Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 55 (2), 2016: 253–260
(Ann. Warsaw Univ. Life Sci. – SGGW, Anim. Sci. 55 (2), 2016)

Association of *CAST* and *RYR1* genes polymorphism with carcass and meat quality in crossbreed pigs with a share of Pietrain breed

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Abstract: *Association of* CAST *and* RYR1 *genes polymorphism with carcass and meat quality in crossbreed pigs with a share of Pietrain breed.* The aim of this study is to determine the effect of the calpastatin (*CAST*/*Taq*I) and ryanodine receptor (*RYR1*) genes polymorphism on carcass and meat quality traits in Pietrain crossbred pigs. The polymorphism in *CAST* and *RYR1* genes was detected using the PCR-RFLP (Restriction Fragment Length Polymorphism Analysis of PCR--Amplified Fragments) method. Two alleles of *CAST* gene were identified – *A* (0.34) and *B* (0.66) and three genotypes – *AA* (0.21), *AB* (0.25) and *BB* (0.54). In relation to carcass and pork quality, no statistically significant differences were found between the *CT* and *CC* genotypes of *RYR1* gene as well as between *AA*, *AB* and *BB* genotypes of *CAST* gene. In addition, no significant interaction was found between *CAST*/*Taq*I × *RYR1* genotypes and all the analyzed carcass and meat quality traits.

Key words: *CAST*, *RYR1*, meat quality, slaughter value, pigs

INTRODUCTION

Work on improving the quality of the pig carcass has been carried out for many years. The most important aspect is to increase the proportion of meat in the carcass in the way that does not decrease the quality of the meat. For example, slaughter performance can be improved by the use of pigs with high meat content, mainly the Pietrain breed. However, intensive breeding work for improvement carcass quality in the crossbred offspring of Pietrain boars has revealed a number of problems, particularly related to the high frequency of the $RYR1^T$ allele in this breed, which causes the occurrence of PSE (pale, soft, exudative) meat (Fiedler et al. 2001).

Calpastatin (*CAST*) is a specific inhibitor of calpain, a Ca^{2+} activated protease family and is considered to be responsible for the initiation of myofibrillar protein degradation in living muscle (Murachi 1989). The calpain system may also affect the number of skeletal muscle cells in domestic animals by altering the rate of myoblast proliferation and by modulating myoblast fusion. An increased rate of skeletal muscle growth can result from a decreased rate of muscle protein degradation, and this is associated with a decrease in activity of the calpain system, principally due to a large increase in calpastatin activity (Goll et al. 1998). The activity of calpastatin is strongly associated with muscle growth rate as well as with the rate of post mor-

tem proteolytic changes that make the meat tender. This is the reason why this protein is so important in relation of pork quality traits (Koćwin-Podsiadła and Kurył 2003, Melody et al. 2004).

The calpastatin gene has been mapped near the centromere of chromosome 2 (SSC2) in the region q2.1-q2.4. The calpastatin molecule consists of L domain, encoded by exons 2–8, and four repetitive domains, each of which is encoded by exons 9–14 (Stearns et al. 2005). Polymorphisms in the calpastatin gene (*CAST*), identified in the sixth intron with three restriction enzymes (*Hinf*I, *Msp*I, and *Rsa*I) were first reported by Ernst et al. (1998). Further analysis showed that these polymorphisms are associated with meat colour, pH, water holding-capacity (WHC) and texture parameters measured in the *longissimus dorsi* and *semimembranosus* muscles in four purebreds used in Polish breeding programmes and one conservative breed (Ropka-Molik et al. 2014).

Ciobanu et al. (2004), however identified a *CAST* gene polymorphism in domains L, 1, and 4, recognized by *Apa*LI, *Hpy*188I, and *Pvu*II enzymes, respectively. After sequencing the 1991bp DNA fragment of the *CAST* gene, a novel polymorphism – C/T transition was also detected in intron 24, which is recognized by *Taq*I enzyme (Wang et al. 1997). To date, it is the only paper containing data concerning *CAST*/*Taq*I genotypes frequency in six pig populations, without presenting their relation to production traits.

