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The effect of *CAST* and *RYR1* polymorphisms on carcass and meat quality traits in Pietrain crossbred pigs

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The aim of this study was to determine the effect of the calpastatin (*CAST*) and ryanodine receptor (*RYR1*) genes polymorphism on carcass and meat quality traits in Pietrain crossbred pigs. No significant differences in the traits examined were identified between pigs with the genotypes *CT* and *CC* at the *locus RYR1*. A significant association occurred between the polymorphisms *CAST*/PvuII and *CAST*/RsaI, and the traits characterizing the quality of carcass and composition of meat. Meat from pigs with the genotype *AB CAST*/*Pvu*II had a significantly higher pH determined 24 and 48 h *post mortem*, lower drip loss, lower yellowness (b*) and a lower protein content compared to meat from pigs with the genotype *AA*. In addition, the meat from pigs with the genotype *EF CAST*/*Rsa*I had a significantly higher pH 48 h *post mortem*, lower drip loss and lower yellowness (b*) than that of pigs with the genotype *EE*. The results indicate that several quality and composition traits of fresh meat from the offspring by Pietrain boars are significantly related to the *CAST* genotype.

KEY WORDS: carcass /*CAST* / meat quality /pigs /polymorphism / *RYR1* / slaughter value

Post mortem proteolysis of myofibrillar proteins is associated with the activity of the calpain system (-μ and -m calpain) and its inhibitor calpastatin [Goll *et al.* 1998, Sensky *et al.* 1999]. The activity of calpastatin correlates significantly with the rate of muscle growth, as well as the rate of proteolytic and *post mortem* tenderization

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[Goll *et al.* 1998]. Thus, calpastatin influences many traits of carcass and meat quality [Melody *et al.* 2004]. It has been documented that calcium channel activity is regulated by calpastatin domain L. The Ca2+ level in the skeletal muscle is also regulated by the *RYR1* gene. *RYR1* encodes the subunit of the Ca2+ release channel of the sarcoplasmic reticulum in the skeletal muscles, *i.e*. the ryanodine receptor [Fujii *et al.* 1991]. The C1843T point mutation in the *RYR1* gene is one of the reasons for the disrupted regulation of intracellular Ca2+ in pig skeletal muscle [Hao *et al.* 2000]. Polymorphism in the calpastatin gene (*CAST*), identified in intron 6 with the restriction enzymes *Hinf*I, *Msp*I and *Rsa*I, was first described by Ernst *et al*. [1998]. Ciobanu *et al.* [2004] identified polymorphisms in the domains L (involved in the reactivation of calcium channels), 1 and 4, which were identified by the enzymes *Apa*LI, *Hpy*188I and *Pvu*II, respectively. The mutations described by Ciobanu *et al*. [2004] cause a change in the amino acid sequence of each domain. Other studies show a significant impact of the polymorphism of *CAST* gene on carcass and meat quality [Koćwin-Podsiadła *et al.* 2003, Kurył *et al.* 2004]. The results obtained by Kurył *et al.* [2004] in Pietrain crossbred pigs show that the incidence of meat with a significant drip loss and a low water-holding capacity in pigs which are not carriers of mutated allele (*CC* at the *locus RYR1*), as well as the traits of meat quality in animals which are carriers of mutated allele (*TT* at the *locus RYR1*) may be the result of a modified impact of the *CAST* genotype on *post mortem* changes of muscle.

The aim of this study was to determine the association between the calpastatin gene (*CAST*/*Rsa*I and *CAST*/*Pvu*II) polymorphisms, the ryanodine receptor gene (*RYR1*) polymorphism and the traits of carcass and meat quality in the crossbred offspring of Pietrain boars.

Material and methods

The study was carried out on 125 porkers (76 gilts and 49 barrows) from a pig farm located in Mecklenburg-Vorpommern (Germany). It was based on the offspring produced by crossing German Landrace × German Large White and also Leicoma × German Large White sows with Pietrain boars. The animals were kept under similar environmental conditions and fed a balanced feed mix to appetite. All the test animals were assembled into one group and taken to the Meat Plant in Szczecin (Poland) in the evening (4 hours transportation over a distance of 250 km), and slaughtered on the next day in the morning (lairage time – 12 hrs). After stunning with CO_2 the pigs were slaughtered and blood was collected to extract DNA for the identification of the *CAST* and *RYR1* genotypes. Subsequently, the lean meat per cent of carcass was estimated as well as the hot carcass weight, the thickness of the *longissimus dorsi* muscle and of backfat between the 3rd and 4th last rib (7 cm laterally from the carcass split line, on the left-hand side of the carcass, with an optic-needle CGM apparatus (SYDEL, France). The mean per cent of lean meat amounted to 55.39% ±0.40 and the hot carcass weight to 87.75 kg ±0.55.

