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THE INFLUENCE OF CAST/MspI, HinfI, RsaI POLYMORPHISM ON PRODUCTION TRAITS IN PIGS

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The aim of this study was to perform the *CAST* gene polymorphism genotyping and to verify its possible influence on the quantitative and qualitative indicators characterizing carcass value in pigs. The study found a significant effect of the *CAST* gene on carcass value. In the case of alelle *A* present in the *CAST*/*Hinf*I gene there was a higher lean meat share (i.e. lower fat content) and therefore the detected quality of pork meat was lower. The signifiant differences were found between the homozygotes *AA* and heterozygotes *AB*, mainly in the amount of fat content ($P \leq 0.05$). Concerning the *CAST/Msp*I gene, it was found that genotype *CD* caused higher lean meat share due to the higher shares of muscles in the main meat parts. However higher lean meat share does lead to lower quality of the meat. Another discovered influence was that of the allele *D*, which was associated with the intramuscular fat content (IMF) in the neck ($P \leq 0.05$). Our results also show signifiant influence ($P \leq 0.05$) of the allele *C* on the qualitative indicators of pork meat (*MS* EC_{50}). Concerning the *CAST/Rsa*I polymorphism, the study proved that this polymorphism doesn't influence any of the monitored qualitative parameters.

Key words: allele, *CAST,* pig, polymorphism, qualitative traits

INTRODUCTION

The crucial phenomenon, determining the competitiveness of the Czech pig production, can be characterized as both quantitative and qualitative parameters of pork meat. Meat quality

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itself is determined by a complex system of parameters such as species, genetic background, the muscle protein complexes metabolism, environment, etc. (CIOBANU *et al.,* 2004). The catabolism of muscle proteins affecting the pork meat quality is influenced mainly by a candidate gene calpastatin (*CAST*), which affects the proteolytic system of meat *p.m.* (KRISTENSEN *et al.,* 2002). Calpastatin affects a number of various sensory and physical characteristics of pork meat such as appearance, color, taste, fat content, texture, pH, electrical conductivity and temperature.

Calpastatin is a specific endogenous inhibitor of calcium-activated proteases known as calpain (μ- and *m*-calpain) and specific polypeptide calpastatin inhibitor, which participate in the muscle cells metabolism. The calpains represent a highly conserved family of non-lysosomal calcium dependent cysteine proteases comprising two ubiquitous isoforms (calpain I and II), several tissue specific isoforms, and a 28kD regulatory subunit (calpain 4). Their activity is linked to the Ca-ions concentration in the cell (MUCHARI 1989).

GOLL *et al.* (2003) demonstrated, that active calpain and calpastatin are essential for cell proliferation and thus for normal growth of the skeletal muscles. In this context KOĆWIN-PODSIADŁA *et al.* (2004) reported, that the growth of skeletal muscle is primarily dependent on the rate of protein synthesis and degradation, as well as on the number and size of muscle fibers. These findings are based on the previous work of these authors (KOĆWIN-PODSIADŁA *et al.* (2003) which showed that the calpastatine activity is associated not only with the speed of proteolytic *p.m.* muscle changes, but also with the muscle growth intensity. MELODY *et al.* (2004) elaborated on this topic and found, that the *p.m.* muscle protein degradation is not only the result of the calpastatin and μ-calpain activity, but it also depends on the type of muscle myofibrils. This information is completed by the work of KOOHMARAIE (2006), who state that calpastatin influences the enzymatic activity of the enzymes μ *m*-calpain.

The gene encoding porcine calpastatin is located on the second chromosome ERNST *et al.* (1998) and it represents a subject of intensive research. The authors CIOBANU *et al.* (2004), ERNST *et al.* (1998) and others identified a number of polymorphisms in some of the CAST gene domains related to carcass value and pork meat quality. This area is also researched by KURYŁ *et al.* (2003) and most recently by RYBARCZYK *et al.* (2010a,b).