Previous studies also indicated that the ryanodine receptor gene (*RYR1*) variants significantly influence the carcass and meat quality traits (Urbański et al. 2013) and could interact with other genes.

The aim of this study is to determine the relationship between calpastatin gene (*CAST*/*Taq*I) and the ryanodine receptor gene (*RYR1*) polymorphism and the carcass and meat quality traits in the crossbred offspring of Pietrain boars.

MATERIAL AND METHODS

Offspring were obtained by crossing German Landrace × German Large White and also Leicoma × German Large White sows with Pietrain boars. The study was carried out on 125 pigs (76 gilts and 49 barrows) from a pig farm located in Mecklenburg-Vorpommern (Germany). All the animals used in the study were kept under similar environmental conditions and fed with a balanced mix of feed *ad libitum*. All the test animals were assembled into one group and taken to the meat plant in Szczecin (Poland) in the evening (4 h transport over a distance of 250 km), and slaughtered the next day in the morning (lairage time – 12 h). After CO_2 stunning, blood was collected from pigs to extract DNA for the identification of the *CAST* and *RYR1* genotypes. Subsequently, following traits were measured: carcass percentage of lean meat, hot carcass weight, thickness of the *longissimus dorsi* muscle (LD) and the back fat between the third and fourth last ribs (7 cm laterally from the carcass split line, on the left-hand side of the carcass, by means of an CGM optic-needle apparatus (Sydel, France).

Two hours after slaughter, during carcass cooling, electric conductivity (EC_2) was measured in the *longissimus dorsi* muscle, between the fourth and fifth

lumbar vertebrae of the right-hand side of the carcass using an LF-Star MATTHÄUS conductometer. After 24 h of carcass cooling, meat samples from the *longissimus dorsi* muscle were collected from the first to fourth, *lumbar vertebrae* section (*longissimus lumborum* – LL) of the right-hand side of the carcass. 24--hour *post mortem*, the meat pH_{24} value (Elmetron CP-411 pH-meter) and the volume of drip loss from the muscle tissue were determined according to Honikel (1987).

Within 48 h *post mortem*, minced samples of LL muscle were measured for pH in a water solution (pH_{48}), and the pork colour parameters, i.e. L* (lightness), a* (redness) and b* (yellowness), were established by means of a Hunter-Lab Mini Scan XE Plus 45/0 with D65 light illuminant and 10° observer. The meat water-holding capacity (WHC) was determined according to Grau and Hamm (1952) as modified by Pohja and Niinivaara (1957). Thermal drip was calculated as the difference of the meat sample weight before and after heating in a water bath at 85°C for 10 min. The water-soluble protein content was determined by the Kotik method (1974). The basic meat chemical composition was estimated in the meat, i.e. total protein, fat, ash and dry matter (AOAC 2003).

Genomic DNA was extracted from the blood sample using a Master Pure Kit (Epicentre Technologies). Genotypes of *RYR1* and *CAST* were identified by PCR--RFLP method. The *CAST* genotypes were identified according to the method used by Wang et al. (1997) by use following primer sequences: *forward* 5'-GTGATGACAAAAAACTTGACG-3' *reverse* 5'-TCATCCTTATCCAAGAGATGTC-3'. After digestion with 3U of the *Taq*I endonuclease (37°C/overnight) and electrophoresis in 2% agarose gels *CAST* genotypes were identified based on following restriction fragments length: *AA* – 963, 806 and 222bp; *AB* – 1028, 963, 806 and 222bp; *BB* – 1028 and 963bp. The *RYR1* genotypes were identified with following primers given by Brenig and Brem (1992): *forward* 5'-GTGCTGGATGTCCTGTGTTCCCT-3' *reverse* 5'-CTGGTGACATAGTTGATGAGGTTTG-3'. Digestion of amplicons with *Hin*6I restriction enzyme (37°C/overnight) and separation in 3% agarose gels allowed to identification two of three genotypes: *CC* – 84 and 50bp; *CT* – 134, 84 and 50bp.

