Animal Science Papers and Reports vol. 28 (2010), no. 3, 253-260
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

The effect of calpastatin polymorphism (*CAST*/*Hinf*I and *CAST*/*Hpy*188I) and its interaction with *RYR1* genotypes on carcass and pork quality of crossbred pigs

Artur Rybarczyk[1]*, Marek Kmieć[2], Filip Napierała[2], Wanda Natalczyk-Szymkowska[1]

[1] Department of Evaluation of Livestock Products, Westpomeranian University of Technology, Doktora Judyma 24, 71-466 Szczecin, Poland

[2] Department of Genetics and Animal Breeding, Westpomeranian University of Technology, Doktora Judyma 6, 71-466 Szczecin, Poland

(Received January 9, 2010; accepted May 21, 2010)

The aim of the study was to recognize the polymorphism in the calpastatine genes (*CAST*/*Hinf*I and *CAST*/*Hpy*188I) and in the ryanodine receptor gene (*RYR1*) as well as to establish a possible linkage between the genes variants and carcass and pork quality traits in crossbreds of German Landrace × German Large White or Leicoma × German Large White sows with Pietrain boars. In terms of carcass and pork quality, no significant differences were found between the genotypes *CT* and *CC* at the *locus RYR1*, as well as between *AA* and *AB* genotypes at the *locus CAST*/*Hpy*188I. On the other hand, a significant effect was identified of the *CAST*/*Hinf*I polymorphism on pork quality traits. The meat of *AB* pigs showed a significantly higher pH, lower drip loss and thermal drip, lower WHC, and lower redness and yellowness of colour as compared to *BB* animals. Furthermore, a significant effect of interaction *CAST*/*Hinf*I × *RYR1* was found in relation to WHC of meat. The results presented indicate that the *CAST* gene polymorphism identified by *Hinf*I enzyme may be considered important in terms of meat quality traits of the analysed crossbreds. A follow-up study is necessary, however, involving a larger population that would represent all possible genetic variants of the *CAST*.

KEY WORDS: calpastatin / carcass / genotype / pigs / pork / *RYR1*

*Corresponding author: artur.rybarczyk@zut.edu.pl

Research shows that, despite belonging to very different breeds, pigs of the same genotype for the *RYR1* gene not only do exhibit a considerable variability in carcass lean content, but also provide pork of varying quality. This may be an effect of other genes that possibly affect both carcass traits and pork quality, modifying the effect of the *RYR1* [Koćwin-Podsiadła and Kurył 2003, Glodek *et al.* 2004]. The gene that has been in focus of many studies is the calpastatin gene (*CAST*), since the calpain–calpastatin system is important for the growth of skeletal muscles during the postnatal life. Active calpain is needed by myoblasts to fuse and by cells to proliferate and grow [Koohmaraie *et al.* 1991, Goll *et al*. 1998]. Activity of calpastatin is strongly associated with muscle growth rate as well as with the rate of *post mortem* proteolytic changes which make the meat tender; that is why it is important in terms of pork quality traits [Koćwin-Podsiadła *et al.* 2003, Melody *et al.* 2004].

The polymorphism in the *CAST* gene as identified in the intron with three restriction enzymes – *Hinf*I, *Msp*I, and *Rsa*I – was first described by Ernst *et al.* [1998]. Ciobanu *et al.* [2004] identified its polymorphism in domains L, 1, and 4 using enzymes *Apa*LI, *Hpy*188I, and *Pvu*II. The results published to date demonstrate a strong influence of the *CAST* gene variants on porcine carcass quality traits and pork quality [Kurył *et al.* 2004].

The aim of this study was to recognize the polymorphism in the calpastatin gene (*CAST*/*Hinf*I and *CAST*/*Hpy*188I) and in the ryanodine receptor gene (*RYR1*) and to establish a possible relation existing between the genes variants and carcass/pork quality traits in crossbred pigs sired by Pietrain boars.

