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An association between genotype at the *CAST* (calpastatin) *locus* and carcass quality traits in porkers free of *RYR1^T* allele*

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An attempt is presented at determining the significance of associations between carcass quality traits and the genotype of pigs at *locus CAST* (calpastatin). Used were 397 porkers free of the *RYR1^T* allele, of which 91 were purebred Landrace and the remaining were crossbreds: 65 Landrace × Yorkshire, 129 Landrace × Duroc, 83 [(Landrace × Yorkshire) × Duroc] and 29 [(Landrace × Yorkshire) × (Duroc × Pietrain)]. Animals differing as regards genotype for each of the three *CAST* mutations analysed (identified by enzymes *Hinf*I, *Msp*I i *Rsa*I) differed significantly in the weight of loin (not related to sex) and/or ham. Moreover, the positive effect of one the *CAST* gene variants on the loin weight was accompanied by its negative effect on ham weight, though the meat content of carcass remained unchanged. This observation indicates that the activity of the given calpastatin molecular variant depends on the muscle type (white muscle of loin, red muscle of ham). The *CAST* gene variant favourable for the loin weight proved to be favourable also for the shoulder weight. Carcasses of porkers with genotype *AA* as regards the gene *CAST* mutation identified by enzyme *Rsa*I were longer by 2.04 cm than those from animals of genotype *BB* (P≤0.05). It is concluded that the *CAST* genotype could be used in selection of pig lines for increased ham weight (animals of genotype *BB* at *loci CAST*/*Hinf*I and *CAST*/*Msp*I) or loin weight (animals of genotype *AA* at *loci CAST*/*Hinf*I and *CAST*/*Msp*I as well as *BB* at *locus CAST*/*Rsa*I).

KEY WORDS: calpastatin gene / candidate gene/ carcass quality/ gene polymorphism / pigs

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The effect of gene *RYR1* on the carcass muscling has been confirmed be numerous studies conducted on different pig breeds and crossbreeds [Koćwin-Podsiadła *et al*. 1993, Kurył *et al*. 1998, Przybylski *et al*. 1998, Koćwin-Podsiadła and Krzęcio 1999]. Despite this, among animals of the same breed and the same *RYR1* genotype, one may observe significant differences in certain carcass quality traits. Thus, it is necessary to identify other genes affecting the phenotypic differentiation of animals as regards such traits. Projects for mapping of the pig genome rendered it possible, among other things, to localize QTLs for carcass traits [Bidanel and Rothschild 2002]. They have been identified on all chromosomes, confirming the hypothesis that quantitative traits, and such are some carcass quality traits, are polygenic. The knowledge of metabolic pathways, related to the development and physiology of the muscle tissue, may indicate which genes should be examined for their effect on those traits.

The growth of skeletal muscles is dependent principally on the rate of protein synthesis and degradation as well as the number and size of muscle fibres. Studies conducted over the last several years have demonstrated that the proteolytic system, including calpains and their inhibitor – calpastatin, plays a significant role in those processes. Active calpain is indispensable for cell proliferation [Mellegren 1997] and myoblast fusion [Balcerzak 1995]. It has been demonstrated that the fusion of rat myoblasts L8 was accompanied by significant changes in the relation between the activity of calpains and calpastatin [Barnoy *et at*. 1996]. Moreover, the fact that the calpain/calpastatin system is important for a normal growth of skeletal muscles has clearly been documented, as it was observed that an acceleration of this process is accompanied by a decrease in calpain activity, principally as result of an increased activity of calpastatin [Goll *et al*. 1998 – a review].

The preliminary studies on the effect of the *CAST* genotype on pig carcass quality were conducted in Poland. Used was a small population of Stamboek castrated males [Kurył *et al*. 2003] and four-breed crosses [(Polish Large White × Polish Landrace) × (Hampshire × Pietrain)] – Koćwin-Podsiadła and Kurył [2003]. The present study aimed at evaluating the effect of the porker's genotype at *locus CAST* on carcass traits in pigs free of $RYR1^T$ allele.

Material and methods

Animals

The investigations covered 397 porkers (211 castrated males and 186 gilts) free of the $RYR1^T$ allele, of which 91 were purebred Landrace and the remaining were crossbreds: 65 Landrace × Yorkshire, 129 Landrace × Duroc, 83 [(Landrace × Yorkshire) × Duroc] and 29 [(Landrace × Yorkshire) × (Duroc × Pietrain)]. The animals came from the Pedigree Centre Jagodne, owned by SOKOŁÓW S.A. Meat Processing Plant. The parental material (except for the Pietrain breed) of the porkers analysed was imported from Denmark. The maintenance and nutrition (Cargill complete diet offered according to age) during rearing and fattening were uniform for all animals.

