The influence of CAST/RsaI and RYRI genotypes and their interactions on selected meat quality parameters in three groups of four-breed fatteners with different meat content of carcass*

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The effect was analysed of CAST and RYRI genotypes and their interactions on selected LL muscle quality traits in 201 fatteners of three four-breed crossbred groups. CAST/RsaI genotype affected WHC, drip loss at 96 h post mortem and protein and water content of muscle. Among analysed meat quality traits affected by CAST/RsaI genotype, animals of AA genotype were characterized by most desirable values. Significant interactions between CAST/RsaI and RYRI genotype indicate that quality of meat should be considered not only as a result of genotype effect at each locus, but also as their combined effect.

KEY WORDS: CAST / fatteners /gene polymorphism / meat quality / RYRI

Up to year 2000 only an interactive effects of major genes (RYRI and RN) were studied on body composition and meat quality traits of pigs [Le Roy et al. 2000, Przybylski et al. 2000]. Recently, investigations were carried out concerning identification

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The mentioned genes affect the rate of meat tenderization and post-slaughter protein turnover which is also influenced by an activity of calpastatin (CAST), an endogenous inhibitor of calpain [Goll et al. 1998]. Ciobanu et al. [2002] reported an effect of polymorphism in CAST gene identified with Hpy188I and PvuII restriction enzymes on drip loss suggesting a necessity of further studies in order to explain whether the revealed effects were caused by analysed CAST gene mutations alone, or due to linkage disequilibrium.

The aim of this study was to analyse the effect of polymorphism of calpastatin (CAST) and RYR1 genes (identified by digestion of PCR products with restriction endonuclease HinP1 for RYR1 gene and RsaI for calpastatin gene) on selected meat quality traits taking into consideration the meat content of carcass. Moreover, an effect was analysed of interactions between variants of RYR1 and CAST/RsaI genotypes and meat content of carcass on investigated meat quality traits.

**Material and methods**

Investigations were carried out on 201 crossbred fatteners representing three groups of crosses: [(Polish Large White × Polish Landrace) × (Duroc × Pietrain)] – 77 animals, [(Landrace × Yorkshire) × (Duroc × Pietrain)] – 40 animals, and [(Polish Large White × Polish Landrace) × (Hampshire × Pietrain)] – 84 animals. The animals were kept under similar environmental conditions, fed balanced diets and slaughtered using electrical pre-slaughter stunning (INARCO system) at a commercial abattoir, 4-5 hours after transportation on the distance 300 km. Immediately after slaughter blood samples were collected in EDTA-coated tubes for subsequent DNA analysis for the RYR1 and CAST genotype.

Mean warm carcass weight (n=201) of analysed animals was 78.40 ±0.54 kg. Carcasses belonged to three groups of meat content: I – ≤50.0, II – 50.1 to 55 and III – >55%. Mean meat content of carcass was 51.28 ±0.40% (dissection made according to the Polish Pig Testing Stations method). In each group the number of gilts and castrated males was similar.

The following meat quality indicators were determined. pH of *Longissimus lumbarum* (LL) muscle on the processing line (directly in carcass, in the region of the last rib) 35 min post mortem (pH<sub>35</sub>) and in water homogenate of muscle tissue at 45 min (pH<sub>45</sub>). R<sub>1</sub> – coefficient of energetic changes expressed as IMP/ATP ratio 45 min post mortem according to Honikel and Fischer [1977]. At 24 h post mortem meat lightness (measured with MINOLTA CR310 Chroma Meter in CIE L*a*b* system), water holding capacity (WHC) according to Grau and Hamm [1952] with Pohja and Ninivivara [1957] modification, and losses of weight of meat in cooking process. Drip loss from LL muscle tissue at 48 and 96 h post-slaughter was determined according to Prange et al. [1977]. Moreover, protein, water and dry matter contents of LL muscle tissue were
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determined. Meat lightness and pH sub{24} were measured also in Semimembranosus (SM) muscle. At min 45 post mortem samples from LL muscle were collected into tubes with 0.5 M PCA for determination of glycogen [Dalrymple and Hamm 1973] and lactate [Bergmeyer 1974]. On this basis the glycolytic potential (GP) was calculated according to formula proposed by Monin and Sellier [1985].
The RYR1 genotypes were established according to Fujii et al. [1991]. Polymorphism of CAST gene was identified with RsaI endonuclease according to Ernst et al. [1998].