Two hrs after slaughter, during carcass cooling, electric conductivity (EC_2) was measured in the *longissimus dorsi* muscle, between the 4th and 5th lumbar vertebra of the right-hand side of the carcass using an LF-Star MATTHÄUS conductometer. After 24 hours of carcass cooling, meat samples from *longissimus dorsi* muscle were collected from the 1st to 4th lumbar vertebra section (*longissimus lumborum* – LL) of the right-hand side of the carcass. Twenty four hours *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 hours *post mortem*, minced samples of LL muscle were measured for pH in water (pH_{48}), and the colour parameters, (L* – lightness, a* – redness and b* – yellowness), were determined with a HunterLab Mini Scan XE Plus 45/0 with light illuminant D65 and observer 10°. 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 according to Kotik [1974]. The meat chemical composition (total protein, fat, ash and dry matter were determined according to AOAC [2003].

Genomic DNA was extracted from the blood sample using a Master Pure kit (EPICENTRE TECHNOLOGIES). Genotypes *RYR1*, *CAST*/*Rsa*I and *CAST/Pvu*II were identified by the PCR/RFLP method according to Fujii *et al.* [1991], Ernst *et al.* [1998] and Ciobanu *et al.* [2004], respectively.

Statistical evaluation aimed at comparing carcass and meat quality traits between pigs of different *CAST* and *RYR1* genotypes, using the least squares method of the GLM procedure (Statistica 9.0 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;
- 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/Rsa*I genotype (k = *EE*, *EF*) or the *CAST/Pvu*II genotype (k = *AA*, *AB*);
- bc_{jk} – interaction (*RYR1* × *CAST/ Rsa*I or *CAST/Pvu*II genotype);
- β – linear regression coefficient for hot carcass weight;
- x_{ijkl} – hot carcass weight of *ijkl*-th individual included as covariable;
- $\bar{x}$ – meean for hot carcass weight;
- e_{ijkl} – random error.

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

Results and discussion

Intensive work on the improvement of the carcass quality of crossbred offspring of Pietrain boars has revealed a number of problems, particularly related to the high frequency of the *RYR1 T* allele in that breed, which results in the occurrence of PSE (pale, soft, exudative) meat [Fiedler *et al.* 2001]. Accordingly, modern crossbreeding programmes involve the production of the crossbred offspring of sows without the *RYR1*^{T} allele and boars which exhibit high meat deposition but which are not always free of this allele [Rosner *et al.* 2003]. These problems were confirmed by the present study, in which the examined pigs were found to have two genotypes at: *CC* and *CT* (Tab. 1). However, there were no significant differences in the quality of carcass and meat quality and composition between *RYR1* genotypes (Tab. 2). This is inconsistent with the results of the other papers on the offspring of Pietrain boars

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

Item	*RYR1* *CC* (n = 71)	*RYR1* *CT* (n = 54)	Total	Frequency of genotypes (%)	Frequency of alleles
CAST/*Rsa*I					
EE	51	41	92	73.6	*E* = 0.87
EF	20	13	33	26.4	*F* = 0.13
CAST/*Pvu*II					
AA	62	46	108	86.4	*A* = 0.93
AB	9	8	17	13.6	*B* = 0.07

Table 2. Effect of *CAST* polymorphism on carcass and meat quality traits in pigs

