean percentage of lean meat amounted to 55.39 ± 0.40 and hot carcass weight to 87.75 ± 0.55 kg (mean value and standard error).

Meat quality and basic meat chemical composition

Two hours after slaughter during carcass cooling, electric conductivity (EC) was measured in the longissimus dorsi muscle, between the 4th and 5th lumbar vertebrae of the right-hand side of the carcass half using an LF-Star MATTHÄUS conductometer. After 24 hours of carcass cooling, meat samples from the longissimus dorsi muscle were collected from the 1st to 4th lumbar vertebra section (longissimus lumborum) of the right-hand side of the carcass half. 24 hours from the slaughter, meat pH value (Elmetron CP-311 pH-meter) and the volume of drip loss from the muscle tissue were determined according to Honikel (1987).

Within 48 hours after slaughter, minced meat samples were measured for pH in water solution, and meat colour traits, i.e. L* (lightness), a* (redness) and b* (yellowness), were established by means of a HunterLab Mini Scan XE Plus 45/0 with light illuminant D65 and observer 10° (CIE 1976). Meat water-holding capacity (WHC) was determined according to Grau and Hamm (1952) as modified by Pohja and Niinivaara (1957), as well as thermal drip from a difference of meat sample weight before and after heating in a water bath at 85°C for 10 minutes. Water-soluble protein content was determined by Kotik method (1974). Marbling (the degree of intramuscular fatness) was determined by a trained 5 person team of panellists, using a 1-5 point scale (1 point – slight muscle fatness; 5 points – strong muscle fatness). The basic meat chemical composition, i.e. total protein, fat, ash and dry matter (AOAC 2003), was estimated.

Genotyping

Genomic DNA was extracted from blood using a Master Pure kit (Epicentre Technologies). Genotypes RYR1, CAST/MspI and CAST/ApaLI were identified by PCR/RFLP method according to Fujii et al. (1991), Ernst et al. (1998) and Ciobanu et al. (2004), respectively.

Statistical analysis

A statistical analysis was performed to compare carcass and meat quality traits and basic chemical composition of meat between pigs of different CAST and RYR1 genotypes, using the least squares method of the GLM procedure (Statistica 8.0 PL) according to the following linear model:

\[ Y_{ijkl} = \mu + a_i + b_j + c_k + bc_{jk} + \beta (x_{ijkl} - \bar{x}) + e_{ijkl} \]

where:
- \( Y_{ijkl} \) - trait measured,
- \( \mu \) - the overall mean,
- \( a_i \) - the effect of sex (i = 1, 2),
- \( b_j \) - the effect of RYR1 genotype (j = CT, CC),
- \( c_k \) - the effect of CAST/MspI genotype (k = CD, DD) or CAST/ApaLI genotype (k = AB, BB);
- \( bc_{jk} \) - interaction (RYR1 × CAST/MspI or CAST/ApaLI genotype),
- \( \beta \) - linear regression coefficient for hot carcass weight;
- \( x_{ijkl} \) - hot carcass weight of ijk-lth individual included as covariable;
- \( \bar{x} \) - mean for hot carcass weight;
- \( e_{ijkl} \) - the random error.
A detailed comparison of mean least squares (LSQ) for the analysed CAST and RYR1 genotypes was done using a Tukey’s test.

Results

The frequencies CAST/MspI, CAST/ApaLI, and RYR1 of alleles and genotypes in Pietrain-sired pigs are presented in Table 1. The significances of association between the genotypes of calpastatin

Table 1. The frequency of CAST and RYR1 alleles and genotypes in analysed pigs.

| (n = 125) | CAST/MspI | CAST/ApaLI | RYR1 |
|-----------|-----------|------------|------|
| CC | CD | DD | AA | AB | BB | CC | CT | TT |
| No. of animals | - | 68 | 57 | - | 95 | 30 | 71 | 54 | - |
| Frequency of alleles | C = 0.27 | D = 0.73 | A = 0.38 | B = 0.62 | C = 0.78 | T = 0.22 |
| Frequency of genotypes (%) | - | 54.4 | 45.6 | - | 76.0 | 24.0 | 56.8 | 43.2 | - |