Material and methods

The investigation was carried out on 125 porkers (76 gilts and 49 castrated males) coming from a pig-producing farm located in Mecklemburg-Vorpommern (Germany). The study comprised the offspring from crossing of German Landrace × German Large White or Leicoma × German Large White sows with Pietrain boars, which was kept under similar environmental conditions and fed with a balanced feed-mix *ad libitum*. All pigs destined for the study were conveyed by one means of transport to the "Agryf" Meat Plant in Szczecin (Poland) in the evening and slaughtered on the next day in the morning (about 12 h lairage time), after 4 h transportation from a distance of 250 km.

During the slaughter, after animals' stunning with CO_2, the blood was withdrawn for identification of *CAST* and *RYR1* genotypes. Subsequently, carcass meat deposition rate was measured, as well as the thickness of *longissimus dorsi* (LD) muscle and backfat between the 3rd and 4th rib, 6 cm from the line of carcass partition into sides, by means of optic-needle (CGM apparatus, Sydel, France), as well as hot carcass weight of pigs established. Mean per cent carcass meat deposition amounted to 55.39±0.40 and hot carcass weight to 87.75±0.55 kg.

During carcass cooling, two hours post slaughter, electric conductivity (EC_2) was measured in the LD muscle, between the 4th and 5th lumbar vertebra of the right carcass-side, using a LF-Star MATTHÄUS conductometer. After 24 hours carcass cooling, meat samples from the LD muscle were collected from 1-4 lumbar vertebrae section (LL) of the right carcass-side. Meat pH_{24} value (ELMETRON CP-311 pH-meter) and the drip loss from the muscle tissue were determined 48 h post-slaughter according to Honikel [1987].

About 48 hours post-slaughter, on the minced meat, pH in water suspension was determined and meat colour traits, *i.e*. L* (lightness), a* (redness) and b* (yellowness), were established by means of a HUNTER Lab Mini Scan XE Plus 45/0 with light illuminant D65 and observer 10° (CIE 1976), and meat water-holding capacity (WHC) was determined according to Grau and Hamm [1952] as modified by Pohja and Niinivaara [1957], as well as thermal drip from a difference of meat sample weight before and after heating in water bath at 85°C for 10 min. Water-soluble protein content was determined after Kotik [1974] and the basic meat chemical composition, *i.e*. total protein, fat, ash and dry matter after AOAC [2003].

Genomic DNA was extracted from blood using Master Pure kit of EPICENTRE Technologies. Genotypes *RYR1*, *CAST*/*Hinf*I and *CAST*/*Hpy*188I were identified with the PCR/RFLP method, according to Fujii *et al.* [1991], Ernst *et al.* [1998] and Ciobanu *et al.* [2004], respectively.

Genetic equilibrium of analysed population was evaluated on the basis of chi-square test. A statistical analysis was performed to compare carcass and meat quality traits and meat basic chemical composition between pigs of different *CAST* and *RYR1* genotypes, using the least squares method of the GLM procedure (Statistica 8.0 PL) according to the following linear model:

$$Y_{ijkl} = \mu + a_i + b_j + c_k + bc_{jk} + \beta\,(x_{ijkl} - \overline{x}) + e_{ijkl}$$

where:

Y_{ijkl} – trait measured;

μ – overall mean;

a_i – the effect of sex (i = 1, 2);

b_j – the effect of *RYR1* genotype (j = *CT*, *CC*);

c_k – the effect of *CAST/Hinf*I genotype (k = *AB*, *BB*) or *CAST/Hpy*188I genotype (k = *AA*, *AB*);

bc_{jk} – interaction (*RYR1* × *CAST/ Hinf*I or *RYR1* × *CAST/Hpy*188I genotype),

β – linear regression coefficient for hot carcass weight;

x_{ijkl} – hot carcass weight of *ijkl*-th individual included as covariable;

$\overline{x}$ – mean for hot carcass weight;

e_{ijkl} – random error.

