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EFFECT OF I199V POLYMORPHISM ON PRKAG3 GENE ON CARCASS AND MEAT QUALITY TRAITS IN SLOVENIAN COMMERCIAL PIGS

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ABSTRACT

*The effect of I199V polymorphism at the PRKAG3 gene on carcass and meat quality of commercial pigs was studied independently from PRKAG3 200Q or RYR1 "n" allele, known to decrease meat quality, i.e., animals were genotyped for RYR1 R615C, PRKAG3 I199V and R200Q substitutions, and only pigs without 200Q and "n" were retained for the analysis of carcass and meat quality traits (*n = *274). Genotype frequencies were 12.0, 57.7 and 30.3% for I/I, I/V and V/V, respectively. The I199V polymorphism affected significantly fat thickness and drip loss and tended to affect ultimate pH. Interestingly, I/V were the fattest and significantly different from V/V, with I/I pigs being intermediate. The ultimate pH of I/I was higher than the one of I/V or V/V pigs. Regarding drip loss, significant difference was detected between I/I and V/V pigs. The heterozygous I/V pigs were intermediate with differences to I/I or V/V tending toward significance.*

PRACTICAL APPLICATIONS

Our results provide new evidence about the significant effect of second polymorphism on the PRKAG3 gene (I199V) on carcass and meat quality. Because of low frequencies of I/I genotype in the majority of modern pig breeds, the available literature data for genotype I/I are scarce. Our results confirm a beneficial impact of 199I allele for pork quality, but indicate possible

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adverse impact for carcass leanness. It would be worthwhile rechecking the interesting position of heterozygous I/V pigs, which turned out to be the fattest. We expect the results to be useful for breeders in search of the compromise between carcass and meat quality.

INTRODUCTION

Many factors affect pork quality; however, as regards the genotype, mainly RYR1 R615C (hal gene) and PRKAG3 R200Q (RN gene) substitutions have been thoroughly investigated. By favoring calcium release in muscle cells, the recessive RYR1 "n" allele influences the rate of pH fall (Gueblez *et al.* 1995; De Smet *et al.* 1996; Larzul *et al.* 1997; Monin *et al.* 1999; Fisher *et al.* 2000), while the dominant allele RN^- (200Q) causes high glycogen levels, and consequently, lower ultimate pH (Le Roy *et al.* 1990; Sellier and Monin 1994; Le Roy *et al.* 2000); both genes consequently exerting impact on meat water-holding capacity. Both alleles, a dominant 200Q (RN^-) and RYR1 "n" allele, exert beneficial impact on leanness (Pommier *et al.* 1992; Leach *et al.* 1996; Hamilton *et al.* 2000; Le Roy *et al.* 2000). Presently, a lot of research is dealing with the PRKAG3 gene, which encodes a muscle specific isoform of the regulatory γ-subunit of the adenosine monophosphate-activated protein kinase, an enzyme that has a key role in regulating energy metabolism. Five nonsynonymous substitutions (T30N, G52S, L53P, I199V and R200Q) have been detected in the PRKAG3 gene (Milan *et al.* 2000; Ciobanu *et al.* 2001). In addition to R200Q substitution, I199V showed the most significant effect on meat quality (i.e. muscle pH and color) with the allele 199I being considered as more favorable (Ciobanu *et al.* 2001; Lindahl *et al.* 2004a,b). There is a need for more information regarding the effect of PRKAG3 codon 199 polymorphism on carcass and meat quality, in particular being separated from the effect of 200Q allele. Therefore, the aim of the present study was to add to the body of knowledge that exists on the effects of polymorphisms on the PRKAG3 gene, providing more evidence on how I199V polymorphism affects carcass and meat quality traits independently from allele 200Q, as well as the RYR1 recessive "n" allele.

MATERIALS AND METHODS

Animals and Harvesting

The present analysis is based on phenotypic data collection from field trials conducted over a period of 2 years and collected from 407 pigs coming

from two herds, and slaughtered in two abattoirs in 11 batches. All animals were genotyped for RYR1 C1843T (R615C) according to Brenig and Brem (1992), and for PRKAG3 I199V and PRKAG3 R200Q substitutions (Milan *et al.* 2000). Pigs having 200Q allele, i.e., RN^- phenotype or carrying RYR1 mutation (N/n) were excluded from the analysis, compliant with the aim of the study. Thus, for carcass and meat quality evaluation only, a subsample of 274 pigs were retained. Pigs were approximately 6-month-old commercial crosses of both genders and came from two herds; herd A, which was producing commercial pigs sired by Pietrain (Pi) or Pietrain × Hampshire (Pi × H) boars, and herd B, which produces commercial pigs sired by Duroc (Du) or Duroc × Hampshire (Du × H) boars (with Landrace × Large white female line). Animals were slaughtered according to the routine abattoir procedure, i.e., CO_2 stunning, vertical exsanguination, vapor scalding, dehairing and evisceration, followed by veterinary inspection and carcass classification. At the end of the slaughter line, small pieces of ear laps were taken for genetic analysis.