KOĆWIN-PODSIADŁA *et al.* (2003, 2004), RYBARCZYK *et al.* (2010a,b), both state that their studies found significant effect of the *CAST/Hin*fI polymorphism on pork meat quality. RYBARCZYK *et al.* (2004) found that the efficiency of molecular variants of calpastatin (inhibiting calpain) depends on the part of the carcass body as well as on the type of muscle fibers. The greatest influence of the *CAST/Hin*fI gene in this respect was demonstrated in the case of the loin and lean meat share (i.e. pig fatness), which was also demonstrated by RYBARCZYK *et al.* (2010a,b). These authors also found a significant interaction between *CAST/Msp*I gene and the loin and shoulder proportions in the carcass.

The influence of the *CAST/Rsa*I gene on the share of main meat parts, lean meat share and fatness was studied by KOĆWIN-PODSIADŁA *et al.* (2004). They demonstrated that this gene causes a notable reduction of the above mentioned carcass characteristics. This gene also significantly affects the glycolytic potential of the muscle, which influences the pH, EC (electrical conductivity), technological meat yield and protein content KRZĘCIO *et al.* (2008).

The aim of this study was to carry out the genotyping of the *CAST* gene polymorphisms (*Msp*I, *Hinf*I, *Rsa*I) and to verify their possible influence on the quantitative and qualitative parameters of the carcass value in pigs.

MATERIALS AND METHODS

Animals

The study was conducted on 709 pigs of 10 hybrid combinations (685) and on 24 pure breed LW pigs (Table 1).

Table 1. Overview of hybrid pig combinations for an association study

Group	Cross combination	Pig frequency (heads)
1	(PNxLW_S) x (LW_DxL)	36
2	(LW_SxD) x (LW_DxL)	72
3	(HxPN) x (LW_DxL)	72
4	(LW_SxPN) x (LW_DxL)	72
5	PN x (LW_DxL)	168
6	LW_S x (LW_DxL)	72
7	PICx (LW_DxL)	25
8	LW_D x L	24
9	PIC x FH	72
10	DanBred	72
11	LW_D	24

PIC-sow, FH - France hybrid boar, DanBred - programme.

During the whole course of the study, the animals were kept under the same conditions at the Experimental Test Station of the Department of Animal Husbandry, Czech University of Life Science, Prague. The pigs were penned the average live weight of 25-30 kg and slaughtered at an average weight of 108 kg. Following the slaughter, the blood of every single animal was collected and used for the isolation of high-molecular- weight DNA.

Nutrition

The nutrition of all the animals participating in the test was carried out with respect to their nutritional needs ŠIMEČEK *et al.* (2000). The fattening was conducted with the use of four-component feed mixture (CFM) based on wheat, barley, soybean meal and premix. Out of the total number 709, 594 animals were fed ad libitum and 115 animals were fed in doses. The CFM at the beginning (and at the end) of the fattening period contained 12.2, 8.7 g LYZ/1kg and 12.9 MJ MEp/1kg CFM.

Monitored phenotypic values of the carcass value

The carcass dissection was carried out according to NISSEN *et al.* (2006). The following quantitative parameters were evaluated:

- quantitative carcass lean meat (with the use of FOM instrument),
- meat share in loin, neck and shoulder without the fat cover including skin (meat+bone),

The following parameters characterizing the body fatness were evaluated:

- average backfat thickness (mm),
- backfat thickness above the last thoracic vertebra (mm),
- fat share cover, including skin, the ham, loin, neck, shoulder (%),

- intramuscular fat share (IMF) in the ham, loin, neck, shoulder STUPKA *et al.* (2004),

The following qualitative parameters characterizing the carcass value were evaluated:

- EC_{50} *MS* (*m.semimembranosus* in mS^{-1}),
- pH_{24} *MLL*T (*m. longissimus lumborum et thoracis*),
- *MLLT* drip loss (% 48 hours *p.m.*).