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

Results and discussion

The frequencies of *CAST*/*Hinf*I, *CAST*/*Hpy*188I, and *RYR1* alleles and genotypes in Pietrain-sired pigs are presented in Table 1. Chi-square test revealed that genotype frequency at the *loci CAST*/*Hpy*188I and *RYR1* did not remain in the Hardy-Weinberg equilibrium. Significance of associations of the genotypes of calpastatin (*CAST*/*Hinf*I and *CAST*/*Hpy*188I) and the ryanodine receptor gene (*RYR1*) with carcass and pork quality traits are presented in Table 2.

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

Item (n=125)	*CAST*/*Hinf*I			*CAST*/*Hpy188*I			*RYR1*		
	AA	*AB*	*BB*	*AA*	*AB*	*BB*	*CC*	*CT*	*TT*
No. of animals	-	11	114	71	54	-	71	54	-
Frequency of alleles		*A* = 0.04 *B* = 0.96			*A* = 0.78 *B* = 0.22			*C* = 0.78 *T* = 0.22	
Frequency of genotypes (%)	-	8.8	91.2	56.8	43.2	-	56.8	43.2	-
Chi square*		0.006			4.859**			4.859**	

*According to Hardy-Weinberg equilibrium.
**Significant at P≤0.05.

No significant differences were found in meat or carcass quality between *RYR1 CC* and *CT* genotypes, being in accordance with other authors who studied carcass [Kusec *et al.* 2005] or pork quality [Koćwin-Podsiadła *et al.* 2003] in Pietrain-crossed pigs.

The frequency analysis of *CAST*/*Hinf*I genotypes of Pietrain-sired crossbreds carried out in this study revealed two genotypes present – *AB* and *BB* – which has also been established in TORHYB-programme pigs – Pietrain × (Polish Large White × Polish Landrace) – as well as in Polish Landrace pigs [Kurył *et al.* 2003]. According to Ernst *et al*. [1998] and Kurył *et al.* [2003], Pietrain pigs were monomorphic as regards the *CAST*/*Hinf*I polymorphism and were of the *BB* genotype. All three possible genotypes were observed among Yorkshire, Large White [Ernst *et al.* 1998], Stamboek (Dutch Large White × Dutch Landrace), and Złotnicka Spotted pigs [Kurył *et al.* 2003].

Our analysis presented here did not reveal an association between the *CAST*/*Hinf*I polymorphism and carcass quality traits, which was also showed by Kurył *et al.* [2003], who tested *RYR1*^{T}-free Stamboek porkers. On the other hand Koćwin-Podsiadła *et al.* [2004] analysing *RYR1*^{T}-free crossbreds found a significant relationships between the *CAST*/*Hinf*I genotype and eight out of 19 analysed carcass traits. They concluded that a *CAST*/*Hinf*I genotype should be used while selecting for a line of pigs towards larger hams (*CAST*/*Hinf*I genotype *BB*) or loin (*AA* genotype).

Table 2. Least squares means (LSM) and standard errors (SE) for analysed traits and relationship between genotypes at the *loci* *CAST*/*Hinf*I and *CAST*/*Hpy*188I and *RYR1* for carcass and meat quality traits in examined pigs

Trait	LSM	SE	Significance of effect of		
			CAST/*Hinf*I	*CAST*/*Hinf*I × *RYR1*	*CAST*/*Hpy*188I × *RYR1*
Meat content of carcass (%)	55.39	0.39	ns	ns	ns
Backfat thickness (mm)	14.90	0.38	ns	ns	ns
Muscle thickness (mm)	56.64	0.58	ns	ns	ns
Total protein (%)	22.40	0.06	ns	ns	ns
Fat (%)	2.52	0.05	ns	ns	ns
Ash (%)	1.18	0.01	ns	ns	ns
Dry matter (%)	26.10	0.07	ns	ns	ns
pH_{24}	5.66	0.01	P≤0.01	ns	ns
pH_{48}	5.57	0.01	P≤0.01	ns	ns
EC_2 (mS/cm)	3.08	0.12	ns	ns	ns
L*	54.74	0.30	ns	ns	ns
a*	9.33	0.11	P≤0.05	ns	ns
b*	16.81	0.12	P≤0.01	ns	ns
Drip loss (%)	7.65	0.23	P≤0.01	ns	ns
WHC (% of free water)	17.42	0.44	P≤0.01	P≤0.05	ns
Thermal drip (%)	25.88	0.25	P≤0.05	ns	ns
Water-soluble protein (%)	8.22	0.08	ns	ns	ns

ns – not significant.

