PRKAG3 Gene and Meat Quality of Hybrid Pigs

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Summary

The study was performed on 89 PIC (Pig Improvement Company) pig carcasses with the aim to investigate influence of I199V polymorphism at PRKAG3 locus on meat quality traits. After the slaughter pH_{45} and pH_{24} in *MS* (*semimembranosus*) and *LD* (*longissimus dorsi*) muscle, CIE-L*a*b*, drip loss, cooking loss and shear force were measured and pigs were genotyped for PRKAG3 Ile199Val polymorphism. Genotype frequencies at 199th codon of the PRKAG3 gene were 80.90%, 11.24% and 7.87% for Val/Val, Ile/Val and Ile/Ile genotype, respectively. Influence of I199V polymorphism was observed for pH_{45} values in *MS* and *LD* muscle, b*, drip loss and shear force values, where Ile/Ile genotype showed preferable values in all investigated traits.

Key words

pig, PRKAG3, frequency, meat quality

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Received: April 1, 2013 | Accepted: July 5, 2013

ACKNOWLEDGEMENTS
Results presented in this paper are the result of the scientific project "Early prediction of Pig Carcass and Meat Quality" investigations. The project was granted by Ministry of Science, Education and Sports, Republic of Croatia.

Aim

The PRKAG3 gene encodes a muscle-specific isoform of the adenosine monophosphate-activated protein kinase (AMPK) gamma subunit. Often described as energy instrument, AMPK is enrolled in regulation of energy sources and energy pathways in organ systems, such as liver, central nervous system, fat tissue and skeletal muscles (Kahn et al., 2005; Hardie et al., 2006). In muscle tissue it regulates metabolism of carbohydrates and lipids by controlling their intake and energy catabolism (Carling et al., 1994; Mitchelhill et al., 1994). The decreased activity of AMPK is characteristic for pigs with mutated PRKAG3 gene (Milan et al., 2000), causing a severe deterioration in meat quality. Out of seven SNPs (single nucleotide polymorphisms) determined in PRKAG3 gene, five of them are causing amino acid substitutions (Thr30Asn, Gly52Ser, Leu53Pro, Ile199Val and Arg200Gln) affecting the regulation of AMPK activity in muscle tissue (Milan et al., 2000; Ciobanu et al., 2001). Most widely studied haplotype is Arg200Gln, causing the formation of "acid meat" characterised by severe drop in ultimate pH values and leading to high water losses, colour defects and low processing yield of the final product. On the other hand, Ile→Val substitution at 199th codon of the PRKAG3 gene is associated with preferable meat and carcass quality characteristics, such as leanness, carcass length, muscle glycogen content, ultimate pH values and water holding capacity (Ciobanu et al., 2001; Enfält et al., 2006; Lindhal et al., 2004). It is assumed that allele responsible for these positive characteristics is 199I (Otto et al., 2007; Škrlep et al., 2008; Ryan et al., 2012), whose frequency is lower than 10% in most modern pig breeds and their crosses (Ciobanu et al., 2001; Lindahl et al., 2004; Škrlep et al., 2008; 2009), except in Berkshire breed (Ciobanu et al., 2001).

Therefore, the aim of this paper was to determine frequencies of the genotypes at I199V of the PRKAG3 locus in PIC (Pig Improvement Company) hybrid pigs and to investigate relationship between these genotypes and pig meat quality traits.

Material and methods

Animals

The investigation was carried out on 89 (43 gilts and 46 barrows) P337xC23 PIC pig carcasses originating from three sires and 22 dams. Animals were reared under commercial conditions and fed standard diets with *ad libitum* access to feed and water. At approximately 110 kg (±2 kg) live weight the animals were transported to a commercial slaughterhouse, where they were slaughtered following stunning with CO2 and dressed according to the conventional procedure.

