THE INFLUENCE OF RYR1 AND CAST/MSPI GENES POLYMORPHISM AND THEIR INTERACTIONS ON SELECTED PORK MEAT QUALITY TRAITS

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Background

Post mortem proteolysis of myofibrillar proteins is associated with activity of the calpain system (-μ and -m calpain) and their inhibitor calpastatin (Sensky et al. 1999, Hao et. al 2000). Calcium channel activity is regulated by domain L of calpastatin (Hao et al. 2000). Ca^{2+} level in sceletal muscle is also regulated by *RYR1* gene. Activity of calpastatin (as endogenous inhibitor of calpain) in sceletal muscle is highly related to rate of meat tenderization and protein turnover after slaughter (Goll et al. 1998). Thus, *CAST* represents an excellent candidate gene for studying variation in pork quality (Ernst et al. 1998).

Objectives

The aim of this study is analysis of the effect of polymorphism of *RYR1*and calpastatin (*CAST*) genes for some meat quality traits taking into consideration group of meatiness. An effect of interactions between variants of *RYR1* and *CAST* gene and group of meatiness for investigated meat quality traits was also analysed.

Materials and methods

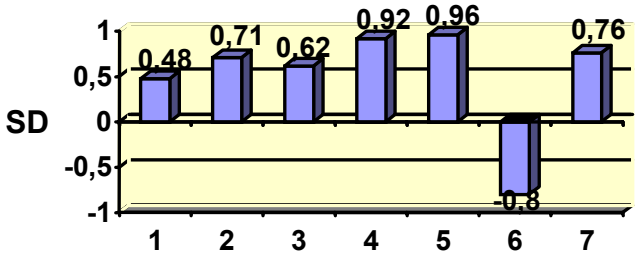
Investigations were carried out on 201 fatteners being crosses of [(Polish Large White x Polish Landrace) x (Duroc x Pietrain)] (77), [(Landrace x Yorkshire) x (Duroc x Pietrain)] (40) and [(Polish Large White x Polish Landrace) x (Hampshire x Pietrain)] (84). The animals were kept in similar environmental conditions, fed balanced mixtures and slaughtered using electrical stunning ("Inarco" system) at 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.
Average warm carcass weight of analysed animals was 78.40±0,54 kg (mean value ± se). Carcasses belonged to three groups of meatiness: I ≤50.0; II from 50.1 to 55 and III >55 percent of meat in carcass respectively. Average meatiness of carcasses analysed population of fatteners was 51.28±0.40 %. In each group was similar number of gilts and castrates.
The following meat quality characteristics immediately after slaughter were determined: pH of *Longissimus Lumborum* (*LL*) muscle tissue (immediately in carcass - pH_{35}) and in water homogenate of muscle tissue (pH_{45}); R_1 expressed as IMP/ATP ratio at 45 min *post mortem* according to Honikel and Fischer (1977). At 24 h *post mortem* pH, meat lightness (measured with Minolta CR310 Chroma Meter in CIE L*a*b* system), water holding capacity (WHC) according to Grau and Hamm (1952) and Pohja and Niniivaara (1957) modification and losses of weight of meat in cooking process were determined. Drip loss from muscle tissue at 48 and 96 h after slaughter was determined according to Prange et al. (1977). Besides, analysis of protein, water and dry matter content in *LL* muscle tissue were determined. Meat lightness and pH_{24} in *Semimembranosus (SM)* muscle were also executed. At 45 min *post mortem* samples from *Longissimus Lumborum* muscle were collected into tubes with 0.5 M PCA for determination of glycogen (Dalrymple and Hamm 1973) and lactate (Bergmeyer, 1974). On the basis of them 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 *Msp*I endonuclease according to Ernst et al. (1998).
Statistical elaboration of the data was executed using three-way non-orthogonal ANOVA. Statistical model comprised: *RYR1* and *CAST* genes polymorphism, group of meatiness and their interactions:
Detailed comparison of average values of analysed groups was made using Tukey' test.