A significant relationship was found in the analysed pigs between the *CAST*/*Hinf*I genotype and the quality traits of pork – pH, yellowness and redness of colour (a* and b*), WHC, drip loss, and thermal drip. The meat of the *AB* pigs had significantly higher pH_{24} and pH_{48}, lower water-holding capacity (WHC), as well as lower drip loss and thermal drip in relation to the *BB* genotypes (Tab. 3). On the other hand, *BB* meat exhibited significantly more intense yellowness of colour (b*), and more intense redness (a*), as compared to the meat of *AB* pigs. Other studies on pigs being carriers of $RYR1^T$ allele (*CT*/*RYR1* genotype) and those free of this mutation (*CC* genotype) also demonstrate an association of the *CAST*/*Hinf*I polymorphism with pork quality traits [Koćwin-Podsiadła *et al.* 2003, Kapelański *et al.* 2004, Kurył *et al.* 2004]. Kapelański *et al.* [2004], who studied pigs of various breeds and their crosses, demonstrated significant relationships between the *CAST*/*Hinf*I genotype and pH_{45}, as well as brightness and saturation of meat colour. Studies on crossbred pigs sired

Table 3. Least squares means (LSM) and standard errors (SE) for meat quality traits in relation to genotypes at the *CAST*/*Hin*fI *locus* in examined pigs

Trait	*CAST*/*Hin*fI genotype			
	AB		*BB*	
	LSM	SE	LSM	SE
No. of animals	11		114	
pH_{24}	5.79^{A}	0.24	5.65^{B}	0.13
pH_{48}	5.75^{A}	0.26	5.55^{B}	0.13
a*	8.60^{a}	0.26	9.40^{b}	0.12
b*	15.63^{A}	0.48	16.92^{B}	0.12
Drip loss (%)	5.13^{A}	0.67	7.91^{B}	0.23
WHC (% of free water)	13.12^{A}	1.82	17.85^{B}	0.44
Thermal drip (%)	24.22^{a}	0.86	26.04^{b}	0.26

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

by Duroc × Pietrain boars revealed association between the polymorphism and pH_{45} [Koćwin-Podsiadła *et al.* 2003] and water-holding capacity of the LL muscle only [Kurył *et al.* 2004]. Krzęcio *et al.* [2004], who analysed the *RYR1*^{T}-free pigs, reported a strict relationship between the *CAST*/*Hin*fI genotype and seven out of 28 pork quality traits and meat chemical characteristics, *i.e.* pH measured in various time periods post-slaughter, ATP-to-IMP degradation rate (R_1), drip loss, and meat tenderness.

In this study no significant relationship was identified between the *CAST*/*Hpy*188I polymorphism and any of the pork quality traits considered. Ciobanu *et al.* [2004] found in Berkshire × Yorkshire crossbreds that *CAST*/*Hpy*188I genotype affected tenderness and sensory properties of pork. Furthermore, they observed that the haplotypes *CAST*/*Hpy*188I - *CAST*/*Pvu*II are strongly associated with cutting force, sensory quality of pork, and free drip loss in the LD muscle.