Carcass and Meat Quality Measurements

Carcass properties were measured on the first day on the slaughter line using a HGP4 Hennessy grading probe (Hennessy Grading Systems Ltd., Auckland, New Zealand) with puncture between the second and third last rib, 7 cm laterally from the carcass split line. One day after the slaughter, further carcass and meat quality measurements were performed. The hind leg was cut off the carcass between the 6th and the 7th lumbar vertebrae, and the shank was removed. The weight of the leg (ham) was recorded before and after the removal of the skin and subcutaneous fat and ham leanness (%) assessed as the ratio between muscle with bones and whole ham weight. A cross section of the carcass was made at the level of the last rib and a digital image of the cross section was taken using a digital photo camera (Canon PowerShot G3, Canon Inc., Tokyo, Japan). *Longissimus dorsi* muscle (LD) area, corresponding fat area were determined on images with aid of LUCIA.NET 1.16.5 software (Laboratory Imaging s.r.o, Prague, Czech Republic). The measurements of color and pH were taken on the freshly cut surface of LD. The color of the LD was assessed using a six-point Japanese color scale (Nakai *et al.* 1975). Color parameter measurements (CIE L^*, a^* and b^*) were taken in triplicate using a Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan) with an 11-mm diameter aperture, D_{65} illuminant, calibrated against a white tile. Muscle pH was determined in two replicates in the central area of the LD using a MP120 Mettler Toledo pH meter (Mettler-Toledo, GmbH, 8603 Schwarzenbach, Switzerland) fitted with a combined glass electrode (InLab427) and previously calibrated at pH 4.0 and 7.0. Also, a 2.5-cm thick slice of LD was

removed from the loin at the level of last rib for drip loss determination according to the EZ drip loss method published by Christensen (2003). Drip loss was determined after 24 and 48 h storage at 4C and expressed as a percentage of the initial weight. The intramuscular fat content of LD was estimated on minced samples with NIRS (NIR System model 6500 Spectrometer, Silver Spring, MD) as described in Prevolnik *et al.* (2005).

Statistical Analysis

Analysis of variance was performed using a statistical package SAS (SAS Inst., Inc., Cary, NC) and the procedure MIXED. The model included fixed effects of herd, PRKAG3 I199V genotypes (I/I, I/V, V/V), the gender, the breed cross nested within herd and the two-way interaction (herd × PRKAG3 I199V). The slaughter batch within the herd was included as a random effect. Significant differences between least square means were evaluated using the option PDIFF. No significant effect of the interaction of herd and PRKAG3 I199V genotypes was found.

RESULTS AND DISCUSSION

Genotype Frequencies

The occurrence of RYR1 and PRKAG3 genotypes in the studied sample of Slovenian commercial pigs is presented in Table 1. In total, our sample

TABLE 1.
FREQUENCY (N) OF GENOTYPES AT TWO GENES (PRKAG3 AND RYR1) IN THE STUDIED SAMPLE OF SLOVENIAN COMMERCIAL PIGS

PRKAG3 codon 200	RYR1	Herd*	PRKAG3 codon 199			Number of pigs
			I/I	I/V	V/V	
rn+/rn+ (R/R)	N/N	A	16	62	40	118
	N/N	B	17	96	43	156
	Total†	A+B	33	158	83	274
	N/n	A	9	38	44	91
RN–/rn+ (Q/R)	N/N	A	0	6	21	27
	N/n	A	0	6	11	17
	Total		42	208	159	409

* Herd A pigs were crosses sired by Pietrain and Pietrain × Hampshire boars while herd B pigs were crosses sired by Duroc and Duroc × Hampshire boars.

