Influence of C489T SNP at MYOD1 Gene on Carcass, Meat Quality Traits and Chemical Composition of Hybrid Pigs

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Summary

Study was carried out on 82 hybrid pigs with the aim to investigate influence of g.489C>T SNP of MYOD1 on carcass traits, meat quality and on chemical composition of *longissimus dorsi* muscle. Slaughter was carried out at approximately 110, 125 and 150 kg live weight. Carcass lengths from *os pubis* to *atlas* and from *os pubis* to 1st rib, ham length (from *os calcaneus* to *os ichium*) and its circumference, pH_{45} and pH_{24} at *semimembranosus* and *longissimus dorsi* muscles and electrical conductivity were determined at slaughter line. Loin eye and fat area according to Comberg (1978), CIE $L^*a^*b^*$ colour coordinates, drip loss by "bag method", cooking loss and shear force were measured in laboratory at *longissimus dorsi* muscle. Contents of intramuscular fat, protein, moisture and collagen were determined by FoodScan™ Meat Analyser. Pig DNA was isolated from animal tissue and genotyped for C489T substitution at MYOD1 gene. Genotype frequencies were 20.73%, 36.58% and 42.68% for CC, TT and CT, respectively. C489T mutation influenced all carcass traits, where CT genotype showed preferable values in carcass lengths, ham length, its circumference and loin eye area indicating its potential use as marker for improved pig carcass traits. Influence of C489T SNP was observed for pH_{45} in *semimembranosus* muscle and both pH_{45} and pH_{24} in *longissimus dorsi* muscle. The same genotype showed favourable values for all three traits.

Key words

pigs, C489T SNP, MYOD1, carcass and meat quality traits, chemical composition

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Aim

Due to the high share of pork in the total meat consumption different animal and food producing industries are faced with various challenges to ensure sufficient pork quantities together with satisfactory quality. Numerous studies suggest that increased pig meat leanness often results in decreased meat quality (Sonesson et al., 1998; Kralik et al., 2002; Kušec et al., 2004). Muscle formation and muscle growth processes have significant effect on meat quality traits (Kuhn et al., 2002; Rehfeldt, 2005; Rehfeldt and Kuhn, 2006). Pork quality is influenced by the muscle fibres number and size (Rehfeldt et al., 2000; Lee et al., 2012b). In addition to non-genetic effects the muscle fibres number is determined by genetic factors which are capable to influence prenatal myogenesis (Rehfeldt et al., 2000). MyoD family of genes encodes highly conserved basic helix-loop-helix proteins that control the embryonic muscle fibers development and their postnatal hypertrophic growth (Olson, 1990). It is consisted of four related genes: MYOD1 (MYF3), MYF4, MYF5 and MYF6. Identification of MYOD1 gene, as the main regulatory gene for skeletal muscle differentiation, enabled the assessment of the role of tissue-specific transcription factors in the regulation of cell differentiation (Tapscott, 2005). Several previous MYOD1 SNP studies showed that MYOD1 can be a very important factor for carcass and meat quality traits (Kapelański et al., 2005; Urbanski et al., 2006; Lee at al., 2012a). Changes in the expression or variations in MYOD1 gene can affect the number of muscle fibers and their thickness, which has a significant impact on carcass and meat quality traits (te Pas et al., 2000; Urbanski et al., 2006; Stupka et al., 2012).

The aim of this study was to investigate associations of C489T SNP, located in the MYOD1 gene, with carcass and meat quality traits, as well as with chemical composition of *m. longissimus dorsi* in hybrid pigs.

Material and methods

The animal material consisted of 82 PIC (Pig Improvement Company; P337xC23) pigs, gilts (n=41) and barrows (n=41) originating from three sires and 21 dams. Pigs were housed under same conditions and fed balanced diets with *ad libitum* access to feed and water. The animals were kept to 110, 125 and 150 kg live weight. When the animals reached planned slaughter weight (±2 kg) they were transported to the slaughterhouse and slaughtered after electrical stunning (225-380 V; 0.5 A; 5-6 s).

