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Original scientific paper

EFFECT OF THE SER638ARG VARIATION IN THE *CAST* GENE AND CAUSAL SNP G.1843C>T IN THE *RYR1* GENE ON CARCASS TRAITS IN CROSBREED PIGS

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The objectives of this investigation was to demonstrate that genotypes of calpastatin (*CAST*) Ser368Arg and ryadonide receptor 1 (*RYR1*) g.1843C>T may affect the carcass traits of pigs. Association analysis of the aforementioned SNPs was performed on 518 pigs included eight commercial crossbreeds and one pure pig breed. All pigs were slaughtered at average body weight of 113kg. Genotypes at the SNPs were determined by PCR–RFLP. There were found only two genotypes of *RYR1* gene: *CC* (428 pigs) and *CT* (90 pigs) in this study. The effect of allele *C* compared to allele T on the higher fat content of pig carcass was confirmed. The allelic frequencies of allele 638Ser (*C*) and allele 638Arg (*A*) were 0.26 and 0.74, respectively. The significant association ($P<0.05$) was occurred between allele *A* and higher fat content, and between *C* allele and higher lean meat content. Our results showed no significant effect of the observed polymorphismSer638Arg on the intramuscular fat content or other indicators of carcass value.

Keywords: Calpastatin, PCR-RFLP, meat quality, pig

INTRODUCTION

The genetic factors of fat tissue accumulation are intensively studied for many reasons. Fatness traits (most frequently backfat thickness and intramuscular fat content) are very important in pig production. Moreover, fat accumulation is intensively studied in humans, due to

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the worldwide spread of the obese phenotype (SWITONSKY *et al.*, 2010). Nowadays, consumers pay more attention to the meat quality,especially in meat for culinary purposes (BORUSZEWSKA *et al.*, 2016). The causal SNP was found in the RYR1 gene, which was related to malignant hyperthermia susceptibility in pigs (MA *et al.*, 2012). This gene defect leads to changed membrane characteristics in the skeletal muscle fibres, especially to an increased release of calcium ions from the sarcoplasmic reticulum as a response to different stress factors (OLIVÁN *et al.*, 2018). In pigs that carry allele *T*, FIEDLER *et al.* (1999) reported increased diameters of the mean fibre types and increased glycolytic metabolic potential, lower pH value and higher drip loss. Although many studies have demonstrated a major influence of the *RYR1* gene on qualitative traits, many authors have reported a significant influence of other candidate genes in stress-resistant populations (RYR^T) (KRZĘCIO *et al.*, 2008).

CAST (calpastatin) is one of the genes which affect muscle cells and its specific inhibitor of calpain enzymes (CHOI *et al.*, 2016). The calpain-calpastatin proteolytic system plays an important role in the normal growth of skeletal muscles during the postnatal period (GOLL *et al.*, 1998). Moreover, calpastatin influences the degradation of muscle protein. Therefore, this gene affects the proteolytic system of meat during the post mort period (KRISTENSEN *et al.*, 2002; ROPKA-MOLIK *et al.*, 2014). The calpastatin gene (*CAST*) is located on chromosome 2 (2q2.1–2q2.4) (ERNST *et al.*, 1998; DAVOLI *et al.*, 2017). CIOBANU *et al.* (2004) reported an association analysis of a missense mutation (Ser638Arg) in the *CAST* gene where quantitative trait loci (QTL) for meat quality such as shear force and tenderness,were described. Several *CAST* polymorphisms have been investigated, including Arg249Lys and Ser638Arg, and some of them have been associated with meat tenderness, pH, colour, and drip loss qualitative traits in pigs (DAVOLI *et al.*,2017; ZHANG *et al.*,2018). Moreover, STALDER *et al.* (2005) found an significant effect of *CAST* gene marker as source of variation for cured moisture content of ham. Research by KÓCWIN- PODSIADŁA *et al.* (2003), KRZĘCIO *et al.* (2005; 2008) also demonstrated the effect of several other (intron) polymorphisms (SNPs) in the *CAST* gene on backfat thickness, weight of the shoulder, meat colour and pH value. However, ŠKRLEP *et al.* (2010) did not find any effect of p.Ser638Arg in the *CAST* gene on the weight of ham and backfat thickness.

MATERIAL AND METHODS

Animals

All procedures described in this study were conducted after obtaining the approval by the Local Ethics Commission, the experiment was conducted in CZ21038206. Association analysis of SNPs in the *CAST* and *RYR1* genes with carcass traits was performed in a commercial crossbred pig population. Data were collected from 518 pigs. This population included eight commercial crossbred combinations and one pure breed of pigs (Table 1). All pigs were maintained in a testing station, in an air-conditioned barn, essentially under the conditions as described by DVOŘÁKOVÁ *et al.* (2012) and housed in pens according to sex. They were fed ad libitum with complete feed mixtures.

