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The influence of *CAST/Rsa*I and *RYR1* genotypes and their interactions on selected meat quality parametres in three groups of four-breed fatteners with different meat content of carcass*

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The effect was analysed of *CAST* and *RYR1* genotypes and their interactions on selected LL muscle quality traits in 201 fatteners of three four-breed crossbred groups. *CAST/Rsa*I 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/Rsa*I genotype, animals of *AA* genotype were characterized by most desirable values.
Significant interactions between *CAST/RsaI* and *RYR1* 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 / *RYR1*

Up to year 2000 only an interactive effects of major genes (*RYR1* 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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and effect of candidate genes [Emnet *et al*. 2000, Malek *et al*. 2001] and their interactions [Koćwin-Podsiadła *et al*. 2003].

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 *Hpy*188I and *Pvu*II 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 *Hin*P1 for *RYR1* gene and *Rsa*I 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/Rsa*I 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 lumborum* (LL) muscle on the processing line (directly in carcass, in the region of the last rib) 35 min *post mortem* (pH_{35}) and in water homogenate of muscle tissue at 45 min (pH_{45}). R_1 – 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 Niniivaara [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

determined. Meat lightness and pH_{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 *Rsa*I 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;
- μ – 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/Rsa*I *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 ($P \leq 0.01$) influence of *RYR1* genotype on lactate level, pH_{35} and pH_{45} and significant on R_1 value ($P \leq 0.05$) were observed. It should be stressed that

Table 1. The frequency of genotypes and alleles at the *CAST/Rsa*I *locus*

RYR1	*CAST/Rsa*I (n=201)		
	AA	*AB*	*BB*
CC (n=114)	20	55	39
CT (n=87)	26	38	23
Total	46	93	62

Frequency of *CAST/Rsa*I alleles (%)	
A	*B*
46.02	53.98

Frequency of *CAST/Rsa*I genotypes (%)			
	AA	*AB*	*BB*
Observed	22.89	46.27	30.84
Expected*	21.18	49.68	29.14
P value	ns	ns	ns

*According to Hardy-Weinberg equilibrium.
ns – difference between observed and expected frequency of genotype not significant.

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_{35}, $pH_{45,}$ R_1 values that are basis of PSE meat classification. As expected, the most desirable values of above mentioned parametres were noted in stress-resistant (*CC*) group of fatteners (Tab. 2).

A significant influence (P≤0.05) of *CAST/Rsa*I 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/Rsa*I genotype shows that *AA* animals were characterized by lower WHC (by 0.90 cm^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/Rsa*I *loci* was noted for pH_{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/Rsa*I and *RYR1* genotype and group of meat content of carcass (Tab.2).

Interaction between all analysed factors (*CAST/Rsa*I, *RYR1* and group of meat content) was significant (P≤0.01) for pH_{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

Table 1. The relationship between polymorphisms at RYR1 and CAST/RsaI loci and muscle meat quality traits in pigs

Trait	CAST/RsaI genotypes: AA	AB	BB	sign.	RYR1 genotypes: CC	CT	sign.	Breed genotypes: <75%	75–87.5%	>87.5%	sign.	Interactions: RYR1 × CAST/RsaI	CAST/RsaI × BG	RYR1 × BG	RYR1 × CAST/RsaI × BG
Lactate level (µmol/g)	[illegible]	[illegible]	[illegible]	ns	[illegible]	[illegible]	**	[illegible]	[illegible]	[illegible]	ns	ns	ns	ns	ns
Glycogen (µmol/g)	[illegible]	[illegible]	[illegible]	ns	[illegible]	[illegible]	ns	[illegible]	[illegible]	[illegible]	ns	ns	ns	ns	ns
Glycolytic potential (µmol/g)	[illegible]	[illegible]	[illegible]	ns	[illegible]	[illegible]	ns	[illegible]	[illegible]	[illegible]	ns	ns	ns	ns	ns
pH_{45} LL	[illegible]	[illegible]	[illegible]	ns	[illegible]	[illegible]	*	[illegible]	[illegible]	[illegible]	ns	ns	ns	ns	*
pH_{3} LL	[illegible]	[illegible]	[illegible]	ns	[illegible]	[illegible]	**	[illegible]	[illegible]	[illegible]	ns	*	ns	ns	ns
pH_{24} LL	[illegible]	[illegible]	[illegible]	ns	[illegible]	[illegible]	ns	[illegible]	[illegible]	[illegible]	***	ns	ns	ns	ns
pH_{24} SM	[illegible]	[illegible]	[illegible]	ns	[illegible]	[illegible]	ns	[illegible]	[illegible]	[illegible]	***	ns	ns	ns	ns
R_1	[illegible]	[illegible]	[illegible]	ns	[illegible]	[illegible]	*	[illegible]	[illegible]	[illegible]	ns	ns	ns	ns	ns
Meat lightness (L*) in muscle LL	[illegible]	[illegible]	[illegible]	ns	[illegible]	[illegible]	ns	[illegible]	[illegible]	[illegible]	**	ns	ns	ns	ns
Meat lightness (L*) in muscle SM	[illegible]	[illegible]	[illegible]	ns	[illegible]	[illegible]	ns	[illegible]	[illegible]	[illegible]	**	ns	ns	ns	ns
Water holding capacity (cm²)	[illegible]	[illegible]	[illegible]	*	[illegible]	[illegible]	ns	[illegible]	[illegible]	[illegible]	***	ns	ns	ns	ns
Losses in cooking of meat (%)	[illegible]	[illegible]	[illegible]	ns	[illegible]	[illegible]	ns	[illegible]	[illegible]	[illegible]	ns	ns	ns	ns	ns
Drip loss at 48 h (%)	[illegible]	[illegible]	[illegible]	ns	[illegible]	[illegible]	ns	[illegible]	[illegible]	[illegible]	ns	*	ns	ns	ns