Trait	LSM	SE	Significance of effect of the *CAST* genotype: *CAST/Rsa*I	*CAST/Pvu*II
Slaughter value indicators				
lean meat deposition (%)	55.39	0.39	ns	ns
backfat thickness (mm)	14.90	0.38	ns	ns
LL muscle thickness (mm)	56.64	0.58	ns	ns
Basic chemical composition of meat				
total protein (%)	22.40	0.06	ns	P≤0.01
fat (%)	2.52	0.05	ns	ns
ash (%)	1.18	0.01	ns	ns
dry matter (%)	26.10	0.07	ns	ns
Meat quality traits				
pH_{24}	5.66	0.01	ns	P≤0.05
pH_{48}	5.57	0.01	P≤0.05	P≤0.01
EC_2 (mS/cm)	3.08	0.12	ns	ns
L*	54.74	0.30	ns	ns
a*	9.33	0.11	ns	ns
b*	16.81	0.12	P≤0.01	P≤0.01
drip loss (%)	7.65	0.23	P≤0.05	P≤0.05
WHC (% of free water)	17.42	0.44	ns	ns
thermal drip (%)	25.88	0.25	ns	ns
water-soluble protein (%)	8.22	0.08	ns	ns

ns – not significant.

and their crossbreds [Koćwin-Podsiadła *et al.* 2003, Otto *et al.* 2007] which reported a higher quality of carcasses and also a lower quality of meat from heterozygous pigs (*CT*) compared to those with the *CC* genotype.

In this study, the analysis of the frequency of the genotypes *CAST/Rsa*I in the offspring of Pietrain boars revealed the presence of two genotypes: *EE* and *EF*, which have also been reported in TORHYB [Pietrain × (Polish Large White × Polish Landrace)] and in Polish Landrace pigs [Kurył *et al.* 2003, Kłosowska *et al.* 2005]. In the study by Kurył *et al.* [2003], Pietrain pigs were monomorphous at the l*ocus CAST/Rsa*I and had the genotype *EE*. All three possible genotypes were observed in Yorkshire and Large White pigs [Ernst *et al.* 1998], Stamboek pigs (Dutch Large White × Dutch Landrace) and Zlotnicka Spotted pigs [Kurył *et al.* 2003, Kłosowska *et al.* 2005].

The activity of calpastatin is highly significantly correlated with muscle growth, the rate of proteolytic changes and the *post-mortem* tenderization of meat [Kristensen *et al.* 2002]. As a result, the calpain-calpastatin system has a significant effect on the number of muscle fibres [Goll *et al.* 1998]. The accelerated growth of the skeletal muscles may be due to reduced protein degradation caused either by the reduced activity of calpain or by a significant increase in the activity of calpastatin, the inhibitor of calpain [Goll *et al.* 1998]. In this study, no association between the polymorphism of *CAST/Rsa*I and *CAST/Pvu*II and the carcass value was found. This is in contrast to other reports on crossbred pigs lacking the *RYR1*^{T} allele. In a study by Kurył *et al.* [2003] on Stamboek pigs a significant association was found between the polymorphism of *CAST/Rsa*I and backfat thickness at certain points of measurement, the surface of the eye muscle and meat content of carcass. In addition, Krzęcio *et al.* [2008] observed in crossbred pigs that the values of five out of 19 measured traits of meat deposition and the composition of the carcass were related to the genotype *CAST/Rsa*I.

The calpain-calpastatin system plays an important role in the *post mortem* proteolysis, thus affecting a number of meat quality traits, including tenderness, water-holding capacity and drip loss [Melody *et al.* 2004]. In the present study, a significant association between the polymorphism *CAST/Rsa*I and a value of certain traits of meat quality was found. The meat of pigs with the genotype *EF* had a significantly higher pH_{48}, lower drip loss ($P \leq 0.05$) and highly significantly lower yellowness (b*) compared to the meat of pigs with the genotype *EE* (Tab. 3). Koćwin-Podsiadła *et al.* [2006] and Krzęcio *et al.* [2008] demonstrated an association between the polymorphism in *CAST/Rsa*I and pH and drip loss during the storage of meat up to 144 h *post mortem*, electrical conductivity (EC), technological yield in the process of curing and heating, and the total protein content of body of pigs which were free of mutated allele at the *RYR1 locus* (*CC* genotype). Additionally, Kapelański *et al.* [2004] found that the polymorphism at the *locus CAST/Rsa*I affects the traits associated with water-holding capacity during the storage of meat and also those related to meat texture. The relationship between the polymorphism of the *CAST* gene (*CAST/Rsa*I) and pH and WHC, observed both in this and other studies, may be associated with the glycolytic potential and the traits of the *longissimus lumborum*

Table 3. The effect of the *CAST*/*Rsa*I and *CAST*/*Pvu*II genotypes on meat quality traits in pigs