Carcass and meat quality traits

Pigs were weighed individually in weekly intervals and feed intake was daily monitored. Based on thus obtained values, average daily weight gain (ADG), feed conversion ratio (FCR) and average daily feed intake (DFI) were calculated. The age and average slaughter weight of pigs at the end of fattening was 164 days and 113kg, respectively. Pigs were slaughtered at a

small commercial abattoir and parameters of carcass traits, the carcass weight, lean meat percentage (FOM) and backfat thickness were measured. Carcass dissection was performed according to WALSTRA and MERKUS (1995). From the qualitative parameters, the pH value (45 min p.m.) and electrical conductivity (50 min p.m.) were measured. Intramuscular fat content (IMF) was measured in chop, shoulder, neck, ham by Soxhlet methods.

Table 1 The groups of testeds animals

Groups	Pig crosses	No.
1	PNx(CLxCLW$_D$)	65
2	CLW$_D$, CLW$_D$xCL, PNx(CLxCLW$_D$)	62 (18+20+24)
3	(CLW$_S$xPN)x(CLxCLW$_D$), PICx(CLxCLW$_D$)	70 (36+34)
4	FHxPIC	62
5	(DxCLW$_S$)x(CLxCLW$_D$)	56
6	(PNxCL)x(CLxCZLW$_D$)	66
7	PNx(CLxCLW$_D$)	66
8	(PNxH)x(CLxCLW$_D$)	71

*PN - pietrain; CL – czech landrase; CLW – czech large white; PIC – programme; FH – France hybrid boar; H - Hampshire

Isolation of DNA and genotyping

Genomic DNA was isolated from blood using a QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). All animals were genotyped for the Ser638Arg in the *CAST* gene and for causal mutation g.1843C>T in the *RYR1* gene inaccordance with to Ciobanu et al. (2004) and BRENIG*et*BREM (1992), respectively. The primers used for amplifying the fragments and PCR conditions are presented in Table 2. Polymerase chain reaction was performed in 25 µl reaction volumes using 100 ng of genomic DNA, standard reaction buffer, 1.0 or 1.5 mM MgCl2 (see 200 µM of each dNTP, 10 pmol of each primer and 1 U Taq polymerase (Top-Bio, Prague, Czech Republic). The PCR profile was 3 min at 95°C, followed by 31 cycles of 60 s at 95°C, 30 s at 62°C, and 30 seconds (see Table 2) at 72°C. There was a final extension of 5 min at 72°C. SNP g.1836C>T was genotyped with H*ha*I (T 134bp/C 84bp+50bp) and Ser638Arg (A 183/C 142+41 was genotyped with P*vu*II. DNA fragments were separated on a 3% agarose gel.

Table 2. Primers and PCR conditions for amplification of RYR1 and CAST fragments

Primer name	Primer sequences (5′ - 3′)	Location	Amplicon length (bp)	DNA polymerase	$MgCl_2$ (mM)	T_a (°C)	Extension (sec)	Note
RYR1-F′	GTCCCAGAAACAACCTAACA	exon 17	134	1 U Taq	1.0	62	30	Brenig
RYR1-R′	TCCTACCCCACAGAAACAATAT							a Brem
								(1992)
CAST-F′	GTCCCAGAAACAACCTAACA	exon 28	183	1 U Taq	1.5	61	30	Cioban
CAST-R′	AGCAACCCCAGTGGTACTGA							u et al.
								(2004)

Statistical analysis

The effect of the missense mutation p.Ser638Arg of the CAST gene and causal mutation g.1843C>T of the *RYR1* gene on quantitative and qualitative traits was analysed using the UNIVARIATE, MEANS, GLM procedures in SAS statistical programme (Statistical Analysis System, Version 9.4, 2012). Differences between selected traits were tested by analysis of variance (Sheffe´s test). The model included the *CAST/RYR1* genotype, a crossbred combination, gender, and the carcass weight as a regression coefficient (the regression coefficient did not use for average daily gain).

$$Y_{ijklm} = \mu + a_i + b_j + c_k + d_l + \beta X_m + e_{ijklm}$$

where $Y_{ijklm}$is the value of the trait; μ is the overall mean; aiis the effect of *CAST* genotype (i = 1, 2, 3); $b_j$is the effect of *RYR1* genotype (j = 1, 2); $c_k$is the effect of crossbred combination (k = 1, 2, 3, 4, 5, 6, 7, 8, 9); $d_l$is the effect of sex (l = 1, 2); β is the regression coefficient on carcass weight; $X_m$is carcass weight of the animal n; $e_{ijklm}$is the random residual.

RESULTS AND DISCUSSION

Only two genotypes of the causal mutation in the *RYR1* gene in the tested population were found in this study (*CC* in 428 and *CT* in 90 pigs). This fact can be explained by the strict negative selection of pigs with the *T* allele. The *T* allele is responsible for PSE manifestation in pork meat (lower pH value, higher drip loss, increased glycolytic metabolits potential, *etc*.; RYBARCZYK *et al.*,2012; DE SMET *et al.*,1996; LEACH *et al.*,1996). However, there were no significant differences in meat quality between RYR1 genotypes.A statistically significant effect on live weight (P<0.01) and on the average daily gain (P<0.02), fat content of belly (P<0.01), and backfat thickness (P<0.04) for the *CC* genotype was found. This fact can be explained as the effect of allele *C* on greater fatness and lower lean meat content. Many authors confirm our results (FIEDLER *et al.*,1999; OTTO *et al.*, 2007; CESAR *et al*., 2017; OLIVÁN *et al.*,2018).