Table 2. The LSQ means of analysed traits and relationship between genotypes at the loci CAST/MspI and CAST/ApaLI and RYR1 for carcass and meat quality in pigs.

| Trait | LSQ | SE | CAST/MspI | CAST/ApaLI | RYR1 | CAST/MspI × RYR1 | CAST/ApaLI × RYR1 |
|-------|-----|----|-----------|------------|------|-----------------|-----------------|
| Slaughter value | | | | | | | |
| Lean meat content (%) | 55.39 | 0.39 | n.s. | p=0.011 | n.s. | p=0.006 | p=0.007 |
| Backfat thickness (mm) | 14.90 | 0.38 | n.s. | p=0.026 | n.s. | p=0.037 | p=0.006 |
| Muscle thickness (mm) | 56.64 | 0.58 | n.s. | p=0.035 | n.s. | p=0.014 | p=0.047 |
| Basic chemical composition | | | | | | | |
| Total protein (%) | 22.40 | 0.06 | n.s. | n.s. | n.s. | n.s. | n.s. |
| Fat (%) | 2.52 | 0.05 | n.s. | n.s. | n.s. | n.s. | n.s. |
| Ash (%) | 1.18 | 0.01 | n.s. | n.s. | n.s. | n.s. | n.s. |
| Dry matter (%) | 26.10 | 0.07 | n.s. | n.s. | n.s. | n.s. | n.s. |
| Marbling (score) | 1.28 | 0.04 | n.s. | p=0.020 | n.s. | n.s. | n.s. |
| Meat quality | | | | | | | |
| pH24 | 5.66 | 0.01 | n.s. | n.s. | n.s. | n.s. | n.s. |
| pH48 | 5.57 | 0.01 | n.s. | n.s. | n.s. | n.s. | n.s. |
| EC2 (mS/cm) | 3.08 | 0.12 | n.s. | n.s. | n.s. | n.s. | n.s. |
| L* | 54.74 | 0.30 | n.s. | n.s. | n.s. | n.s. | n.s. |
| a* | 9.33 | 0.11 | n.s. | n.s. | n.s. | n.s. | n.s. |
| b* | 16.81 | 0.12 | n.s. | n.s. | n.s. | n.s. | n.s. |
| Drip loss (%) | 7.65 | 0.23 | n.s. | n.s. | n.s. | n.s. | n.s. |
| WHC (% of free water) | 17.42 | 0.44 | n.s. | n.s. | n.s. | n.s. | n.s. |
| Thermal drip (%) | 25.88 | 0.25 | n.s. | n.s. | n.s. | n.s. | n.s. |
| Water-soluble protein (%) | 8.22 | 0.08 | n.s. | n.s. | n.s. | n.s. | n.s. |

n.s. - statistically not significant.
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CAST/MspI and CAST/ApaLI, RYR1 and carcass and meat quality traits of the pigs are presented in Table 2.

We did not find any significant differences between the genotypes CC and CT of the RYR1 in terms of carcass percentage of lean meat, backfat and LD muscle thickness, as well as in meat quality and basic chemical composition determined in the longissimus lumborum muscle.

Significant differences were found in carcass slaughter performance and meat marbling between pigs of genotypes AB and BB at the locus CAST/ApaLI (Table 3). The AB pigs had significantly higher percentage of lean meat, thinner backfat, thicker LD muscle, and lower marbling of the meat as compared with the BB genotype (p ≤ 0.05). No significant association, however, was found between the CAST/ApaLI genotype and meat quality traits of the pigs. Moreover, significant CAST/ApaLI × RYR1 interactions were found in terms of carcass quality. Significantly higher percentage of lean meat and thinner backfat (p ≤ 0.01), as well as thicker LD muscle (p ≤ 0.05), were found in the AB/CT, AB/CC, and BB/CT genotypes in relation to BB/CC pigs (Table 4).