Genotyping

The collected blood was first stabilized and consequently the high-molecular-weight DNA was isolated. The DNA sample was then mixed with 25µl of reaction solution containing 100 ng genomic DNA, standard PCR buffer, 1.5 mM $MgCl_2$, 200 µM of each of dNTPs, 10 pmol primer, 2% DMSO and 1.0 U LA DNA polymerase (Top Bio, Prague, CR). In order to carry out the genotyping the suitable primers according to ERNST *et al.* (1998) were used. The DNA cycling conditions were as follows - 2 min at 95°C, followed by 32 cycles: 95°C (1 min), 58.5ºC (1 min), 68°C (1 min) and final elongation at 68°C (7 min). The PCR product was then cleaved with the use of three restriction enzymes, resulting in fragments with an approximate length of *Msp*I (~C – 760/D – 370), *Hinf*I (~A – 790/B – 500) and *Rsa*I (~E – 360/F – 250).

Statistical analysis

The obtained results were interpreted with the use of a mathematical and statistical application SAS® (2001). A following model with fixed effects (*CAST/RYR1* genotypes, nutrition, sex) was used, with the carcass weight values representing the regression coefficient. Because the production traits are significantly influenced by sex STUPKA *et al.* (2008), BAHELKA *et al.* (2007) and *RYR1* gene KOĆWIN-PODSIADŁA *et al.* (2003), KRZĘCIO *et al.* (2007), these effects were also included in the model. The model was:

$Y_{ijklmn} = \mu + a_i + b_j + c_k + d_l + e_m + \beta X_n + e_{ijklmn}$, where:

Y_{ijkmn} = measured value of the carcass value,
μ = overall average,
a_i = effect of the *CAST* genotype (i = 1, 2, 3),
b_j = effect of the *RYR1* genotype (j = 1, 2),
c_k = effect of the hybrid pig combination (k=1, 2, 3, 4, 5, 6, 7, 8,9,10,11),
d_l = effect of the sex (l = 1, 2),
e_m = effect of the nutrition (m = 1, 2),
β = regression coefficient of the carcass weight,
X_n = carcass weight n,
e_{ijklmn} = residual error

RESULTS AND DISCUSSIONS

The analysis of the three possible polymorphisms of the CAST gene (*CAST/Msp*I, *CAST/Hinf*I and *CAST/Rsa*I) required the acquisiton of fragments with the expected length of:

- *Msp*I (cleaved allele *C* - 275+407+749, uncleaved allele *D* - 275+407+105+369+275)
- *Hin*fI (cleaved allele *A* - 163+513+755, uncleaved allele *B* - 163+513+174+370+211)
- *Rsa*I (cleaved allele *E* - 998+345+89, uncleaved allele *F* - 998+163+182+89)

The results, describing the frequency of allels and the frequency of individual genotypes of the monitored animals, are presented in Table 2. As it is evident, all three studied *CAST* polymorphisms included all three observed genotypes (Table 2).

*Table 2. The frequency of genotypes in the CAST gene polymorphism (CAST/Hinf*I*, Msp*I*, Rsa*I*)*

Item	CAST/HinfI			CAST/MspI			CAST/RsaI		
N=564	AA	AB	BB	CC	CD	DD	EE	EF	FF
The number of alleles	56	250	258	134	273	157	205	253	106
Allele frequency	A=032 B=068			C=048 D=052			E=059 F=041		
Genotype frequency (%)	9.9	44.3	45.8	23.8	48.4	27.8	36.3	44.9	18.8

Table 3 then shows the relationship between the *CAST/Hinf*I gene polymorphism and the selected parameters describing the carcass value in pigs (Table 3).