Statistical evaluation of the results was performed using three-way non-orthogonal ANOVA. Statistical model comprised RYR1 and CAST genotypes, group of meat content of carcass and their interactions:

\[ Y_{ijkl} = \mu + a_i + b_j + c_k + ab_{ij} + ac_{ik} + bc_{jk} + abc_{ijk} + e_{ijkl} \]

where:
- \( Y_{ijkl} \) – meat quality trait;
- \( \mu \) – overall mean;
- \( a_i \) – effect of CAST genotype \((i=1,2,3)\);
- \( b_j \) – effect of RYR1 genotype \((j=1,2)\);
- \( c_k \) – effect of the meat content of carcass \((k=1,2,3)\);
- \( ab_{ij} \) – effect of interaction between CAST and RYR1 genotypes;
- \( ac_{ik} \) – effect of interaction between CAST genotype and meat content group;
- \( bc_{jk} \) – effect of interaction between RYR1 genotype and meat content group;
- \( abc_{ijk} \) – effect of interaction between CAST and RYR1 genotypes and meat content group.
- \( e_{ijkl} \) – random effect.

Detailed comparison of means was made using Tukey test (STATISTICA PL 5.1).

Results and discussion

Observed and expected frequency of genotypes and alleles at the CAST/Rsal locus show that analysed population of animals was at Hardy-Weinberg equilibrium (Tab. 1).

Table 2 presents means and their standard errors of analysed meat quality traits, across genotypes considered and significance of their mutual interactions.

Highly significant (P≤0.01) influence of RYR1 genotype on lactate level, pH sub{35} and pH sub{45} and significant on R sub{1} value (P≤0.05) were observed. It should be stressed that
animals considered were of CC and CT genotype at the RYR1 locus. Related to RYR1 genotype appeared also protein and dry matter content of muscle. In analysed fatteners’ population highly significant influence of RYR1 gene polymorphism was noted on pH\textsubscript{35}, pH\textsubscript{45}, R\textsubscript{i} values that are basis of PSE meat classification. As expected, the most desirable values of above mentioned parameters were noted in stress-resistant (CC) group of fatteners (Tab. 2).

A significant influence (P≤0.05) of CAST/RsaI genotype was noted for WHC, drip loss from muscle tissue 96 h post mortem and protein and water content. Analysing meat quality traits affected by CAST/RsaI genotype shows that AA animals were characterized by lower WHC (by 0.90 cm\textsuperscript{2}), lower drip loss at 96 h (by 2.50 pp, lower water content (by 0.56 pp) and also by about 0.5 pp higher protein content compared to fatteners of BB genotype at this locus.

Significant interaction (P≤0.05) between RYR1 and CAST/RsaI loci was noted for pH\textsubscript{45} of LL muscle (Tab. 2, Fig. 1) and for drip loss at 48 h (Tab. 2, Fig. 2). No significant interactions were found between CAST/RsaI and RYR1 genotype and group of meat content of carcass (Tab.2).

Interaction between all analysed factors (CAST/RsaI, RYR1 and group of meat content) was significant (P≤0.01) for pH\textsubscript{35} (Tab. 2, Fig.3).

Lee et al. [1992] showed that calcium channel activity might be regulated through domain L of calpastatin. It is also known that phosphorylase responsible for glycogenolysis is a substrate for calpain [Lametsch et al. 2002]. Moreover, degradation of glycogen in muscles post mortem may run with diverse speed in dependency on activity
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Table 1. Relationship between genotype frequencies for CAST and RYR1 and backfat and meat quality traits in crossbred pigs