No significant effect of the CAST/MspI polymorphism on carcass and meat quality or meat basic chemical composition was observed. Significant CAST/MspI × RYR1 interactions, however, were found as regards carcass quality of the pigs.

| Table 3. The relationship between genotypes at the CAST/ApaLI locus and carcass quality traits in pigs. |
|---------------------------------------------------------------|
| Item | CAST/ApaLI genotypes | AB | BB |
| No. of animals | 95 | 30 |
| Lean meat content (%) | 55.93±0.43 | 53.70±0.91 |
| Backfat thickness (mm) | 14.46±0.40 | 16.30±0.92 |
| Muscle thickness (mm) | 57.28±0.66 | 54.57±1.14 |
| Marbling (score) | 1.24±0.04 | 1.43±0.10 |

Results in the table are given as LSQ mean ± standard error

| Table 4. Effect of interaction between CAST and RYR1 genotypes and carcass quality traits in pigs. |
|---------------------------------------------------------------|
| Item | CAST/ApaLI and RYR1 genotypes | AB/CT | BB/CT | AB/CC | BB/CC |
| No. of animals | 34 | 20 | 61 | 10 |
| Lean meat content (%) | 56.18A±0.66 | 55.28A±0.81 | 55.78A±0.56 | 50.56B±1.88 |
| Backfat thickness (mm) | 13.94A±0.66 | 14.80A±0.81 | 14.75A±0.51 | 19.30B±1.97 |
| Muscle thickness (mm) | 56.53A±0.90 | 56.40A±1.32 | 57.70A±0.90 | 50.90B±1.70 |

| Item | CAST/MspI and RYR1 genotypes | CD/CT | DD/CT | CD/CC | DD/CC |
| No. of animals | 31 | 23 | 37 | 34 |
| Lean meat content (%) | 56.16A±0.75 | 55.41A±0.65 | 53.61A±0.85 | 56.61A±0.71 |
| Backfat thickness (mm) | 14.13A±0.75 | 14.43A±0.67 | 16.43A±0.82 | 14.26A±0.67 |
| Muscle thickness (mm) | 57.26A±1.07 | 55.43A±0.95 | 54.78A±1.12 | 58.88A±1.22 |

Results in the table are given as LSQ mean ± standard error

A,B Mean values marked by different capital letters differ significantly at p≤0.01.

a,b Mean values marked by different small letters differ significantly at p≤0.05.
The DD/CC and CD/CT pigs had significantly higher percentage of lean meat ($p \leq 0.01$) and thinner backfat ($p \leq 0.05$) as compared with the CD/CC genotype. Moreover, DD/CC pigs had a significantly thicker LD in relation to CD/CC ($p \leq 0.05$).

**Discussion**

The presented study on Pietrain-sired pigs of unknown family structure did not reveal significant differences in carcass or meat quality between CC and CT genotype at the locus RYRI. Results by other authors who studied carcass and meat quality in the same genotypes of pigs with Pietrain genes are not unambiguous. Busk et al. (2000) stated that the carcasses of CT pigs contained more lean and had worse meat quality as compared with the CC pigs. Kusec et al. (2005) did not observe significant differences in butchery value of carcasses between the CC and CT pigs; the authors found, however, that meat quality of heterozygous pigs (CT) was worse. Also Krzęcio et al. (2005) and Kuhn et al. (2003) demonstrated a relationship between the LD and percentage of lean meat. It is known that the proteolytic calpain-calpastatin system has its role in the processes of muscle growth and development. An increased rate of skeletal muscles development may be a result of a reduced rate of protein degradation in the muscle, which is associated with a reduced activity of the calpain system resulting mainly from a CAST/MspI polymorphism and some carcass quality traits in RYRT-free Stamboek hogs. DD pigs had thinner backfat at some measurement points and a larger rib eye area in relation to CC pigs. Also Koćwin-Podziadła et al. (2004) observed that crossbred, RYRT-free fatteners revealed significant effect of association between the CAST/MspI genotype and five out of 19 analyzed carcass traits. The authors report that activity of a given molecular type of calpastatin depends on the muscle, since BB-genotype pigs at CAST/MspI had a larger ham weight, whereas larger loins were cut from AA pigs.