*Table 3. The relationship between the CAST/Hinf*I *polymorphism and carcass value traits in pigs*

Indicator	*CAST/Hinf*I		
	AA	*AB*	*BB*
Lean meat share (%)	55.99±0.54	55.26±0.31	55.15±0.29
Meat share (%) in the			
ham	22.12±0.36	22.33±0.22	22.10±0.21
loin	13.28±0.21	13.19±0.13	13.00±0.12
neck	7.06±0.13	6.79±0.08	6.84±0.07
shoulder	9.87±0.15	9.94±0.09	9.98±0.09
Backfat thickness (mm)			
average	25.89±0.68	26.83±0.45	26.70±0.42
above the last thoracic vertebra (mm)	19.34±0.71[a]	20.73±.48[a]	20.48±0.44
Fat share cover including skin (%) in the			
ham	5.56±0.27	5.61±0.17	5.71±0.16
loin	4.88±0.18	4.98±0.11	4.94±0.11
neck	1.30±0.07	1.27±0.04	1.30±0.04
shoulder	3.15±0.10	3.36±0.06	3.39±0.06
IMF share (%) in the			
loin	1.82±0.17	1.91±0.11	1.97±0.11
neck	4.71±0.84	3.0±0.45	4.17±0.41
shoulder	2.08±0.22	2.21±0.11	2.31±0.10
ham	3.08±0.36	3.21±0.22	3.66±0.19
EC_{50} *MS (*mS^{-1})	4.26±0.16	4.10±0.09	3.94±0.09
pH_{24} *MLLT*	5.47±0.07	5.57±0.02	5.55±0.02
MLLT drip loss (%)	9.23±0.63	9.28±0.44	8.95±0.43

[a,b] - inter groups diferences (P≤0,05)

As it is apparent from Table 3, in the presence of allele *A* of the *CAST/Hinf*I gene, the important production traits lean meat share and fatness are influenced the most. There is a significant tendency of a higher lean meat share and lower fatness, which ultimately lowers the quality of the meat. There is also a signifiant difference (P≤0.05) in the backfat thickness above the last thoracic vertebra between the homozygotes *AA* and heterozygotes *AB*.

A similar finding was reached by KOĆWIN-PODSIADŁA *et al.* (2004) who demonstrated that homozygotes *AA* (as compared to homozygotes *BB* and heterozygotes *AB*) show the higher main meat parts proportion, namely the loin, which positively affects the overall meat share. Table 3 also demonstrates that pigs carrying the allele *B* have a tendency to show slightly higher IMF content.

RYBARCZYK *et al.* (2010a) identified a significant impact of the CAST/HinfI gene polymorphism on the quality of porcine meat. They found a significant effect of the *AB* genotype on higher pH_{24} values and less driploss (as compared to the genotype *BB*). The same findings were also confirmed by KURYŁ *et al.* (2004). It is then obvious, that the *AB* CAST/HinfI genotype significantly influences the measure of driploss. With regards to the measured phenotypic values of the pork meat quality, the *AB* genotype tends to lead to higher driploss, which also affects pH45. Similar findings were also published by KOĆWIN-PODSIADŁA *et al.* (2003).

The next purpose of this study was to observe te effect of the *CAST/Msp*I polymorphism on the selected production traits in pigs. Our fading are presented in Table 4.

*Table 4. The relationship between the CAST/Msp*I *polymorphism and carcass value traits in pigs*

Indicator	*CAST/Msp*I		
	CC	*CD*	*DD*
Lean meat share (%)	55.11±0.37	55.52±0.30	55.24±0.32
Meat share (%) in the			
ham	22.23±0.25	22.28±0.21	22.04±0.23
loin	13.04±0.15	13.16±0.12	13.00±0.14
neck	6.77±0.09	6.88±0.08	6.88±0.09
shoulder	9.84±0.11	10.02±0.09	9.96±0.10
Backfat thickness (mm)			
average	26.68±0.52	26.62±0.43	26.69±0.48
above the last thoracic vertebra (mm)	21.05±0.54	20.22±0.54	20.20±0.50
Fat share cover including skin (%) in the			
ham	5.75±0.19	5.59±0.16	5.61±0.18
loin	5.02±0.13	4.93±0.11	4.88±0.12
neck	1.31±0.04	1.24±0.04	1.33±0.04
shoulder	3.32±0.07	3.37±0.06	3.38±0.07
IMF share (%) in the			
loin	1.59±0.13	1.90±0.11	1.99±0.12
neck	$3.36±0.54^{a}$	$3.86±0.40^{b}$	$4.76±0.46^{ab}$
shoulder	2.07±0.13	2.29±0.10	2.35±0.11
ham	3.16±0.25	3.36±0.20	3.66±0.21
EV_{50} *MS (*mS^{-1})	$4.15±0.12^{b}$	$4.09±0.09^{a}$	$3.83±0.11^{ab}$
pH_{24} *MLLT*	5.59±0.02	5.56±0.02	5.54±0.03
(%)	9.58±0.49	9.06±0.42	8.80±0.47