Statistical analysis was performed to compare carcass and meat quality traits and also the meat chemical composition between pigs with the different *CAST* and *RYR1* genotypes using the least squares method of the GLM procedure (Statistica 9.1 PL) according to the following linear model:

$$Y_{ijkl} = \mu + a_i + b_j + c_k + bc_{jk} + e_{ijkl}$$

where:
Y_{ijkl} – trait measured;
μ – overall mean;
a_i – effect of sex ($i = 1, 2$);
b_j – effect of the *RYR1* genotype ($j = CT, CC$);
c_k – effect of the *CAST/Taq*I genotype ($k = AA, AB, BB$);
bc_{jk} – interaction (*RYR1* × *CAST/Taq*I genotype)
e_{ijkl} – random error.

A detailed comparison of the mean least squares (*LSQ*) for the analysed *CAST* and *RYR1* genotypes was conducted using a Tukey's test.

RESULTS AND DISCUSSION

The frequency of the alleles and genotypes of *CAST*/*Taq*I and *RYR1* genes in Pietrain-sired pigs are presented in Table 1. Two different alleles of *CAST* gene were identified – allele *A* (0.34) and *B* (0.66) and three genotypes – *AA* (0.21), *AB* (0.25) and *BB* (0.54). Two genotypes of *RYR1* gene, however were observed – *CT* (0.43) and *CC* (0.57).

The association between the genotypes of *CAST*/*Taq*I and *RYR1* and carcass and meat quality traits of the pigs are presented in Tables 2, 3 and 4. We did not find any significant differences between the genotypes *AA*, *AB*, and *BB* of the *CAST* in relation to 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. Moreover, no significant effect of the *RYR1* polymorphism on carcass and meat quality or meat basic chemical composition was observed.

Based on the conducted research on the analyzed groups of pigs we found all three possible *CAST* genotypes. The presence of the three *CAST*/*Taq*I genotypes was also observed by Wang et al. (2007) in Sutai pigs, PIC hybrids, and in crosses of Duroc, Landrace and Yorkshire. Similar to present study, the *BB* genotype characterized higher frequency followed by *AB* then *AA* genotypes. In the Yorkshire × Sutai crosses the above mentioned authors noticed a higher frequency of heterozygotes (*AB*) than homozygotes, while in the Meishan breed

TABLE 1. The frequency of *CAST* and *RYR1* alleles and genotypes in analyzed pigs

Polymorphism	Allele frequency		Genotype frequency		
CAST/*Taq*I	*A*	*B*	*AA*	*AB*	*BB*
	0.34	0.66	0.21	0.25	0.54
RYR1/*Hin*6I	*C*	*T*	*CC*	*CT*	*TT*
	0.21	0.79	0.57	0.43	–

TABLE 2. Effect of the *CAST*/*Taq*I and *RYR1* genotypes on carcass quality traits in pigs

Trait	*CAST*/*Taq*I genotypes			*RYR1* genotypes		Significance of interaction influence
	AA	*AB*	*BB*	*CC*	*CT*	
Hot carcass weight (kg)	88.66 ±7.14	86.44 ±5.52	87.99 ±5.92	87.88 ±6.06	87.83 ±6.45	n.s.
Meatiness (%)	54.44 ±4.54	55.70 ±4.20	55.63 ±4.54	55.05 ±4.91	55.83 ±3.73	n.s.
Backfat thickness (mm)	15.85 ±4.36	14.65 ±4.28	14.64 ±4.25	15.39 ±4.60	14.27 ±3.71	n.s.
Thickness muscle (mm)	54.81 ±6.13	57.39 ±6.53	57.01 ±6.57	56.75 ±7.21	56.51 ±5.40	n.s.

n.s. – statistically not significant.