The analyzed pigs did not show a significant association between the CAST/MspI genotype and meat quality as well as basic chemical composition of meat in the longissimus lumborum muscle. Also Koćwin-Podziadła et al. (2003) and Kurył et al. (2004), who studied crossbreds sired by Duroc × Pietrain boars, failed to demonstrate a relationship between the CAST/MspI genotype and meat quality traits, except for loin efficiency during smoking. Koćwin-Podziadła et al. (2005), on the other hand, observed significant effects of the CAST/MspI polymorphism on the level of lactic acid in the longissimus lumborum muscle tissue 45 minutes post mortem, index of energy metabolism intensity $R_1$, water holding capacity (WHC), drip loss from muscle tissue at 48 and 96 hours post mortem, as well as meat protein and water content. Moreover, Koćwin-Podziadła and Kurył (2003) demonstrate that polymorphism at the locus CAST/MspI relatively strongly correlates with the incidence of increased drip loss from meat in the group of pigs with the gene RYRT.

We have demonstrated here a significant association between the CAST/ApaL1 genotype and carcass quality traits and meat marbling. Bonferroni’s correction revealed a significant linkage between the CAST/ApaL1 and percentage of lean meat. It is known that the proteolytic calpain-calpastatin system has its role in the processes of muscle growth and development. Results of experiments show that the calpain system is important for the proper development of the skeletal muscles. An increased rate of skeletal muscles development may be a result of a reduced rate of protein degradation in the muscle, which is associated with a reduced activity of the calpain system resulting mainly from a...
considerable increase in calpastatin activity (Goll et al. 1998). The presented results may indicate that calpastatin as calpains inhibitor may behave differently depending on the genetic variant. Moreover, analysis found significant evidence of QTL on SSC 2 associated with backfat thickness, longissimus muscle area and fat percent (Malek et al. 2001; Stearns et al. 2005).

The analysis of the pigs revealed significant effect of interactions, CAST/ApaLI × RYR1 and CAST/MspI × RYR1, in relation to carcass quality traits only, i.e. percentage of lean meat, backfat and LD muscle thickness. Bonferroni’s correction revealed significant interactions between CAST/MspI × RYR1 in relation to percentage of lean meat and muscle thickness, as well as between CAST/ApaLI × RYR1 in relation to percentage of lean meat and backfat thickness. On the other hand, Krzęcio et al. (2005), who studied a group of crossbred pigs, observed a significant effect of interaction between the RYR1 and CAST/MspI genotype for muscle tissue acidity 24 hours post mortem and drip loss from the LL tissue 48 hours post mortem. In the studies on crossbred pigs sired by Duroc × Pietrain boars, an interaction between the CAST/MspI and RYR1 genotypes was significant only for loin efficiency during smoking (Koćwin-Podsiadla et al. 2003) and drip loss measured at 48 hours post mortem (Kurył et al. 2004). The authors (Kurył et al. 2004; Krzęcio et al. 2005) conclude that the frequency of meat with high drip loss in pigs free of the stress sensibility gene (genotype CC at the locus RYR1), as well as that of normal meat in carriers of the gene (genotype CT at the locus RYR1) may be a result of a joint modifying effect of the CAST gene on the post mortem changes in the muscle tissue.

Conclusions

No significant differences in the analyzed pigs sired by Pietrain boars were found between genotypes CC and CT at the RYR1 locus and CD and DD at the CAST/MspI locus in terms of carcass and meat quality. However, a significant influence was found of the CAST/ApaLI polymorphism on carcass quality and meat marbling. The carcasses of AB pigs had significantly higher percentage of lean meat, thinner backfat, and thicker LD muscle, as well as lower meat marbling, as compared with the BB pigs. Furthermore, interactions CAST/MspI × RYR1 and CAST/ApaLI × RYR1 were found significant in relation to all the studied carcass traits, i.e. percentage of lean meat, backfat thickness, and LD thickness. The results presented here imply that the CAST gene recognized with ApaLI may be considered as important in terms of the way it affects porcine carcass quality traits. Moreover, research has revealed an association between CAST and RYR1 genotypes as regards formation of carcass traits in pigs. Follow-up studies, however, should be carried out on larger populations representing all possible CAST genotypes.

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