^{a,b} - inter groups diferences (P≤0,05)

As it is evident, the *CD* genotype is associated with the trend of a higher lean meat share and higher main meat parts share, which leads to decreased quality of pork. Similar findings were also published by RYBARCZYK *et al.* (2010b). Conversely, KOĆWIN-PODSIADŁA *et al.* (2004) state, that the *CAST/Msp*I gene has a notable influence on higher proportion of the loin and the shoulder (in favour of the *CC* genotype). However the same authors simultaneously declare, that animals of the *DD* genotype show a higher proportion of ham in the carcass.

Regarding our own findings, we observed a higher proportion of fat in the ham, loin and neck in the animals of the *CC* genotype. On the other hand there were higher IMF levels found in favour of the allele *D* (in all of the main meat parts of the carcass). However the significance of this interaction was confirmed only in the neck ($P \leq 0.05$). In this kontext, RYBARCZYK *et al.* (2010b) refer the influence of the *CAST/Msp*I gene (in the *D* allele) on the backfat thickness. Furthermore KRZĘCIO *et al.* (2005) observed the influence of the *CAST/Msp*I gene on the lactic acid concentration in the *MLLT* (i.e. pH_{45}), on the driploss 48 and 96 hours *p.m.*, as well as on the number of muscle fibers. In our study we observed a positive influence on the qualitative indicators in the animals with the *D* allele.

Table 4 also shows the effect of the *C* allele on the driploss, *MLLT* pH_{24} and on the *MS* EC_{50} values ($P \leq 0.05$). According to KOĆWIN-PODSIADŁA *et al.* (2003), the amount of muscle glycogen in the *MLLT* $_{45}$ directly correlates with the *CAST/Msp*I gene.

*Table 5. The relationship between the CAST/Rsa*I *gene polymorphism and the carcass value traits in pigs*

Indicator	*CAST/Rsa*I		
	EE	*EF*	*FF*
Lean meat share (%)	55.12±0.35	55.36±0.30	55.41±0.43
Meat share (%) in the			
ham	22.41±0.26	22.24±0.21	22.17±0.30
loin	13.10±0.15	13.08±0.12	12.93±0.17
neck	6.81±0.10	6.81±0.08	6.81±0.11
shoulder	9.94±0.11	10.03±0.09	9.92±0.13
Backfat thickness (mm)			
average	26.96±0.51	26.67±0.44	26.85±0.58
above the last thoracic vertebra (mm)	20.95±0.54	20.33±0.45	20.84±0.15
Fat share cover including skin (%) in the			
ham	5.56±0.20	5.69±0.16	5.73±0.23
loin	4.84±0.13	4.88±0.11	4.96±0.15
neck	1.32±0.05	1.26±0.04	1.30±0.05
shoulder	3.42±0.08	3.40±0.06	3.31±0.09
IMF share (%) in the			
loin	1.97±0.13	1.85±0.10	2.03±0.14
neck	4.99±0.54	3.95±0.41	3.49±0.64
shoulder	2.39±0.13	2.24±0.10	2.00±0.16
ham	3.49±0.27	3.42±0.19	3.16±0.31
EV_{50} *MS* (mS^{-1})	3.97±0.12	4.04±0.09	4.16±0.13
pH_{24} *MLLT*	5.60±0.02	5.56±0.02	5.53±0.05
MLLT (%)	8.75±0.51	9.09±0.43	9.04±0.59