TABLE 3. Effect of the *CAST*/*Taq*I and *RYR1* genotypes on meat quality traits in pigs

Trait	*CAST*/*Taq*I genotypes			*RYR1* genotypes		Significance of interaction influence
	AA	*AB*	*BB*	*CC*	*CT*	
pH_{24}	5.63 ±0.10	5.63 ±0.13	5.69 ±0.17	5.68 ±0.14	5.64 ±0.16	n.s.
pH_{48}	5.53 ±0.09	5.54 ±0.12	5.60 ±0.19	5.58 ±0.14	5.56 ±0.18	n.s.
EC_2 (mS/cm)	3.05 ±1.46	3.08 ±1.57	3.12 ±1.26	2.98 ±1.35	3.22 ±1.41	n.s.
L*	55.14 ±3.39	55.12 ±3.29	54.40 ±3.48	54.59 ±3.09	54.94 ±3.77	n.s.
a*	9.45 ±1.41	9.44 ±1.34	9.22 ±1.22	9.42 ±1.23	9.26 ±1.33	n.s.
b*	17.10 ±1.28	17.05 ±1.27	16.57 ±1.37	16.76 ±1.26	16.88 ±1.44	n.s.
Drip loss (%)	7.93 ±2.70	8.30 ±2.36	7.26 ±2.62	7.67 ±2.51	7.63 ±2.71	n.s.
WHC (% of free water)	18.35 ±5.55	18.19 ±4.63	16.71 ±4.87	17.00 ±4.62	17.96 ±5.39	n.s.
Thermal drip (%)	25.83 ±3.27	26.78 ±2.74	25.49 ±2.69	25.48 ±2.68	26.41 ±3.01	n.s.
Water-soluble protein (%)	8.38 ±0.90	8.03 ±1.01	8.24 ±0.93	8.27 ±0.89	8.15 ±1.00	n.s.

n.s. – statistically not significant.

TABLE 4. Effect of the *CAST*/*Taq*I and *RYR1* genotypes on basic chemical composition of meat in pigs

Trait	*CAST*/*Taq*I genotypes			*RYR1* genotypes		Significance of interaction influence
	AA	*AB*	*BB*	*CC*	*CT*	
Total protein (%)	22.51 ±0.57	22.35 ±0.80	22.39 ±0.69	22.34 ±0.74	22.47 ±0.63	n.s.
Fat (%)	2.51 ±0.55	2.46 ±0.52	2.54 ±0.66	2.50 ±0.55	2.55 ±0.66	n.s.
Ash (%)	1.19 ±0.07	1.18 ±0.07	1.18 ±0.08	1.18 ±0.07	1.18 ±0.08	n.s.
Dry matter (%)	26.21 ±0.66	26.00 ±0.71	26.11 ±0.79	26.02 ±0.73	26.21 ±0.74	n.s.

n.s. – statistically not significant.

they found no *AA* homozygotes while in Landrace × Sutai crosses no *BB* homozygotes. In the analyzed offspring of the Pietrain boars we found two *RYR1* genotypes – *CT* and *CC*, which is consistent with the approved German programme of animals qualification for crossing, where the sows should be free from the susceptibility to stress allele ($RYR1^T$), while boars do not (Rosner et al. 2003).

In the present study *CAST*/*Taq*I genotypes had no effect on the carcass quality traits, meat content, backfat thickness and LD muscle thickness at a similar warm carcasses weight. Kluzáková et al. (2014) found a significant effect of the *CAST*/*Hinf*I variants on lean meat share (i.e. lower fat content) and *CAST*/*Msp*I variants on lean meat share due to higher proportions of muscles mass in the main meat parts. Concerning the *CAST*/*Rsa*I polymorphism, the study showed that this polymorphism does not influence any of the quantitative parameters under analysis.