Results and discussion

In analysed population of animals a highly significant influence of *RYR1* genotype on pH value measured at 35 min *post mortem* immediately in *Longissimus lumborum* muscle (pH_{35}) as well as at 45 min *post mortem* in water homogenate of muscle tissue (pH_{45}) and on lightness of *LL* muscle ($P \leq 0.01$) was observed. The *RYR1* gene polymorphism affected also lactate level in *LL* muscle tissue, R_1 value, WHC and drip loss in both terms of measurements ($P \leq 0.05$). It should be stressed that analysed animals were of *CC* and *CT* genotype of the *RYR1* locus. Most profitable values of above mentioned parameters in stress resistant (*CC*) group of fatteners were noted.

A significant influence of *CAST* gene polymorphism identified with *Msp*I enzyme (*CAST/Msp*I) was noted for R_1 coefficient value, drip loss from muscle tissue at 48 and 96 h *post mortem* ($P \leq 0.01$) and for lactate level, WHC and protein and water content ($P \leq 0.05$).

Fig. 1. Differences between mean values of meat quality traits of animals with AA and BB genotypes at the CAST/MspI locus, expressed in SD units of the traits



1-Lactate level; 2-R_1; 3-WHC; 4-drip loss at 48h; 5-drip loss at 96h; 6-% of protein; 7-% of water in LL muscle

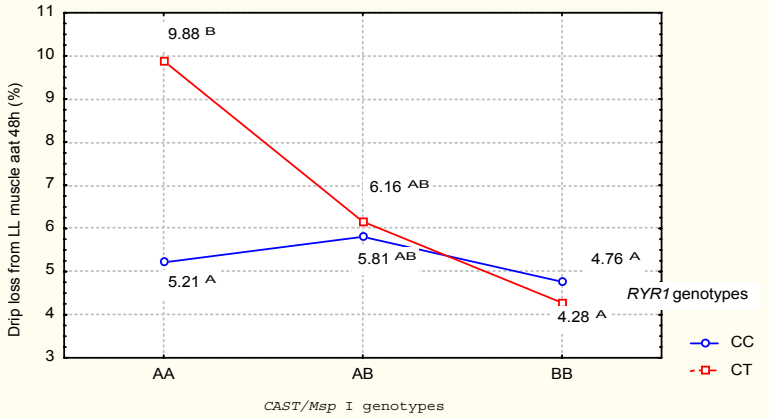
We have observed that animals with *BB* genotype at this locus were characterised by most profitable values of all these traits. High and significant influence of *CAST/Msp*I gene on drip loss from muscle tissue obtained in these investigations is especially interesting, taking into consideration important problem concerning a high variation of this trait (from 2 to 16%) signalised by several authors (Honkavaara 1997, Bertram et al. 2000, Schäfer et al. 2002).

Among analysed meat quality traits affected by *CAST* genotype, differences between average phenotypic values of *AA* and *BB* homozygotes in relation to *CAST/MspI* locus were close to 1 SD unit for drip loss at 48 h as well as at 96 h *post mortem* (0,92 and 0,96 SD respectively) (Fig. 1).

This indicates that *CAST/Msp*I genotype has an effect close to the major effect for drip loss from *LL* muscle tissue.

Statistically significant interaction between *RYR1* and *CAST/Msp*I loci was noted for: drip loss at 48 h post mortem ($P \leq 0.01$) (Fig. 2), and pH_{24} of *LL* muscle ($P \leq 0.05$) (Fig. 3). Value of drip loss at 48 h *post mortem* was differentiated between *CC* and *CT* animals at *RYR1* locus being *AA* homozygotes at *CAST/Msp*I locus. Among animals of *CT* genotype at *RYR1* locus carrying *AA* or *BB* genotypes at *CAST/Msp*I locus drip loss at 48 h *post mortem* differed significantly (9,88 and 4,28% respectively). Conducted additionally one-way ANOVA analysis showed that animals with *AA*/*CT* genotype at *CAST/MspI* and *RYR1* loci, respectively, showed significantly highest drip loss at 48 h *post mortem* (9,88 %), higher drip loss at 96 h and R_1 value, worse WHC and protein content than *BB/CT* animals, whereas meatiness of these two groups did not statistically differ.