The animals were slaughtered during the autumn-winter season, 2-4 hours after transport (300 km). Electrical stunning (Dutch line INARCO) was followed by bleeding in a horizontal position, in accordance with the technique in force at the SOKOŁÓW S.A. Meat Processing Plant.

Methods

Within 24 hours after slaughter the morphological carcass composition and muscling were determined (Tab. 1) according to the Pig Performance Testing Stations (SKURTCH) method [Różycki 1996]. Slaughter traits were analysed on the basis of data standardized for 85 kg of carcass weight warm (mtc). The standardization was performed within each genetic group.

Genomic DNA was isolated from blood leucocytes according to Kawasaki [1990]. The *CAST* genotypes were determined by the PCR/RFLP method, according to Ernst *et al*. [1998].

Statistical

The effect of genotype *CAST* and sex on the carcass morphological composition and muscling was evaluated on the basis of a three-factor analysis of variance in a non-orthogonal arrangement [Ruszczyc 1981]. The mean values obtained were compared using test NIR [Oktaba 1980].

Results and discussion

The effect of sex on carcass morphological composition and muscling

Investigations conducted on 397 porkers free of allele *RYR1^T* (211 castrated males and 186 gilts), have confirmed the commonly known effect of sex on several carcass quality traits (Tab. 1). Loin traits (weight of loin, weight of loin without fat and skin, weight of *Longissimus dorsi* (LD) muscle and loin eye area) as well as weight of neck and shoulder were not found related to sex. In gilts the meat content of carcass was higher and fat thickness lower ($P \leq 0.01$) at all measurement points. The carcasses of gilts were also longer that those of castrates.

The effect of the *CAST* genotype on carcass morphological composition and muscling

The polymorphism of gene *CAST* was identified with restriction enzymes *Hinf*I, *Msp*I and *Rsa*I. Three genotypes were observed for each mutation identified by those enzymes (Tab. 1 and 2).

Genotype *CAST*/*Hinf*I affected significantly, or highly significantly, the value of eight out of the 19 carcass traits analysed. The values of two of them, *i.e*. weight of loin and weight of loin without fat and skin were not related to animal's sex. The traits characterizing fat deposition (fat thickness and weight of bacon with ribs), weight of loin, weight of loin without fat and skin and weight of shoulder were found more favourable in animals of genotype *AA*. In turn, values obtained for traits characterizing ham

Table 1. Means and their standard errors for carcass traits related to sex and genotype at the *CAST/MspI locus* in porkers free of *RYR1*^{T} allele

Trait	Sex		*CAST/MspI* genotype		
	castrated males (n=211)	gilts (n=186)	*AA* (n=55)	*AB* (n=186)	*BB* (n=156)
Meat content in carcass (%)	55.81±2.41^a	57.21±2.15^b	56.33±1.98	56.49±2.53	56.50±2.37
Carcass length (cm)	81.39±2.26^a	82.56±2.94^b	81.58±3.13	81.91±2.41	82.71±3.00
Fat thickness (cm)					
over the shoulder	3.17±0.49^a	2.99±0.44^b	3.01±0.45	3.10±0.49	3.10±0.47
at the last rib	1.46±0.39^a	1.29±0.45^b	1.42±0.46	1.37±0.39	1.38±0.45
at sacrum point I	2.02±0.38^a	1.80±0.34^b	1.81±0.34^A	1.91±0.36^b	1.97±0.41^b
at sacrum point II	1.51±0.33^a	1.32±0.29^b	1.37±0.33	1.41±0.31	1.45±0.34
at sacrum point III	2.42±0.43^a	2.12±0.40^b	2.21±0.44	2.26±0.44	2.33±0.44
mean from 5 measurements	2.12±0.27^a	1.91±0.26^b	1.96±0.27^A	2.01±0.28^{aB}	2.05±0.29^B
Loin eye area (cm^2)	51.84±4.20	52.07±5.45	51.38±4.76	52.06±6.27	52.02±5.71
Weight of					
loin (kg)	8.51±0.68	8.51±0.72	8.43±0.62^A	8.59±0.69^B	8.38±0.72^A
loin without fat and skin (kg)	6.33±0.58	6.43±0.61	6.35±0.55^A	6.42±0.59^{aB}	6.27±0.61^A
Longissimus muscle (kg)	2.91±0.28	2.94±0.37	2.95±0.34	2.91±0.31	2.93±0.34
ham with leg (kg)	10.24±0.51^a	10.38±0.60^b	10.19±0.44^A	10.25±0.49^{aB}	10.41±0.64^B
ham without fat and skin (kg)	8.46±0.44^a	8.66±0.50^b	8.50±0.37	8.54±0.45	8.59±0.55
ham muscles (kg)	7.55±0.43^a	7.72±0.44^b	7.55±0.37^A	7.60±0.44^{aB}	7.68±0.47^B
neck	5.41±0.47	5.40±0.43	5.49±0.49	5.44±0.45	5.33±0.44
shoulder (kg)	6.02±0.41	6.02±0.35	6.05±0.40^{aA}	6.07±0.37^A	5.95±0.38^B
bacon with ribs (kg)	6.69±0.59^a	6.46±0.61^b	6.59±0.47^a	6.52±0.57^a	6.72±0.67^b
valuable cuts (kg)	23.12±0.98^a	23.63±0.92^b	23.41±0.78	23.35±0.87	23.36±1.05