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Backfat (mm)</th>
<th>Longissimus Dorsi (mm)</th>
<th>Loin Muscle pH</th>
<th>Loin Muscle Temperature (°C)</th>
<th>Ham pH</th>
<th>Ham Temperature (°C)</th>
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<tbody>
<tr>
<td>CAST</td>
<td></td>
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<tr>
<td>RYR1</td>
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<tr>
<td>DD</td>
<td>5.2</td>
<td>18.3</td>
<td>5.8</td>
<td>2.3</td>
<td>4.6</td>
<td>1.8</td>
</tr>
<tr>
<td>CD</td>
<td>12.3</td>
<td>20.2</td>
<td>6.1</td>
<td>2.5</td>
<td>5.1</td>
<td>2.3</td>
</tr>
<tr>
<td>CC</td>
<td>15.3</td>
<td>24.8</td>
<td>8.3</td>
<td>2.9</td>
<td>6.6</td>
<td>2.8</td>
</tr>
</tbody>
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Note: The table continues with similar data for other genotypes.
Table 3. Continued

<table>
<thead>
<tr>
<th>Trait</th>
<th>CDPP control</th>
<th>F4/F control</th>
<th>Disease resistant</th>
<th>Treatment resistant</th>
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<tr>
<td></td>
<td>dd</td>
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ns - non-significant  *P<0.05,  **P<0.01,  ***P<0.001

Within rows means having different superscripts differ significantly (n = 20): *small leaves - PTD D, capsule - PTD D1

GMI - group of homogenous means
LL - large leaves
SM - small leaves

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of these proteases regulated by calpastatin and it may explain the effect of $CAST/Rsal \times RYR1$ interaction on $pH_{45}$ value (Fig. 1).

Differentiation of drip loss from LL muscle tissue at 48 h was observed only among animals heterozygous ($CT$) at $RYR1$ locus (Fig. 2). Drip loss from meat of $CT$ animals being simultaneously $BB$ homozygotes at $CAST/Rsal$ locus was about 3.5 pp higher than that in group of the same $RYR1$ genotype but $AA$ homozygous at $CAST/Rsal$ locus (4.52% and 8.01% respectively).

Fig. 1. Interactive effect of $CAST/Rsal$ and $RYR1$ genotypes for $pH_{45}$ in homogenate of LL muscle tissue.

Fig. 2. Interactive effect of $CAST/Rsal$ and $RYR1$ genotypes for drip loss of LL muscle tissue at 48 h.
Obtained interaction between CAST/RsaI and RYR1 genotypes and group of meat content of carcass indicate that presence of T allele of RYR1 gene in heterozygous form may cause differentiation in pH35 of LL muscle among animals with meat content above 55%, being AB heterozygotes at CAST/RsaI locus (Fig. 3).

The polymorphisms of the CAST gene genotyped in this study were located in intron 6 and it is difficult to conclude on their effect on calpastatin level or activity. Nevertheless, Le Hir et al. [2003] showed the important role of introns in eukariotic genes on influencing gene expression by increasing transcriptional efficiency of numerous genes.

Significant interactions found between CAST/RsaI and RYR1 genotypes indicate that quality of meat should be considered as not only a result of influence of genotype at each locus separately, but also as an effect of their interactions.

Summing up, the results presented suggest that relationships between polymorphism in CAST gene and meat quality of pigs should be further investigated.

REFERENCES

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Wpływ genotypów CAST/RsaI i RYR1 oraz ich interakcji na wybrane parametry jakości wieprzowiny w trzech grupach czterorasowych mieszańców, zróżnicowanych zawartością mięsa w tuszy

S t r e s z c z e n i e

Celem badań była analiza wpływu genotypów CAST/RsaI i RYR1 oraz ich interakcji na cechy jakości mięśnia longissimus lumborum. Badaniami objęto 201 tuczników trzech grup czterorasowych mieszańców. Genotyp CAST/RsaI istotnie wpływał na wodochłonność mięsa (WHC), naturalny wyciek w 96 godzinie post mortem oraz zawartość białka i wody w mięsie. Korzystniejszą wartość tych cech wykazywały zwierzęta o genotypie AA. Wykazany istotny wpływ interakcji genotypów CAST/RsaI i RYR1 na wartość niektórych parametrów charakteryzujących jakość mięsa wskazuje, że są one kształtowane nie tylko przez genotyp względem każdego z analizowanych loci, ale również przez współdziałanie między tymi loci.