In the pigs considered in this study a significant effect of interaction *CAST*/*Hin*fI × *RYR1* in relation to WHC was found (Tab. 4). The meat of *AB*/*CC* pigs showed significantly higher WHC (lower percentage of free water) than of *AB*/*CT*, *BB*/*CT* and *BB*/*CC* genotypes. A significant effect of *CAST*/*Hin*fI × *RYR1* interaction was

Table 4. Interactive effect of *CAST*/*Hin*fI and *RYR1* genotypes for meat quality in examined pigs

Trait	*CAST* and *RYR1* genotype							
	AB/*CT*		*BB*/*CT*		*AB*/*CC*		*BB*/*CC*	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
No. of animals	5		49		6		65	
WHC (% of free water)	17.12^{a}	2.92	18.09^{a}	0.77	9.80^{b}	1.28	17.66^{a}	0.52

^{ab}Means in rows bearing different superscripts are significantly different at P≤0.05.

also found in crossbreds sired by Duroc × Pietrain boars as regards pork WHC [Kurył *et al.* 2004] and drip loss from the LL [Koćwin-Podsiadła *et al.* 2003]. Kurył *et al.* [2004] conclude that the presence of the allele *A* in the heterozygous genotype *AB*/*CAST*/*Hinf*I is highly significantly associated with meat WHC of stress gene-free pigs (*CC*/*RYR1*). This conclusion is also reflected in our results, which demonstrate significantly higher WHC of pork in *AB*/*CC* compared to *BB*/*CC*, *AB*/*CT* and *AB*/*CC* pigs (*CAST*/*Hinf*I × *RYR1*).

It may be concluded that in terms of carcass and pork quality, no significant differences were found between the genotypes *CT*/*RYR1* and *CC*/*RYR1*, as well as between *AA* and *AB* genotypes for the *locus CAST*/*Hpy*188I. On the other hand, a significant influence was found of the *CAST*/*Hinf*I polymorphism on pork quality traits. The meat of *AB* pigs had a significantly higher pH, lower drip loss and thermal drip, lower WHC, and lower redness and yellowness of colour as compared to the meat of *BB* pigs. Furthermore, a significant effect of interaction *CAST*/*Hinf*I × *RYR1* was found in relation to WHC of meat. The results presented indicate that the *CAST* gene polymorphism identified by *Hinf*I enzyme may be considered as important in terms of meat quality traits of the analysed pigs. A follow-up study is necessary, however, involving a larger population that would represent all possible genetic variants of the *CAST* and their number sufficient for statistical analysis.

REFERENCES

1. AOAC (Association of Official Analytical Chemists), 2003 – Official Methods of Analysis, 17 th Edition., Revision 2, AOAC International, Gaithersburg, USA.
2. CIOBANU D.C., BASTIAANSEN J.W.M., LONERGAN S.M., THOMSEN H., DEKKERS J.C.M., PLASTOW G.S., ROTHSCHILD M.F., 2004 – New alleles in calpastatin gene are associated with meat quality traits in pigs. ***Journal of Animal Science*** 82, 2829-2839.
3. CIE – COMMISSION INTERNATIONAL DE I'ECLAIRAGE (COLORIMETRY), 1976 – Official Recommendations of the International Commission on Illumination. Publication CIE, No. 15 (E-1.3.1). Bureau Central de la CIE, Paris, France.
4. ERNST C.W., ROBIC A., YERLE M., WANG L., ROTHSCHILD M. F., 1998 – Mapping of calpastatin and three microsatellites to porcine chromosome 2q2.1-q2.4. ***Animal Genetics*** 29, 212-215.
5. FUJII J., OTSU K., ZORZATO F., DE LEON S., KHANNA V.K., WEILER J.E., OBRIEN P.J., MACLENNAN D.H., 1991 - Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. ***Science*** 253, 448-451.
6. GLODEK P., KRATZ R., SCHULZ E., FLACHOWSKY G., 2004 – Effect of sire breeds in commercial pig crosses on growth, carcass composition, meat and fat quality. ***Archiv für Tierzucht*** Dummerstorf 47, 59-74.
7. GOLL D.E., THOMPSON V.F., TAYLOR R.G., OUALI A., 1998 – The calpain system and skeletal muscle growth. ***Canadian Journal of Animal Science*** 78, 503-512.
8. GRAU R., HAMM R., 1952 – Eine einfache Methode zur Bestimmung der Wasserbindung in Fleisch. ***Fleischwirtschaft*** 4, 295-297.
9. HONIKEL K.O., 1987 – pH and water binding of meat. ***Fleischwirtschaft*** 67, 1098-1102.