Table 1 Continued

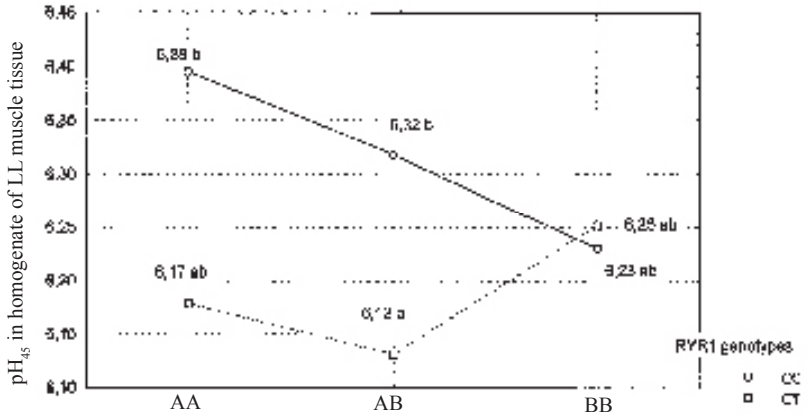

Fig. 1. Interactive effect of *CAST/Rsa*I and *RYR1* genotypes for pH_{45} in homogenate of LL muscle tissue.

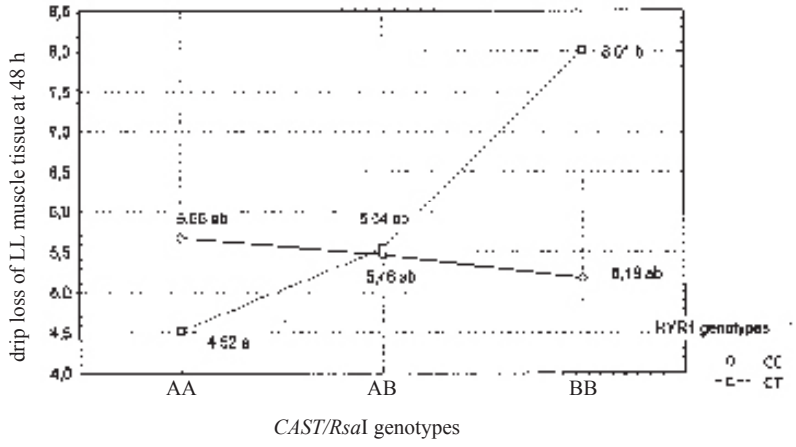

Fig. 2. Interactive effect of *CAST/Rsa*I and *RYR1* genotypes for drip loss of LL muscle tissue at 48 h.

of these proteases regulated by calpastatin and it may explain the effect of *CAST/Rsa*I × *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/Rsa*I *locus* was about 3.5 pp higher than that in group of the same *RYR1* genotype but *AA* homozygous at *CAST/Rsa*I *locus* (4.52% and 8.01% respectively).

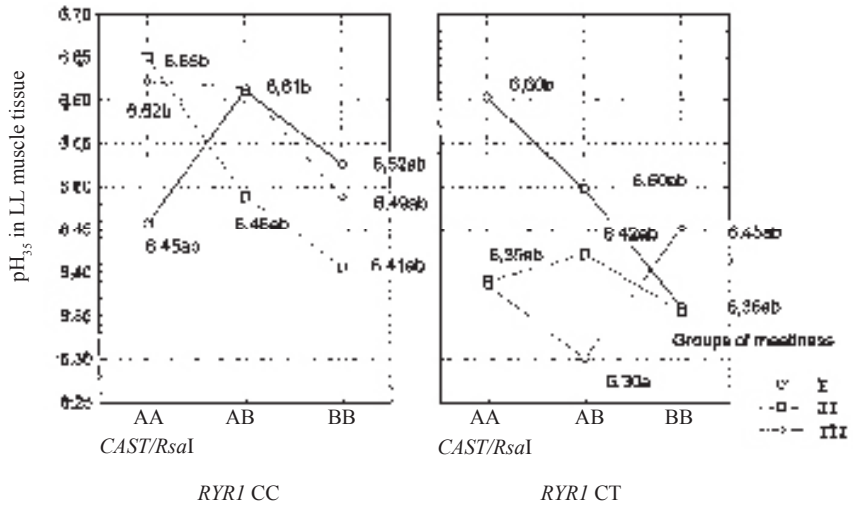

Fig. 3. Interactive effect of *CAST/Rsa*I and *RYR1* genotypes with group of meatiness for pH_{35} in LL muscle tissue.

Obtained interaction between *CAST/Rsa*I 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 pH_{35} of LL muscle among animals with meat content above 55%, being *AB* heterozygotes at *CAST/Rsa*I *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.

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Wpływ genotypów *CAST/Rsa*I 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*/*Rsa*I i *RYR1* oraz ich interakcji na cechy jakości mięśnia *longissimus lumborum*. Badaniami objęto 201 tuczników trzech grup czterorasowych mieszańców. Genotyp *CAST*/*Rsa*I 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*/*Rsa*I 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*.