The following Table 5 illustrates the results of the *CAST*/*Rsa*I gene analysis (Table 5). If the authors KOĆWIN-PODSIADŁA *et al.* (2004) state that the *CAST/Rsa*I gene, in the presence of the allele *E*, significantly affects the loin and shoulder proportion in the carcass, our results can only confirm this trend. Regarding the fatness of the animals, the decrease of this value is connected to the *FF* genotype, which is also confirmed by our results. As it is shown in the Table 5, there is a possibility of a positive interaction between the *EE* genotype, higher IMF share, higher neck, shoulder and ham proportion and also higher amount of fat cover of the neck and shoulder. Although KOĆWIN-PODSIADŁA *et al.* (2003), indicate that they found a significant influence of the *CAST/Rsa*I gene on the pH_{45} and driploss, our research did not confirm this result. However the relationship between the allele *F* and lower values of *MLLT* pH_{24}, higher *MLLT* driploss and higher *MS* EV_{50} was demonstrated. Regarding this topic KRZĘCIO *et al.* (2008) reported, that the *CAST/Rsa*I gene affects the pH of pork meat.

As it is evident from the Table 5, there were no significant interactions found between the monitored production traits and the *CAST/Rsa*I genotypes in pigs.

CONCLUSION

The study found a relationship between the *CAST* gene and the quantitative and qualitative indicators of the carcass value in pigs. On the base of all obtained results it can be stated, that in the case of allele *A,* present in the *CAST*/*Hinf*I gene, there was a higher lean meat share (i.e. lower fat content) and therefore the detected quality of pork meat was lower. There were signifiant differences found between the homozygotes *AA* and heterozygotes *AB*, mainly in the amount of fat content ($P \leq 0.05$). Concerning the *CAST/Msp*I gene, it was found that the genotype *CD* caused higher lean meat share as well as lowering the pork meat quality. The study also showed a signifiant influence of the allele *D* on the meat share of the main meat parts, i.e. on the IMF content in the neck ($P \leq 0.05$).

The obtained results also show a significant effect ($P \leq 0.05$) of the allele *C* on the quality of pork meat (MS EV_{50}). Another finding was that the *CAST/Rsa*I polymorphism has practically no effect whatsoever on the monitored carcass value characteristics in pigs.

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UTICAJ POLIMORFIZMA GENA *CAST/Msp*I, *Hin*fI, *Rsa*I NA KVALITATIVNA SVOJSTVA PRASADI

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Izvod

Cilj ovog istraživanja je bilo izvršenje tipizacije genova polimorfizma gena *CAST/Msp*I, *Hinf*I, *Rsa*I i proveravanje njihovog mogućeg uticaja na kvantitativne i kvalitativne indikátore koji karakterišu proizvodnu vrijednost prasadi. Utvrđen je značajan uticaj gena CAST na indikatore proizvodne vrednosti prasadi. U slučaju prisutnosti alele *A* kod gena *CAST/Hinf*I je bila otkrivena sklonost za veći dio nemasnog mesa, odnosno manje masti i niži kvalitet svinjskog mesa. Utvrdjene su statistički značajne razlike izmedju homozigota *AA* i heterozigota *AB* naročito kod količinei ($P \leq 0,05$). Kod gena *CAST/Msp*I je utvrdjen uticaj genotipa *CD* na věci deo nemasnog mesna kod prasadi zbog većeg udela muskulature glavnih mesnih dijelova. Medjutim, veći dio nemasnog mesa prasadi vodi do nižeg kvaliteta mesa. Utvrdjen je uticaj alela *D* na sadržinu IMT u delu vrata ($P \leq 0,05$). Rezultati potvrđuju značajan uticaj ($P \leq 0,05$) alela *C* na kvalitativne karakteristike svinjskog mesa (*MS* EC_{50}*)*. U pogledu polimorfizma *CAST/Rsa*I istraživanje je potvrdilo da ovaj polimorfizam ne utiče na kvalitativne parametre proizvodne vrijednosti prasadi.

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