In our study we found no relation between the *CAST*/*Taq*I gene polymorphism and meat quality traits and its basic chemical composition. Other study on the offspring of Hampshire × Pietrain boars showed associations between *CAST*/*/Rsa*I polymorphism and the concentration of glycogen and glycolytic potential of the LL muscle, between *CAST/Hinf*I and pH_{45} as well as between *CAST*/*Msp*I and loin weight in the curing process (Koćwin-Podsiadła et al. 2003). Study of Ropka-Molik et al. (2014), performed on the common breeds maintained in Poland suggests that the most informative polymorphisms in *CAST* gene are *CAST*/*Hpa*II and *CAST*/*Rsa*I. They had the significant effect on WHC regardless of the breed analyzed and on meat pH, firmness and toughness for most breeds. Interestingly for almost all breeds, the significant effect of both polymorphism on intramuscular fat content (IMF) was observed.

In the present study on the offspring of Pietrain boars, there were no statistically significant differences between the *CC* and *CT* genotypes of the gene *RYR1* regarding carcass and meat quality traits or the basic chemical composition of the meat, which was also confirmed in the research of Koćwin-Podsiadła et al. (2003). Other studies on the offspring of Pietrain boars, however showed that individuals with the *CT* genotype had a poor quality of meat compared to pigs with the *CC* genotype, which is reflected in a higher percentage of carcasses with PSE meat (Krzęcio et al. 2005).

We found no significant interactions between the genotypes of *RYR1* and *CAST*/*Taq*I in values of carcass and meat quality traits, nor in the basic chemical composition of the meat in the analyzed population of pigs. In studies on crossbred Duroc × Pietrain or Hampshire × × Pietrain boars, the interactions between the *CAST* and *RYR1* genotypes were significant for muscle acidity (pH_{24}) and drip loss (Kurył et al. 2004, Krzęcio et al. 2005). In the study of Kluzáková et al. (2012) an important interaction was found between the identified polymorphisms *CAST*/*Hinf*I, *CAST*/*Msp*I, *CAST*/*/Rsa*I and the *RYR1* gene in relation to lean meat, ham share and main meat parts as well as meat quality traits, namely for pH_{45} and drip loss. The results obtained by Kurył et al. (2004) in Pietrain crossbred pigs showed that the presence of meat with a significant drip loss and

a low water-holding capacity in pigs with *CC* genotype of *RYR1* gene, as well as the traits of meat quality in animals with *TT* genotype may results from modified impact of the *CAST* gene on *post mortem* changes in muscle.

To sum up, in the offspring of Pietrain boars we found no significant differences between the identified genotypes of *CAST*/*Taq*I (*AA*, *AB*, *BB*) and *RYR1* (*CC*, *CT*) in relation to the carcass and meat quality traits, nor the basic chemical composition of the meat. Moreover, we did not find any significant interaction between the *CAST*/*Taq*I and *RYR1* genes in relation to the quantitative and qualitative indicators of carcass value in the selected pig crossbreds.

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Streszczenie: *Zależność polimorfizmu genów* CAST *i* RYR1 *z cechami jakości tuszy i mięsa u świń mieszańców z udziałem rasy pietrain*. Celem niniejszych badań jest określenie wpływu polimorfizmu genów kalpastatyny (*CAST*/*Taq*I) i receptora ryanodiny (*RYR1*) na cechy jakości tuszy i mięsa u świń mieszańców rasy pietrain. Polimorfizm genów *CAST i RYR1* określono za pomocą metody PCR-RFLP (analiza polimorfizmu fragmentów restrykcyjnych amplifikowanych metodą PCR). Zidentyfikowano dwie allele – *A* (0,34) i *B* (0,66) oraz trzy genotypy genu *CAST* – *AA* (0,21), *AB* (0,25) i *BB* (0,54). W odniesieniu do jakości tuszy i mięsa nie zaobserwowano statystycznie istotnych różnic pomiędzy genotypami *CT* i *CC* genu *RYR1* jak również genotypami *AA*, *AB* i *BB* genu *CAST*. Dodatkowo nie stwierdzono istotnych interakcji między genotypami *CAST*/*Taq*I × *RYR1*, a wszystkimi ocenianymi cechami jakości tuszy i mięsa.

Słowa kluczowe: *CAST*, *RYR1*, jakość mięsa, wartość rzeźna, świnie

MS received 10.06.2016
MS accepted 05.09.2016

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