Meat quality

The measurements of muscle pH were taken at *semimembranosus* (*SM*) and *longissimus dorsi* (*LD*) muscles 45 minutes and 24h *post mortem* with "Mettler MP 120-B" portable pH meter. Drip loss was determined by bag method according to Honikel (1987) after 48h of cooling the samples at 4°C. CIE-L*a*b* colour values were obtained by Minolta CR-300 colorimeter (Minolta Camera Co. Ltd., Osaka Japan) with a D65 light source and two-degree standard observer. For instrumental tenderness evaluation, a 2.54 cm thick chops of *LD* muscle were frozen for two weeks, defrosted for 24h at 4°C, sealed in vacuum bags, cooked in water bath to 73°C internal temperature and cooled at 4°C overnight. Shear force was measured on at least six 1.27 mm thick cores using a TA.XTplus Texture Analyser equiped with a 1 mm thick Warner-Bratzler shear attachment. Cooking loss was assesed from *LD* chops used for instrumental tenderness evaluation. It was calculated from weights taken before and after cooking and expressed as a percentage.

DNA analysis

Total genomic DNA was isolated from 50 mg of muscle tissue using a commercial High Pure PCR Template Kit (Roche, Germany). A 465 bp fragment of exon 3 (GeneBank; NM_214077.1) of the PRKAG3 gene was amplified using a forward primer P1 (5'-GGA GCA AAT GTG CAG ACA AG -3') and a reverse primer P2 (5'-CCC ACG AAG CTC TGC TTC CTT-3'). The PCR reaction contained 5 µL of genomic DNA, 0.3 µL 10mM dNTPs, 0.45 µL 50mM MgCl2 (Invitrogen), 1 µL of 10 µM P1 and P2 primers, 0.2 µL 5U/ µL Platinum Taq DNA polymerase (Invitrogen) and 11.5 µL PCR pure water (Applied Biosystems) in 20 µL volume. The PCR was performed on Gold-plated 96-well GeneAmp PCR System 9700 (Applied Biosystems, USA) at following temperature conditions: an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 40 sec and extension at 72°C for 30 sec, with final extension step at 72°C for 7 minutes. The obtained PCR products were purified with 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. Sequence analysis was performed using DNA Baser (Heracle BioSoft S.R.L.) and Prim 2 – version 0.5 (www.animalgenom.org/cgi-bin/expeditor/prim2) sequence alignment programs. Using these computer software following triplets were resolved: ATT, ATC and ATA for Ile; and GTT, GTC and GTA for Val.

Statistical analysis

Associations between different genotypes at I199V locus and meat quality traits were evaluated using Kruskal-Wallis ANOVA followed by Mann-Whitney test (Statistica 8.0, StatSoft Inc.), where $p<0.05$ was classified as significant difference and $p<0.1$ as tendency.

Results and discussion

The frequency of three investigated genotypes at 199th codon of the PRKAG3 gene are presented in Figure 1. Of 89 investigated pigs, 80.90% were homozygous Val/Val, 7.87% were homozygous Ile/Ile and 11.24% were heterozygous. The low frequency of Ile/Ile genotype is in accordance with previous reports (Ciobanu et al., 2001; Lindahl et al., 2004; Škrlep et al., 2008; 2009; 2010; Chen et al., 2008). However, Škrlep et al. (2008; 2009; 2010) reported the highest frequency of Ile/Val genotype, which is not in accordance with results of this study. A high frequency of Val/Val genotype was also reported by Rothschild et al. (2002) in Landrace (75.0%) and Large White (62.0%) pig populations and by Chen et al. (2008) in Landrace (89.0%), Large White, Landrace and crossbreeding population between Large White and Landrace (80.0%), Meishan (100.0%), as well as in crossbreeding populations between Large White and Meishan (85.0%)