10. KAPELAŃSKI W., GRAJEWSKA S., KURYŁ J., BOCIAN M., JANKOWIAK H., WIŚNIEWSKA J., 2004 – Calpastatin (*CAST*) gene polymorphism and selected meat quality traits in pigs. ***Animal Science Papers and Reports*** 22, 435-441.
11. KOĆWIN-PODSIADŁA M., KURYŁ J., 2003 – The effect of interaction between genotypes at *loci CAST*, *RYR1* and *RN* on pig carcass quality and pork traits – a review. ***Animal Science Papers and Reports*** 21, Supplement, 61-75.
12. KOĆWIN-PODSIADŁA M., KURYŁ J., KRZĘCIO E., ANTOSIK K., ZYBERT A., SIECZKOWSKA H., 2004 – An association between genotype at the *CAST* (calpastatin) locus and carcass quality traits in porkers free of *RYR1*^{T} allele. ***Animal Science Papers and Reports*** 22, 497-505.
13. KOĆWIN-PODSIADŁA M., KURYŁ J. KRZĘCIO E., ZYBERT A., PRZYBYLSKI W., 2003 – The interaction between calpastatin and RYR1 genes for some pork quality traits. ***Meat Science*** 65, 731-735.
14. KOOHMARAIE M., WHIPPLE G., KRETCHMAR D.H., CROUSE J.D., MERSMANN H.J., 1991 – Postmortem proteolysis in longissimus muscle from beef, lamb, and pork carcasses. ***Journal of Animal Science*** 69, 617-624.
15. KOTIK T., 1974 – Zawartość białka w ekstraktach wodnych mięsa jako wskaźnik jego jakości (Protein content in water extracts of meat as an index of its quality) In Polish, summary in English. ***Roczniki Instytutu Przemysłu Mięsnego*** 12, 47-52.
16. KRZĘCIO E., KOĆWIN-PODSIADŁA M., KURYŁ J., ANTOSIK K., ZYBERT A., SIECZKOWSKA H., POSPIECH E., ŁYCZYŃSKI A., GRZEŚ B., 2004 – An association between genotype at the *CAST locus* (calpastatin) and meat quality traits in porkers free of *RYR1*^{T} allele. ***Animal Science Papers and Reports*** 22, 489-496.
17. KURYŁ J., KAPELAŃSKI W., PIERZCHAŁA M., GRAJEWSKA S., BOCIAN M., 2003 – Preliminary observations on the effect of calpastatin gene (*CAST*) polymorphism on carcass traits in pigs. ***Animal Science Papers and Reports*** 21, 87-95.
18. KURYŁ J., KRZĘCIO E. KOĆWIN-PODSIADŁA M., MONIN G., 2004 – The influence of CAST and RYR1 genes polymorphism and their interactions on selected meat quality parameters in four-breed fatteners. ***Animal Science Papers and Reports*** 22, 479-488.
19. KUSEC G., BAULAIN U., HENNING M., KÖHLER P., KALLWEIT E., 2005 – Fattening, carcass, and meat quality traits of hybrid pigs as influenced by MHS genotype and feeding systems. ***Archiv für Tierzucht*** Dummerstorf 48, 40-49.
20. MELODY J.L., LONERGAN S.M., ROWE L.J., HUIATT T.W., MAYES M.S. HUFF-LONERGAN E., 2004 – Early post mortem biochemical factors influence tenderness and water-holding capacity of three porcine muscle. ***Journal of Animal Science*** 82, 1195-1205.
21. POHJA M.S., NIINIVAARA F.P., 1957 – Die Bestimmung der Wasserbindung des Fleisches mittels der Konstantdrückmethode. ***Fleischwirtschaft*** **9**, 193-195.