THE EFFECT OF CALPASTATIN (*CAST/MSP*I AND *CAST/HINF*I AND *CAST/RSA*I) AND ITS INTERACTION WITH RYR1GENOTYPES ON CARCASS AND MEAT QUALITY OF CROSSBRED PIGS

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Abstract

The aim of this study was to determine the interactions between gene *CAST* (*MspI, Hinf, RsaI*) and gene *RYR1* and indicators characterizing the quantitative and qualitative aspects of carcass value in pigs.

The study showed a significant interaction between three polymorphisms *CAST/HinfI, MspI, RsaI* and *RYR1* gene in relation to proportion of lean meat as well as the weight of ham ($P \leq 0.01$) and main meat parts ($P \leq 0.05$). Regarding the fatness of pigs, a clear interaction between *CAST/RsaI* polymorphism and the amount of fat covering the loin was discovered. Also for all observed polymorphisms (*Hinf*I, *RsaI* $P \leq 0,05$; *MspI* $P \leq 0,01$) and *RYR1* there were significant interactions regarding the amount of fat covering the shoulder. Another important discovered effect was that of the *CAST/ MspI* ($P \leq 0.05$) and *RYR1* gene on intramuscular fat in the neck areas. The study showed significant interactions ($P \leq 0.01$) between *CAST* gene polymorphisms *Hinf*I, *Msp*I, *Rsa*I, as well as interactions between the *RYR1* gene and meat quality traits (as pH45 *MLLT* was shown). Another important observation concerned *CAST/Hinf*I polymorphism and drip loss.

Key Words: Pig, *CAST, RYR1,* polymorphism, allele, meat quality.

As with any production process, including pork, production is market driven. In this case the demand, which is a priority, is meat quality.

Ciobanu et al. (2004) reported that the quality of porcine meat is influenced by a complex process that depends on the animal species, genetic background, postmortem (*p.m.*) metabolism, protein-muscle complex, environment, etc.

The muscle biochemistry is significantly affected by the *CAST* (*calpastatin*) gene through the *p.m.* meat proteolytic system (Kristensen et al., 2002). Calpastatin is an endogenous inhibitor of calcium-activated protease known as calpain (m and m-calpain) and polypeptide specific calpastatin inhibitor. These proteases participate in regulation of the muscle cells activities. Their activity depends on the cell concentration of calcium ions.

Authors Koćwin-Podsiadła et al. (2004) reported that the growth of skeletal muscle primarily depends on the rate of protein synthesis and degradation, as well as the muscle fibres frequency and size. In this context Mellegren (1997) and Goll et al. (1998, 2003) demonstrated that active calpain and calpastatin are essential for cell proliferation, and thus for normal growth of the skeletal muscles. This fact was well demonstrated by Koćwin-Podsiadła et al. (2003), who found that, as the muscle proteolytic rate changes *p.m.*, the calpastatin activity is significantly associated with growth intensity.

In pigs the *CAST* gene is located on the second chromosome (Ernst et al., 1998). It consists of 30 exons and its length exceeds 160 kb (*Ensembl* database). With the use of restriction enzymes Ernst et al. (1998) identified here discussed polymorphisms *MspI, Hinf* and *RsaI*. Authors Kurył et al. (2003), Rybarczyk et al. (2010 a, b) demonstrated the influence of porcine *CAST* gene on carcass characteristics, including its quality. These findings confirm the studies of Koćwin-Podsiadły et al. (2003), Krzęcio et al. (2004) and Ciobanu et al. (2004), in which the authors also showed that there are significant interactions between *CAST* genes, *RYR1*, skeletal muscle formation, the quality of porcine meat and the weight of the loin, as well as the lean meat share (pig fatness). The relationship between *CAST* and *RYR1* genes was also confirmed by Rybarczyk et al. (2010) and Koćwin-Podsiadła et al. (2003). Rybarczyk et al. (2010) describes a significant interaction between the *CAST / Hinf*, *RYR1* genes and meat quality, particularly with respect to pH, heat loss and drip loss, which was also demonstrated by Koćwin-Podsiadła et al. (2003) and Krzęcio et al. (2005). Significant interactions between *CAST/HinfI, RYR1* and quantitative indicators of carcass value are shown for lean meat share, backfat thickness and loin weight (Stearns et al., 2005; Koćwin-Podsiadła et al., 2004). These authors also demonstrated a relationship between the *CAST/MspI* polymorphism and the weight of shoulder and loin. The effect of *CAST/RsaI* for lean meat share and meat quality was also observed by Koćwin-Podsiadła et al. (2004) and Krzęcio et al. (2008). The objective of the study was to find an interaction between *CAST* gene *(MspI, HinfI, RsaI*) and *RYR1* gene and indicators characterizing the quantitative and qualitative aspects of the carcass value in pigs.