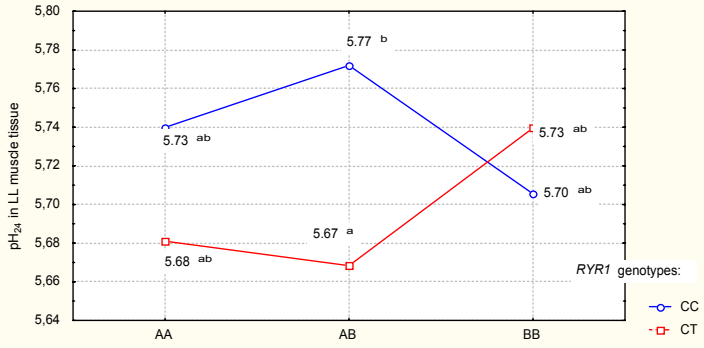
Fig. 2. Interactive effect of *CAST/Msp*I and *RYR1* genotypes for drip loss from LL muscle at 48h



Explanations: A,B mean values showed at the plot, signed by different capital letter differ significantly at P<

It is known that animals with *TT* or *CT* genotype at the *RYR1* locus show higher level of Ca^{2+} ions released from cells as a result of defective functioning of calcium channels under stress conditions. Next, proteolytic activity of calpain system (calpains and their inhibitor - calpastatin) is significantly dependent on Ca^{2+} ions level. One should suppose that different variants of calpastatin conditioned by polymorphism of *CAST* gene could have different sensibility to level of Ca^{2+} ions, and the same different activity stopping proteolytic activity of calpain. This may explain the differences in drip loss and pH values noted between fatteners with the same *RYR1* genotype but differentiated by *CAST/Msp*I genotypes.

Fig. 3. Interactive effect of *Cast/Msp* I and *RYR1* genotypes for pH_{24} in LL muscle tissue



Explanations: a, b mean values showed at the plot, signed by different small letter differ significantly at P<=0.05

Investigations carried out by Koćwin-Podsiadła et al. (2003) in similar scheme but only on (Polish Large White x Polish Landrace) x (Hampshire x Pietrain) crossbreeds showed significant interaction between *RYR1* and *CAST/Hinf*I genotypes for drip loss from *Longissimus Lumborum* muscle at 48 h *post mortem*.

Ciobanu et al. (2002) confirmed the effect of polymorphism of *CAST* gene identified with *Hpy*188I and *Pvu*II restriction enzymes on drip loss.

The polymorphisms of the *CAST* gene genotyped in this study were located in intron 7 and it is difficult to conclude their effect on calpastatin level or activity. The effect of analysed mutations on meat quality traits may be due to the linkage to any other mutation within the coding or regulatory regions of the *CAST* gene being the causal mutation.

Conclusions

In analysed fatteners' population highly significant influence of *RYR1* gene polymorphism on pH_{35}, pH_{45}, R_1 values that are basis of PSE meat classification and also on lactate level in *Longissimus Lumborum* muscle was noted.

Fatteners with *BB* genotype at the *Cast/Msp*I locus by lower lactate level (7.59 µmol/g), R_1 value (0.05), WHC (0.93 cm^2), drip loss at 48 h (3.03%) and at 96 h *post mortem* (3.36%) values, lower water content (1.31%) and also by about 0.5% higher protein content were characterized in comparison to fatteners with *AA* genotype at this locus. Relationships between polymorphism at the *CAST* locus and some meat quality traits, especially the high effect of *CAST/Msp*I polymorphism on drip loss of muscle tissue at 48 h as well as at 96 h *post mortem* (0,92 and 0,96 SD respectively) suggest that effect of the *CAST* gene is close to a major effect for this trait.

Interactions between genotypes *CAST/Msp*I and *RYR1* indicate that quality of meat influenced by *RYR1* genotype may be modifying by simultaneous influence of genotype as regards *CAST* locus. The polymorphism at the *CAST/Msp*I locus was closely related with drip loss from LL muscle tissue at 48 h among animals with *CT* genotype at the *RYR1* locus (with similar meat content in carcass) whereas this relationship among stress resistant (*CC*) animals was not confirm.

Obtained interesting results indicate that relationships between polymorphism of *CAST* gene and meat quality of pigs should be further investigated.

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