At the slaughter-line hot carcass weight (HCW), carcass lengths "a" (from *os pubis* to *atlas*) and "b" (from *os pubis* to 1st rib), ham length (from *os calcaneus* to *os ichium*) and circumference (at its widest part), were measured. pH measurements were taken 45 minutes and 24h *post mortem* at *m. longissimus dorsi* (*LD*) and *m. semimembranosus* (*MS*). Measures of electric conductivity (EC) were taken at the same time and in the same places as for pH. Backfat and muscle areas were measured by geometric procedure (Comberg, 1978) and expressed as fat/loin eye ratio (%). Meat colour was determined using Minolta CR-300 chromameter (Minolta Camera Co. Ltd., Osaka Japan) calibrated against white plate (L^*=93.30; a^*=0.32 and 1.8; b^*=0.33) with a D65 light source and 2° standard observer. The colour values were expressed as CIE $L^*a^*b^*$ (Commission Internationale de l'Eclairage, 1976). Drip loss was measured according to Honikel (1987) after 48h of cooling the samples at 4°C. Instrumental tenderness (shear force) was measured on TA.XT*plus* Texture Analyser fitted with a 1 mm thick Warner-Bratzler shear attachment after cooking the meat in a water bath to internal temperature of 73°C and cooling it for 24h at 4°C.

The chemical composition of *LD* muscle (share of protein, moisture, fat and collagen) was determined using MeatScan™ NIR analyser (Foss, Denmark).

The genomic DNA was isolated from muscle tissue with the "High Pure PCR Template Preparation Kit" (Roche Diagnostics GmbH). After DNA isolation PCR analysis was performed with primer sequences reported by Urbanski et al. (2004). PCR was performed using the "HotStar Taq® Plus Master Mix Kit" (Qiagen Sample & Assay Technologies GmbH; Hilden, Germany) in a total volume of 20 µL containing 7.5 mL HotStar Taq® Plus Master Mix, 9.5 µL ddH_2O, 1µL of each primer and 1µL DNA (200 ng/µL concentration). PCR amplification was performed in a GeneAmp PCR System 2700 (Applied Biosystems) according to the following temperature regime: initial denaturation at 95°C for 15 minutes, followed by 35 cycles of denaturation at 95°C for 60 seconds, annealing at 55°C for 30 seconds and elongation at 72°C for 60 seconds, with the final elongation step at 72°C for 7 minutes. The obtained PCR products were purified using High Pure PCR Clean Up Micro Kit (Roche, Germany) and sequenced with 96-capillary "ABI3730xl DNA Analyzer" (Applied Biosystems, USA) sequencer using BigDye Terminator Kit and fluorescent automatic sequencing protocol. SNP identification was performed by DNA Baser Sequence Aligner v3.5.2, EditSeq 5.01 and MegAlign 5.01 (DNASTAR Lasergene Core Suite).

The relationship between genotypes at MYOD1 gene and carcass and meat quality traits was performed using mixed model ANCOVA in Statistica 10 (StatSoft Inc.), where sex and genotype were treated as fixed and date of slaughter as random effect, while hot carcass was included as covariate, when significant for all traits. The differences between genotypes were evaluated using Fisher LSD test, where $p<0.05$ was classified as significant difference and $p<0.1$ as tendency.

Results and discussion

The results of sequence analysis showed three genotypes on C489T locus of MYOD1 gene: CC, CT and TT, respectively. Of 82 animals investigated 20.73% were homozygous CC and 36.58% were TT genotype, while 42.68% were heterozygous. Urbański et al. (2006b) reported 55.5% of CT genotype, 23.3% of CC genotype and 21.2% of TT genotype in Yorkshire breed. In Landrace pig breed these frequencies were 51.3%, 37.4% and 11.3% for CT, CC and TT genotypes, respectively. Lee et al. (2012a) reported frequencies of 8.0% for CC, 50.0% for CT and 33.0% for genotype in Berkshire breed and 23.0% for CC, 43.0% for CT and 33.0% in Yorkshire breed, respectively. In hybrid gilts belonging to Line 990 the frequencies were 15.5% for CC, 52.4% for CT and 32.1% for TT genotype (Urbański et al., 2005), while in Torhyb line crossbreed fatteners 34.4% of the animals belonged to CC genotype, 53.8% belonged to CT and 11.8% to TT genotype (Urbański et al. 2007).

Table 1. LS means ± Sd for investigated carcass traits of pigs as related to C489T SNP at MYOD1 locus

Trait	Genotype		
	CC	CT	TT
N	17	35	30
Carcass length „a" (cm)	95.24^{a}±3.83	95.77^{a}±4.39	93.03^{b}±3.46
Carcass length „b" (cm)	112.35^{a}±4.33	113.60^{a}±3.52	109.90^{b}±3.71
Ham length (cm)	34.82^{b}±1.51	35.46^{a}±1.01	34.93^{b}±1.70
Ham circumference (cm)	75.12^{a}±3.30	75.34^{a}±4.11	73.47^{b}±4.01
Loin eye area (cm^2)	52.84^{B}±6.66	55.90^{A}±6.13	53.84^{AB}±7.52
Fat area (cm^2)	22.04^{A}±5.97	21.86^{B}±5.88	20.24^{C}±5.47
Fat/meat ratio (%)	41.53^{A}±9.84	39.27^{A}±11.11	37.73^{B}±9.61

^{a,b}Mean values in the same row with different superscript statistically differ ($p<0.05$); $^{A,B}p<0.1$. Length "a" (*os pubis - atlas*); length "b" (*os pubis* - 1st rib); ham length (*os calcaneus - os ichium*).