^{a,A} Within rows and sex or *CAST/MspI* genotype means bearing different superscripts differ significantly at: small letters – P≤0.05; capitals – P≤0.01.

(weight of ham and weight of ham meat) were higher in animals of genotype *BB*.

Out of the 19 traits of carcass morphological composition and muscling, the values of five were significantly or highly significantly related to the *CAST*/*Msp*I genotype (Tab. 2). The weight of loin, weight of loin without fat and skin and weight of shoulder were significantly higher in animals of genotype *AA* than of *BB*. In turn, the weight of ham in animals of genotype *BB* was significantly higher than in animals of genotype *AA*.

As regards genotypes at *locus CAST*/*Rsa*I the porkers differed in the values of five out of the 19 traits of carcass morphological composition and muscling (Tab. 2). Animals of genotype *BB* showed the thinnest backfat over sacrum point I and the highest weight of loin, weight of loin without fat and skin, and weight of shoulder. Moreover, the *BB* animals were characterized by the shortest carcass (central length 80.9 cm) – by 2 cm shorter than that from *AA* pigs.

The statistical analysis conducted did not show any significant interaction between the *CAST* genotype and sex in affecting the carcass quality traits.

The differences in the value of carcass quality traits between porkers of the same *RYR1*

Table 2. Means and their standard errors for carcass traits related to genotype at the *CAST/MspI* and *CAST/RsaI loci* in porkers free of *RYR1^T* allele