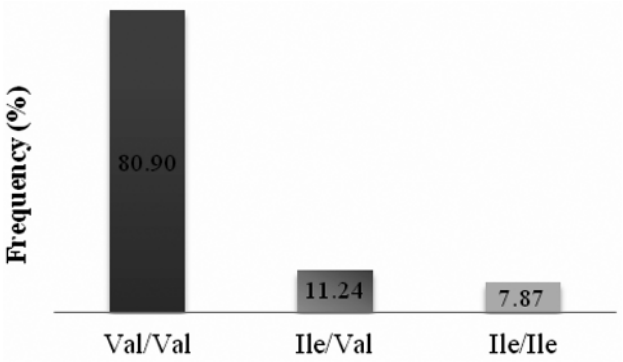

Figure 1. Frequency distribution of genotypes at 199th codon of the PRKAG3 locus

Table 1. Means ± standard deviations for meat quality of investigated I199V polymorphism

Trait	Genotype		
	Val/Val	Ile/Val	Ile/Ile
N	72	10	7
pH_{45}, *MS*	6.32±0.18	6.22±0.20	6.36±0.21
pH_{45}, *LD*	6.32^{b}±0.20	6.32^{b}±0.14	6.48^{a}±0.20
pH_{24}, *MS*	5.88±0.23	5.87±0.24	5.88±0.24
pH_{24}, *LD*	5.75±0.15	5.73±0.17	5.75±0.15
Drip loss (%)	4.24^{A}±2.25	4.43^{AB}±2.44	2.87^{B}±1.63
L^*	50.34±3.51	50.29±2.83	48.69±3.27
a^*	7.03±1.36	7.22±1.42	7.00±1.36
b^*	2.80^{a}±1.18	2.95^{a}±0.92	1.91^{b}±0.30
Cooking loss (%)	33.11±2.87	32.94±2.15	30.15±7.65
Shear force (N)	47.71^{AB}±7.84	48.25^{A}±5.92	43.37^{B}±3.98

^{a,b}Means with different superscripts in the same row differ at $p<0.05$; $^{AB}p<0.1$; *MS-m. semimembranosus; LD-m. longissimus dorsi*

Genotypes differed (Table 1; $p<0.05$) in pH values measured 45 min *post mortem* and in yellowness level (b*), whereas tendency ($p<0.1$) is detected for drip loss and shear force values. Homozygous genotype Ile/Ile showed high initial pH relative to Val/Val and Ile/Val, which is in agreement with previous reports (Ciobanu et al., 2001; Otto et al., 2007; Fontanesi et al., 2008). Contrary to our results, Chen et al. (2008) reported low initial pH values in Ile/Ile genotype and high in Val/Val, while Škrlep et al. (2008) did not observe significant genotype differences in initial and ultimate pH values. Ciobanu et al. (2001) and Ryan et al. (2012) reported a significant association of the investigated polymorphisms with ultimate pH values measured in both *MS* and *LD* muscles. We could not, however, observe such a relationship. Limited affects observed on pH values could be a reflection of animal handling procedures, which include transport time (3h) and physiological discomforts, such as fear and disruption in hierarchy. Also, pH values are affected by number of genes with different roles in specific stages and specific muscles, such as Hal gene, which could possibly interact with PRKAG3 in this trait. Ile/Ile genotype at PRKAG3 had the lowest drip loss values, which is consistent with other literature findings (Otto et al., 2007; Ryan et al., 2012). Consistent with results by Lindahl et al. (2004), genotype Ile/Ile exhibited the lowest b* values. Investigations of Ciobanu et al. (2001), Otto et al. (2007) and Škrlep et al. (2008) evidenced that meat from homozygous Ile/Ile genotype was darker (L*) than two other genotypes. Although we could not statistically detect such effect, Ile/Ile had the lowest L* (Table 1). However, this findings need to be confirmed in a study on a larger number of animals. Results presented in Table 1 show that I199V polymorphism affected instrumental tenderness. Again the homozygous Ile/Ile genotype exhibited the lowest shear force values compared to other two investigated genotypes.

Conclusions

Results of this study indicate that I199V polymorphism affects all technological meat quality traits of economical importance, such as pH values, drip loss and Minolta colour. The homozygous Ile/Ile genotype showed most favourable values for these traits illustrating its potential value as genetic marker for technological meat quality.

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