Table 3. Association analysis of genotypes at Ser638Arg in the CAST gene with production traits

Traits	*AA*	*AC*	*CC*	P value
Live weight (kg)	113±0.22	112±0.25	111±0.85	NS
Carcass yield (%)	81.37±0.17	81.53±0.21	82.01±0.63	NS
Average daily gain (g)	887±5.07	877±5.76	890±11.11	NS
Feed conversion ratio (g)	2.82±0.04	2.87±0.04	3.02±0.11	NS
Fat of the belly (%)	$32.38±0.89^{A}$	$29.80±1.13^{B}$	$26.80±3.43^{B}$	**0.02**
IMT content in the chop (%)	1.81±0.10	1.75±0.11	1.61±0.29	NS
IMT content in the shoulder (%)	2.36±0.10	2.28±0.12	2.63±0.34	NS
IMT content in the neck (%)	3.97±0.25	4.24±0.30	4.18±0.84	NS
IMT content in the ham (%)	3.41±0.29	3.47±0.33	3.67±1.22	NS
Lean meat share (%)	$54.86±0.32^{A}$	$55.33±0.35^{A}$	$57.28±0.68^{B}$	**0.003**
Backfat thickness (mm)	$17.61±0.39^{A}$	$16.97±0.44^{B}$	$14.81±0.85^{C}$	**0.006**
Meat depth (mm)	$60.53±0.65^{A}$	$60.04±0.73^{B}$	$62.97±1.41^{B}$	**0.01**
EV_{50} (mS^{-1})	3.9±0.08	3.83±0.1	4.02±0.24	NS
pH_{45}	6.37±0.03	6.4±0.03	6.34±0.007	NS

A-B - values with different superscripts differ significantly at least at P < 0.05

NS – non significant

CAST polymorphism Ser638Arg frequencies of genotypes did not differ significantly within all analyzed crossbreeds combination. The frequency of all genotypes was 0.26 (allel *A*) and 0.74 (allel *C*) in the *CAST* gene, (*AA* in 297, *AC* in 187, *CC* in 34 pigs). The significant effect of Ser638Arg in the *CAST* gene on the lean meat content ($P<0.003$) and backfat thickness ($P<0.006$) was demonstarated in this study. On the contrary, we did not find any effect on the live weight and carcass yield as shown in Table 3. These findings corresponded with the conclusions of research by STALDER *et al.* (2005) and ŠKRLEP *et al.* (2010). The significant effect of this mutation has also been observed on the fat content of belly ($P<0.02$) for genotype *AA* (638Agr). However, ŠKRLEP *et al.* (2010) did not find any significant differences between the *CAST* genotype and backfat thickness.

Although calpastatin influences the fusion of myoblasts via the enzyme m-calpain (COTTIN *et al.*, 1994; HUFF-LONERGAN *et al.*, 2005), the effect of this substitution mutation on the average daily gain was non significant.Significant differences were found between *CAST* genotypes for average daily gain in purebreed pig of Polish landrace and Polish large white in URBANSKI *et al.* (2015). Also DAVOLI *et al.* (2017) described the conclusive effect of the *CC* genotype on carcass weight.

CONCLUSION

The effect of the *CAST* gene was observed in fatness and lean meat content. We assume an effect of allele 638Arg on greater fatness and allele 638Ser on higher lean meat content. Moreover, a clear effect of causal mutation g.1843C>T in the *RYR1* gene on growth rate and fatness was demonstrated.

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EFEKAT VARIJACIJE SER638ARG KOD *CAST* GENA I UZROČNOG SNP G.1843C>T KOD *RYR1* GENA ZA OSOBINE TRUPA U UKRŠTANJIMA SVINJA

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Izvod

Ciljevi ovog istraživanja su bili da se pokaže da genotipovi CAST Ser368Arg i riadonide receptor 1 (RIR1) g.1843C> T mogu uticati na osobine trupa kod svinja. Asocijativna analiza pomenutih SNP-ova izvršena je na 518 svinja, uključujući osam komercijalnih ukrštanja i jednu čistu svinjsku rasu. Sve svinje su zaklane pri prosečnoj telesnoj težini od 113 kg. U ovom radu nađena su samo dva genotipa sa RYR1 genom: CC (428 svinja) i CT (90svinja). Potvrđen je efekat alela C u odnosu na alel T na viši sadržaj masti kod svinjske polutke. Frekvencija alela 638Ser (C) i alela 638Arg (A) bila je 0.26, odnosno 0.74. Značajna korelacija ($P<0.05$) utvrđena je između alela A i višeg sadržaja masti, i između alela C i višeg sadržaja nemasnog mesa. Naši rezultati su pokazali da nema značajnog efekta proučavanog polimorfizma Ser638Arg na sadržaj intramuskularne masnoće ili druge parametre kvaliteta svinjske polutke.

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