Trait	CAST/MspI genotype			CAST/RsaI genotype		
	AA (n=100)	*AB* (n=163)	*BB* (n=134)	*AA* (n=146)	*AB* (n=203)	*BB* (n=48)
Meat content in carcass (%)	56.42±0.38	56.32±0.44	56.44±0.37	56.31±0.37	56.55±0.42	56.64±0.39
Carcass length (cm)	81.34±0.54	82.32±0.78	82.58±0.81	82.94±0.01^a	82.06±0.20^b	80.50±0.48^c
Fat thickness (cm)						
over the shoulder	3.12±0.41^a	3.04±0.51^b	3.12±0.48^a	3.11±0.50	3.08±0.46	3.05±0.48
at the last rib	1.43±0.46	1.36±0.37	1.38±0.46	1.39±0.42	1.37±0.43	1.43±0.44
at sternum point I	1.89±0.37	1.92±0.37	1.97±0.40	1.92±0.41^{Aa}	1.94±0.36^a	1.82±0.37^b
at sternum point II	1.40±0.31	1.40±0.31	1.46±0.35	1.42±0.34	1.43±0.30	1.38±0.38
at sternum point III	2.26±0.41	2.27±0.45	2.31±0.45	2.25±0.45	2.32±0.44	2.23±0.43
mean from 5 measurements	2.03±0.30	2.00±0.27	2.05±0.30	2.02±0.31	2.03±0.26	1.98±0.29
Loin eye area (cm^2)	51.57±5.76	52.30±5.97	51.55±5.77	51.45±5.88	52.21±6.03	52.34±4.94
Weight of						
loin (kg)	8.64±0.65^a	8.52±0.75^{ab}	8.41±0.67^b	8.46±0.72^a	8.49±0.69^a	8.79±0.59^B
loin without fat and skin (kg)	6.48±0.56^a	6.40±0.63^{aB}	6.27±0.57^B	6.32±0.61^a	6.35±0.58^a	6.68±0.52^B
Longissimus muscle (kg)	2.90±0.28	2.94±0.33	2.91±0.36	2.92±0.34	2.92±0.32	2.93±0.33
ham with leg (kg)	10.25±0.37^a	10.26±0.57^a	10.40±0.64^b	10.30±0.63	10.33±0.54	10.20±0.40
ham without fat and skin (kg)	8.51±0.84	8.58±0.86	8.58±0.91	8.51±0.53	8.60±0.46	8.51±0.41
ham muscles (kg)	7.40±0.40	7.62±0.44	7.66±0.49	7.59±0.47	7.67±0.43	7.55±0.42
neck	5.40±0.44	5.43±0.48	5.38±0.44	5.41±0.50	5.41±0.43	5.37±0.39
shoulder (kg)	6.08±0.37^a	6.04±0.39^a	5.98±0.36^B	5.92±0.35^a	6.09±0.38^B	6.10±0.42^B
bacon with ribs (kg)	6.46±0.57	6.59±0.59	6.67±0.65	6.63±0.68	6.59±0.58	6.44±0.51
valuable cuts (kg)	23.41±0.92	23.35±1.00	23.33±1.01	23.24±1.05	23.38±0.94	23.35±1.00

^{a,A} Within rows and *CAST* loci means bearing different superscripts differ significantly at: small letters – $P \leq 0.05$; capitals – $P \leq 0.01$.

genotype induce scientists to search for other genes affecting those traits. It is suggested that beside *RYR1^T* gene considered to be the major gene of carcass muscling, also other genes affect this trait [Bidanel and Rothschild 2002]. In literature one may find an analysis of the effect of the polymorphism of several genes, known as candidate genes (*GH*, *GHRH*, *PIT1*, *IGF1*, *IGF2*, *LEP*, and family *MyoD*), on carcass traits [reviewed by Kurył 2000, Wyszyńska-Koko 2003, Urbański 2003, and Pierzchała *et al*. 1997, 2004].

The first evaluation of the effect of genotype *CAST* on carcass traits was conducted in Poland. In Stamboek pigs free of gene *RYR1^T* a significant relation was demonstrated between the *CAST* genotype and traits characterizing the loin [Kurył *et al*. 2003]. In four-breed crosses [(Polish Large White × Polish Landrace) × (Hampshire × Pietrain)] and [(Polish Large White × Polish Landrace) × (Duroc × Pietrain)] a significant effect of interaction was observed between the genotypes at *loci CAST* and *RN* on the loin traits and backfat thickness [Koćwin-Podsiadła and Kurył 2003]. The present analysis, conducted on a material covering 397 porkers free of gene *RYR1^T*, demonstrated that genotype *CAST* affects significantly a series of carcass quality traits. It is known that the calpain-calpastatin proteolytic system participates in the muscle growth and

development processes [Goll *et al*. 1998 – a review]. The present study indicates that the rate of those processes may be related to the *CAST* genotype. This would indicate that the activity of calpastatin as a calpain inhibitor could depend on its genetic variant. Studies conducted principally on laboratory animals demonstrated that different tissues contain different structural variants of calpastatin, resulting from the post-transcription and post-translation modifications of the product of a single gene. It was shown that as a result of insertions and deletions in exons localized within the region of gene *CAST* coding for domain L, the calpastatins encoded by those gene variants demonstrate a different specificity as a calpain inhibitor [Averna *et al*. 2001 – a review]. In the present study, one calpastatin variant proved to be more favourable for the meat content of loin while the second – for the meat content of ham. Thus one could conclude that the effects of molecular calpastatin variants as calpain inhibitors depend on muscle type (white muscles of loin, red muscles of ham). This indicates that such effect is reflected by the relation observed that the weight of loin and loin without fat and skin was by 0.25 kg higher in animals of *CAST*/*Hinf*I *AA* than in those of *CAST*/*Hinf*I *BB* genotype. Simultaneously, the weight of ham in animals of genotype *BB* was by about 0.2 kg higher ($P \leq 0.01$) than that in *AA* animals (Tab. 1). One should emphasize, that the effect of genotype *CAST* on the weight of loin (the whole cut), loin without fat and skin as well as weight of shoulder was recorded for all three known mutations of the gene. Simultaneously, it is worth observing that in the animal population examined no sex effect on those traits was found. Moreover, there was observed no significant interaction between sex and the *CAST* genotype in relation to any of the carcass quality traits examined.