Trait	*CAST*/*Rsa*I genotypes		*CAST*/*Pvu*II genotypes	
	EE	*EF*	*AA*	*AB*
No. of animals	92	33	108	17
Total protein (%)			22.47^{A}±0.07	21.99^{B}±0.16
pH_{24}			5.65^{a}±0.01	5.74^{b}±0.05
pH_{48}	5.55^{a}±0.01	5.63^{b}±0.04	5.56^{A}±0.01	5.68^{B}±0.06
Drip loss (%)	7.92^{a}±0.27	6.94^{b}±0.45	7.83^{a}±0.24	6.61^{b}±0.80
b*	17.00^{A}±0.13	16.26^{B}±0.26	16.93^{A}±0.12	16.02^{B}±1.39

$^{aA...}$Means in rows bearing different superscripts are significantly different: small letters – P≤0.05, capitals – P≤0.01.

(LL) muscle microstructure. Studies on Pietrain crossbred pigs showed an association between the *CAST/Rsa*I polymorphism and the concentration of glycogen and the glycolytic potential in the LL muscle [Koćwin-Podsiadła *et al.* 2003]. According to Kłosowska *et al.* [2005], the diameters of the STO (slow twitch oxidative), FTO (fast twitch oxidative) and FTG (fast twitch glycolytic) fibres, and also the percentage of FTG fibres and pathologically altered fibres in the crossbred offspring of Pietrain boars were related to the genotype at the *locus CAST/Rsa*I.

In one of the few studies on the association between the polymorphism of the *CAST* gene identified with the enzymes *Apa*LI, *Hpy*188I and *Pvu*II, and meat quality traits, Ciobanu *et al.* [2004] showed that the haplotypes *CAST/Hpy*188I-*CAST*/*Pvu*II have a significant impact on tenderness and the sensory attributes of porcine meat, as well as on the size of the drip loss from the *longissimus dorsi* muscle. In the present study there was also a significant association between the polymorphism *CAST/Pvu*II and meat quality traits and composition. Pigs with the *AB* genotype produced meat with significantly higher pH_{48}, lower yellowness (b*) and lower protein content (P≤0.01), higher pH_{24} and lower drip loss (P≤0.05) compared to those with the *AA* genotype. In a study by Škrlep *et al.* [2010], the polymorphism *CAST/Pvu*II affected the green colour grade of ham from French crossbred pigs. Stadler *et al.* [2005] showed that the *CAST/Pvu*II gene polymorphism was a source of significant variation in the moisture content of cured ham and tended to be a source of significant changes in yield, ham weight loss, salt content and Minolta colour.

In the present study, regardless of the genetic variant, a higher pH was accompanied by a significantly lower drip loss and lower yellowness of meat (b*). Huff-Lonergan *et al.* [2002] and Krzęcio *et al.* [2005] also demonstrated a significant inverse correlation between the ultimate (24 hrs and 48 hrs *post mortem*) pH in the *longissimus* muscle and drip loss (*r* = from -0.28 to -0.43). Brewer *et al.* [2001] found that a value of pH_{24} was significantly and negatively correlated with the colour parameter b* (*r* = -0.69) measured in *longissimus lumborum* and *longissimus thoracis* muscles.

In this study an influence of interaction between the genotypes *CAST* and *RYR1* (*CAST*/*Pvu*II × *RYR1* and *CAST*/*Rsa*I × *RYR1*) on carcass and meat quality traits appeared to nonsignificant. Other studies on Pietrain crossbred pigs have shown a significant effect of interaction between genotypes *CAST*/*Rsa*I and *RYR1* on drip loss from the LL muscle [Kurył *et al.* 2004], and pH_{45} [Koćwin-Podsiadła *et al.* 2003].

The results of the present study performed on the offspring of Pietrain boars and crossbred sows indicated that the differences between pigs with genotypes *CC* and *CT* at the *RYR1 locus* regarding a value of carcass and meat quality traits were not significant. Moreover, the *CAST* genotypes identified with the *Pvu*II and *Rsa*I endonucleases significantly influenced important fresh pork quality traits, *i.e.* pH, drip loss and yelowness (b*).

It is concluded that selecting pigs for a favourable *CAST* genotype would render it possible to control the quality of fresh meat.

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