Materials and Methods

Animals

The testing was conducted on 709 animals of hybrid pig combinations commonly used in the Czech Republic. All animals were fattened under the same experimental conditions at the Experimental Test Station of the Czech University of Life Science, Prague. The fattening of pigs was conducted from an average live weight of 30 kg, to slaughter at an average weight of 108 kg. Following which, the slaughter blood was collected from each animal and subsequently high-molecular DNA was isolated.

Nutrition

The nutrition was carried out in keeping with the nutritional needs of pigs (Šimecek et al., 2000). The fattening process was executed with the use of complete feed mixture (CFM) ad-libitum (594 heads) and controlled (115 heads).

Phenotypic values of the carcass value

The carcass analysis was carried out according to Walstra, Mercus (1995) and the dissection according to Stupka et al. (2004). The following quantitative carcass characteristic were monitored:

- lean meat share (%),
- loin eye area (mm^2) from 60 kg of live weight in seven-day intervals to the end of the test) with the help of the ALOKA SSD – MICRUS instrument,
- ham, loin, neck, shoulder and belly share (%),
- main meat parts share (%)
- average backfat thickness (mm) measured in the dividing cutting of the carcass,
- the amount of fat covering the ham, loin, shoulder and neck, including skin (%),
- intamuscular fat (IMF) content in the ham, loin, shoulder and neck (%),
- *p. m.* MLLT pH_{45} and pH_{24} (loin eye area),
- MLLT drip loss 48 hours *p. m.* (%).

Genotyping

For the process of genotyping CAST gene primers were used according to Ernst et al. (1998). Collected blood was first stabilized by EDET and consequently the high molecular DNA was isolated. The DNA was exposed to the reaction mixture of 25µl volume containing 100 ng genomic DNA, standard PCR buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 10 pmol primers, 2% DMSO and 1.0 U LA DNA polymerase (Top Bio, Prague, CR). The DNA cycling conditions were carried out 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). PCR product was split with the use of three restriction enzymes and the resulting fragments were obtained, each with an approximate length of MspI (~ -760 C / D -370) HINFO (~ -790 A / B -500) and RsaI (E ~ -360 / -250 F).

Statistical Analysis

The obtained results were evaluated with the use of mathematical and statistical software (SAS Institute 9.1) by GLM-procedure. A model with fixed effects (*CAST/ RYR1* genotypes, nutrition, sex) was utilised, with the use of the carcass weight standing in the place of the regression coefficient. The resulting formula was as follows:

$$Y_{ijklmn} = \mu + a_i + b_j + c_k + d_l + e_m + \beta X_n + e_{ijklmn}$$, 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 carcass weight,
X_n = animal´s carcass weight n,
e_{ijklmn} = residual error.

Results and Discussion

The analysis of three gene polymorphisms *CAST* (*CAST/ Msp*I, *CAST/Hinf*I, *CAST/Rsa*I) and *RYR1* gene was carried out and the interactions between these genes were established. The results of the analysis of alleles frequency and genotype frequencies of individual *CAST* gene in animals are illustrated in Table 1.

As it is evident from Table 1, all three genotypes in all tested gene polymorphisms *CAST* were represented, while the genotypes in the tested animal population were identified at locus *CAS/HinfI (AA, AB, BB), CAST/MspI (CC, CD, DD)* and *CAST/RsaI (EE, EF, FF)*. The *RYR1* gene is absent of any pigs with genotype *TT*.

The effect of polymorphism between genotypes *CAST/ HinfI, MspI, RsaI* and *RYR1* and carcass characteristics in pigs is shown in Table 2.