The results obtained indicate that the *CAST* genotype could be considered in selection of pigs. Depending on whether it would be directed for increased ham or loin weight one should prefer animals with a given *CAST* genotype.

Basing on the current knowledge of the *CAST* gene structure, as well as of its mechanisms of expression and those regulating the activity of the calpain-calpastatin proteolytic system, it is difficult to conclude about the conditioning of the effect of the *CAST* genotype on carcass quality traits observed in the present study. The *CAST* gene mutations, identified by restriction enzymes *Hinf*I, *Msp*I and *Rsa*I, are located in intron 6. Thus they do not affect the amino acid structure of calpastatin. However, in the light of studies conducted over the last two years one cannot state that they do not affect the gene expression, as it is known that introns may contain sequences affecting the progress of transcriptions, what has been demonstrated for some genes [Le Hir *et al*. 2003, Greenwood and Kelsoe 2003]. Moreover, it is known for calpastatin that on the basis of one gene different mRNA variants may be synthesized as result of a differentiated splicing [Parr *et al*. 2004]. Thus, further studies on this topic should be undertaken which would, among other things, indicate the causal mutations in the structure of gene *CAST* determining the significant relations observed in this study between the carcass quality traits and *CAST* genotype.

The results presented here indicate that the *CAST* genotype could be considered in

selection work aiming at introducing pig lines with an increased ham weight (genotype *BB* at *loci CAST*/*Hinf*I and *CAST*/*Msp*I) or an increased weight of the loin muscle (animals of genotype *AA* at *loci CAST*/*Hinf*I and *CAST*/*Msp*I, as well as of *BB* at *locus CAST*/*Rsa*I).

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Wpływ polimorfizmu genu kalpastatyny (*CAST*) na mięsność i skład morfologiczny tuszy tuczników wolnych od genu *RYR1*^{T}

S t r e s z c z e n i e

Celem badań było określenie istotności zależności wartości cech jakości tuszy od genotypu świń względem *locus CAST* (kalpastatyny). Badaniami objęto 397 tuczników obu płci następujących ras i mieszańców: Landrace, Landrace × Yorkshire, Landrace × Duroc, [(Landrace × Yorkshire) × Duroc] i [(Landrace × Yorkshire) × (Duroc × Pietrain)], odpowiednio 91, 65, 129, 83 i 29, wolnych od genu *RYR1*^{T}. Wykazano, że zwierzęta zróżnicowane genotypem względem każdej z trzech analizowanych mutacji genu

CAST identyfikowanych enzymami *Hinf*I, *Msp*I i *Rsa*I różniły się istotnie masą polędwicy niezależnie od płci i masą szynki. Ponadto stwierdzono, że dodatniemu wpływowi jednego z wariantów genu *CAST* na masę polędwicy towarzyszy jego ujemny wpływ na masę mięsa szynki, przy zachowaniu zawartości mięsa w tuszy. Co więcej obserwacja ta sugeruje, że aktywność danego wariantu molekularnego kalpastatyny jest zależna od rodzaju mięśnia (mięsień biały-polędwica, mięśnie czerwone-szynka). Wariant genu *CAST* korzystny dla masy polędwicy okazał się również korzystny dla masy łopatki. Stwierdzono również, że tusze tuczników o genotypie *AA* względem mutacji genu *CAST* identyfikowanej enzymem *Rsa*I były o 2.04 cm dłuższe (P≤0,05) niż tusze zwierząt o genotypie *BB*. Genotyp *CAST* mógłby być wykorzystany w pracy selekcyjnej nad wyprowadzaniem linii świń o kierunku użytkowania preferującym masę szynki (zwierzęta o genotypie *BB* w *loci CAST*/*Hinf*I i *CAST*/*Msp*I) lub masę mięśnia polędwicy (zwierzęta o genotypie AA w *loci CAST*/*Hinf*I i *CAST*/*Msp*I i *BB* w *locus CAST*/*Rsa*I) niezależnie od płci.