Table 2. LS means ± Sd for meat quality traits and chemical properties of *longissimus dorsi* muscle as related to C489T SNP at MYoD1 locus

Trait	Genotype		
	CC	CT	TT
N	17	35	30
pH_{45}, *MS*	6.29^{B}±0.16	6.37^{A}±0.21	6.30^{B}±0.32
pH_{45}.*LD*	6.25±0.22	6.20±0.33	6.32±0.26
pH_{24}.*MS*	5.67^{AB}±0.13	5.69^{A}±0.26	5.60^{B}±0.10
pH_{24}.*LD*	5.63^{ab}±0.14	5.69^{a}±0.15	5.62^{b}±0.13
EC_{45}.*MS* (mS/cm^2)	5.01±1.05	5.52±1.32	5.15±1.64
EC_{45}.*LD* (mS/cm^2)	4.62±0.96	4.63±1.19	4.37±1.53
EC_{24}.*MS* (mS/cm^2)	8.25±2.16	8.55±1.62	7.93±1.73
EC_{24}.*LD* (mS/cm^2)	4.84±1.48	4.99±1.92	4.78±1.80
CIE L^*	49.79±3.45	50.26±2.69	51.22±3.42
CIE a^*	7.47±1.26	7.58±1.38	7.25±1.03
CIE b^*	3.21±0.86	3.29±0.86	3.18±0.91
Drip loss (%)	5.64±2.78	5.26±3.01	5.76±2.86
Cooking loss (%)	33.06±1.80	33.41±1.85	33.40±2.40
Shear force (N)	49.45±7.64	48.08±6.27	46.87±8.01
IMF (%)	2.80±0.72	2.78±0.76	2.82±0.86
Moisture (%)	73.00±0.60	73.24±0.87	73.00±0.85
Protein (%)	23.80±0.45	23.66±0.69	23.81±0.49
Collagen (%)	1.01±0.17	0.99±0.21	0.96±0.20

^{a,b}Mean values in the same row with different superscript statistically differ ($p<0.05$); $^{A,B}p<0.1$.*MS – m. semimembranosus*; *LD – m. longissimus dorsi*; IMF – intramuscular fat

CT genotype exhibited the longest carcases (both "a" and "b" lengths) together with ham length and its circumference (Table 1). The same genotype had the biggest loin eye area.

This is in accordance with investigations of Lee et al. (2012a), who determined a positive influence of CT genotype on loin eye area and meat quality traits suggesting that the transition C489T in exon 1 of MYOD1 gene can be useful DNA marker for improvement of economically important pig carcass traits. Contrary to our results, Urbański et al. (2006a) reported that in Polish Large White x Polish Landrace fatteners the biggest loin eye area was observed in CC genotype, while in (Polish Large White x Polish Landrace) x Pietrain population loin eye area was not under significant influence of C>T transition at MYOD1 locus.

Association of obtained genotypes with meat quality traits and chemical components of *LD* muscle can be observed in Table 2. Differences between genotypes were found for pH_{45} in *MS* muscle and pH_{24} in both *MS* and *LD* muscles. Favourable values for all three traits were observed in CT genotypes. C>T transition at MYOD1 locus did not influence any other investigated meat quality trait nor the chemical composition of *LD* muscle. Similar to our results, Han et al. (2012) reported a significant effect of g.257A>C SNP located in exon 1 of MYOD1 on muscle pH in Large White, while Kapelański et al. (2005) determined a significant effect of g.489C>T on final pH values, colour score and ash content. Contrary to our results, Lee et al. (2012a) determined a positive relationship between C>T substitution at

MYOD1 locus on colour lightness ($p=0.07$). Lack of effect on colour values can be a consequence of animal handling before, during and after the transport as well as transport and lairage time. Furthermore, meat quality traits are regulated by number of genes, such as RYR1 and PRKAG3, which influence cannot be excluded. Kapelański et al. (2004) and Liu et al. (2008) concluded that meat quality traits, such as meat colour, electric conductivity and drip loss are under bigger influence of RYR1 gene than MyoD gene family. For that reason we recommend to repeat investigation with RYR1 and PRKAG3 genes included.

Conclusions

g.489C>T influenced all measured carcass traits and both initial and ultimate pH value, where heterozygous genotype exhibited favourable values in all traits, indicating its potential use in selection aimed at improvement of economically important carcass and meat quality traits.

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