Table 1. The genotype frequencies of the gene CAST/ HinfI, MspI, RsaI

Item	*CAST/Hinf*I			*CAST/Msp*I			*CAST/Rsa*I		
N=564	*AA*	*AB*	*BB*	*CC*	*CD*	*DD*	*EE*	*EF*	*FF*
rate of alleles	56	250	258	134	273	157	205	253	106
frequency of alleles	*A*=0,32 *B*=0,68			*C*=0,48 *D*=0,52			*E*=0,59 *F*=0,41		
frequency of genotypes (%)	9.9	44.3	45.8	23.8	48.4	27.8	36.3	44.9	18.8

Table 2. Results of the analysis of the variance between genotypes of gene CAST/HinfI, MspI, RsaI and RYR1 gene and selected characteristics of the carcass value in pigs

Item	LS-MEAN	MSE	*CAST/Hinf*I $F_{emp.}$	*RYR1* F_{emp}	*CAST/Msp*I F_{emp}	*RYR1* F_{emp}	*CAST/Rsa*I F_{emp}	*RYR1* F_{emp}
Lean meat share - FOM (%)	55.59	2.65	1.20 (NS)	5.52**	0.95 (NS)	5.56**	0.37 (NS)	4.29*
Loin eye area - *MLLT* (mm^2)	4966	469	0.47 (NS)	2.48 (NS)	0.45 (NS)	2.11 (NS)	0.81 (NS)	1.97 (NS)
Ham share (%)	21.76	1.60	0.76 (NS)	7.09**	0.64 (NS)	7.52**	0.36 (NS)	7.78**
Loin share (%)	12.79	0.93	1.79 (NS)	0.97 (NS)	1.03 (NS)	1.01 (NS)	0.51 (NS)	1.13 (NS)
Neck share (%)	6.72	0.59	2.35 (NS)	2.20 (NS)	0.78 (NS)	1.97 (NS)	0.00 (NS)	1.14 (NS)
Shoulder share (%)	10.02	0.68	0.28 (NS)	0.10 (NS)	1.66 (NS)	0.07 (NS)	0.95 (NS)	0.23 (NS)
Main meat parts share (%)	66.47	1.49	0.39 (NS)	4.75*	0.46 (NS)	5.05*	0.32 (NS)	4.44*
Average backfat thickness (mm)	28.12	3.85	1.20 (NS)	1.19 (NS)	0.02 (NS)	1.39 (NS)	0.28 (NS)	0.78 (NS)
Fat covering of the ham (%)	5.30	1.21	0.27 (NS)	0.04 (NS)	0.42 (NS)	0.09 (NS)	0.34 (NS)	0.19 (NS)
Fat covering of the loin (%)	5.12	0.83	0.17 (NS)	3.85 (NS)	0.49 (NS)	3.09 (NS)	0.29 (NS)	3.78*
Fat covering of the neck (%)	1.24	0.31	0.36 (NS)	0.00 (NS)	2.81 (NS)	0.00 (NS)	1.08 (NS)	0.17 (NS)
Fat covering of the shoulder (%)	3.35	0.48	2.61 (NS)	5.93*	0.33 (NS)	6.08**	0.77 (NS)	5.10*
Belly share (%)	17.57	1.07	1.32 (NS)	1.27 (NS)	2.39 (NS)	0.20 (NS)	0.67 (NS)	1.45 (NS)
IMF content in the ham (%)	3.74	1.39	1.96 (NS)	0.74 (NS)	1.31 (NS)	0.97 (NS)	0.43 (NS)	0.85 (NS)
IMF content in the loin (%)	2.05	0.74	0.38 (NS)	1.76 (NS)	0.35 (NS)	1.36 (NS)	1.43 (NS)	1.52 (NS)
IMF content in the neck (%)	6.01	2.69	0.82 (NS)	1.70 (NS)	3.12*	2.40 (NS)	2.82 (NS)	1.54 (NS)
IMF content in the shoulder (%)	2.46	0.69	0.64 (NS)	3.51 (NS)	1.93 (NS)	3.41 (NS)	2.47 (NS)	3.11 (NS)
p. m. MLLT pH_{45}	6.22	0.28	0.07 (NS)	8.56**	0.12 (NS)	10.98**	0.26 (NS)	9.62**
p. m. MLLT pH_{24}	5.54	0.09	0.77 (NS)	0.79 (NS)	0.67 (NS)	1.20 (NS)	0.81 (NS)	2.13 (NS)
MLLT driploss (%)	7.90	2.86	0.28 (NS)	4.97**	0.99 (NS)	4.65 (NS)	0.27 (NS)	2.61 (NS)

NS – not significant, ** significant $P \leq 0.01$, * significant $P \leq 0.05$,
LS-MEAN – Least squares means, MSE – Standard error, F_{emp} – calculated F-value

As it is evident, the table shows a significant interaction between the three studied polymorphisms gene CAST/ HinfI, MspI, RsaI and gene RYR1 in relation to the lean meat share, ham ($P \leq 0.01$) and the main meat parts ($P \leq 0.05$). Regarding the indicators of the pig fatness, an interaction was demonstrated between the fat covering of the loin and CAST/RsaI polymorphism. For fat covering of the shoulder significant interactions were found in all the observed polymorphisms (HinfI, RsaI $P \leq 0.05$, MspI $P \leq 0.01$) and RYR1.

A significant effect of the *CAST/MspI* ($P \leq 0.05$) gene polymorphism and *RYR1* gene on IMT content in the neck was also discovered. Author Rybarczyk et al. (2010 b) demonstrated the influence of the *CAST/MspI* and *RYR1* genes on a higher lean meat share. Conversely, Koćwin-Podsiadła et al. (2004) reported that the *CAST/MspI* gene is associated with higher proportions of loin, shoulder, and backfat thickness. They also showed that the *CAST/RsaI* gene significantly affects the proportions of loin, shoulder and backfat thickness.

Important interactions between *CAST/Hinfl, MspI, RsaI* polymorphisms, *RYR1* gene and *pH45 MLLT* (P≤0.01) were also discovered in this study. The same interaction was also detected between the *CAST/Hinfl* polymorphism and drip loss.

A significant effect of *CAST/Hinf*l polymorphism on porcine meat quality was also shown by Rybarczyk et al. (2010 a). They found a notable effect of this polymorphism on higher pH_{24} as well as drip loss. The same results were also reported by Kurył et al. (2004). The above mentioned authors state that the interaction between *CAST* and *RYR1* genes in relation to drip loss results with action of the *RYR1* gene or activation of calpain-calpastatin system to Ca^{2+} ions.

Furthermore Krzęcio et al. (2005) documented the influence of the *CAST/MspI* polymorphism on the lactic acid level in *MLLT*45´*p.m.*, drip loss 48 and 96 hours *p.m.*, and on the amount of muscle protein. In this context, Koćwin-Podsiadła et al. (2003) found that the glycogen level in the *MLLT* 45´ *p.m.* is directly related to the *CAST/MspI* genotype. Although Koćwin-Polsiadła et al. (2003) state that there is a significant influence of *CAST/RsaI* gene on pH45 and drip loss, the findings in this study did not confirm that hypothesis. However Krzęcio et al. (2008) confirms that the pH is affected by *CAST/RsaI*.

Conclusion

Significant effects of the *CAST* gene on quantitative and qualitative indicators of the carcass value were found in pigs. An important interaction was found among monitored polymorphisms *CAST/HinfI, MspI, RsaI* and *RYR1* gene in relation to the lean meat and ham share (P≤0.01) as well as main meat parts (P≤0.05). Regarding the indicators characterizing pig fatness, only the interaction (P≤0.05) between fat covering of the loin and *CAST/RsaI* was demonstrated. In case of shoulder-fatness, a significant interaction for all tested polymorphisms (*Hinf*I, *Rsa*I P≤0.05; *Msp*I P≤0.01) was shown. Another substantial interaction was found (P≤0.05) between *CAST/MspI, RYR1* polymorphism and IMT content in the neck. Futhermore there were another important interactions (P≤0.01) found between the *CAST/Hinf*I, *Msp*I, *Rsa*I, *RYR1* polymorphisms and meat quality traits, namely for pH_{45} and drip loss.

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