† The subsample of pigs retained for the analysis of I199V polymorphism effect on carcass and meat quality traits.

comprised 409 pigs, out of which 26.4% were carriers of RYR1 "n" allele (N/n), and 10.8% ($n = 44$) were RN^- pigs (Q/R at the PRKAG3 200 codon), while the observed frequencies of PRKAG3 codon 199 genotypes were 9.8% (I/I), 51.1% (I/V) and 39.1% (V/V). After the exclusion of carriers of mutant alleles on RYR1 (n) and PRKAG3 (200Q), our subsample comprised 12.0% (I/I), 57.7% (I/V) and 30.3% (V/V). The frequencies of genotypes differed according to herd, given that herd A was using Pi and Pi × H, and herd B was using Du and Du × H sires. Thus, herd A had pigs with both mutant alleles (n, 200Q), whereas herd B only had pigs free of the mentioned mutations. Our results show low incidence of I/I genotype, which corroborates the available literature reports for different modern breeds or crosses (Ciobanu *et al.* 2001; Josell *et al.* 2003; Huang *et al.* 2004; Lindahl *et al.* 2004a,b; Stalder *et al.* 2005; Otto *et al.* 2007). The only breed for which the reported frequency of I/I genotype is considerably higher (74%) is the Berkshire breed (Ciobanu *et al.* 2001). In agreement with the literature, I/I genotype was always associated with 200R allele. Milan *et al.* (2000) showed that because of the absence of recombination between two neighboring codons (199 and 200), only three haplotypes are present in the domestic pig; 199I-200R and 199V-200R are considered as ancestral haplotypes, and were identified in most of the breeds, including wild boar, while 199V-200Q is considered as most recent since it was identified only in Hampshire breed. According to Ciobanu *et al.* (2001), the RN^- phenotype appear to be a combined effect of haplotype 199V-200Q rather than a mere result of R200Q substitution, which was the reason for our decision to investigate the effect of codon 199 polymorphism without interference of 200Q allele. For the same reason, we also excluded carriers of RYR1 gene "n" allele in our analysis of carcass and meat quality.

Carcass Traits

In the present study, the I199V polymorphism on the PRKAG3 gene showed a significant effect on subcutaneous fat thickness (Table 2). It affected backfat thickness measured with HGP probe at the level of 2/3 last rib ($P = 0.05$) and fat area over LD muscle measured at the cross section at the level of last rib ($P = 0.05$). On the contrary, no significant effect of I199V polymorphism was observed on ham leanness. Interestingly, heterozygous (I/V) pigs were the fattest (the thickest backfat, largest fat area), and V/V pigs the leanest, while I/I pigs showed an intermediate position. In our opinion, these results indicate that 199I allele might be less favorable for leanness. There is not much data in the literature about the effect of I199V PRKAG3 polymorphisms on carcass composition. In particular, the information for I/I genotype is very limited. The available studies of Enfält *et al.* (2006), Lindahl *et al.* (2004a,b) and Josell *et al.* (2003) pooled the results of I/I and I/V

TABLE 2.
LEAST SQUARE MEANS (SE) OF I199V GENOTYPES FOR CARCASS TRAITS

	PRKAG3 codon 199			*P*
	I/I	I/V	V/V	
Number of pigs	33	158	83	
Carcass weight (kg)	93.6 (2.3)	94.1 (2.0)	93.5 (2.0)	0.87
HGP fat (mm)†	14.6^{a,b} (0.7)	15.2^{a} (0.5)	14.2^{b} (0.6)	0.05
HGP muscle (mm)†	59.6 (1.6)	61.9 (1.3)	61.1 (1.3)	0.21
HGP meat (%)†	60.0 (0.7)	59.7 (0.5)	60.3 (0.5)	0.45
LD area (cm^2)‡	50.0 (1.4)	51.9 (1.2)	50.9 (1.2)	0.13
Fat area (cm^2)‡	14.6^{a,b} (0.9)	15.2^{a} (0.7)	14.0^{b} (0.7)	0.05
Ham (muscle + bones) (kg)	9.7 (0.3)	10.0 (0.3)	9.8 (0.3)	0.33
Ham (kg)	11.8 (0.4)	12.1 (0.3)	11.9 (0.3)	0.21
Ham leanness (%)	83.1 (1.0)	82.4 (0.8)	82.3 (0.8)	0.63

a,b Least squares means within a row and followed by a different letter are significantly ($P < 0.05$) different.
† Measured with Hennessy grading probe between 2nd and 3rd last rib.
‡ Measured at the cross section of *Longissimus dorsi* muscle at the level of last rib.

genotype into one genotype group due to, as they explained, very low frequencies of I/I genotype and the absence of important differences between I/I and I/V pigs for most of the measured traits. Despite that, Enfält *et al.* (2006), in their conclusion, also indicated that the presence of allele 199I decreased the lean meat content compared to two other alleles (199V-200Q and 199V-200R).

Meat Quality

The effect of PRKAG3 codon 199 polymorphisms on some meat quality traits proved to be important (Table 3). A significant effect was observed in the water-holding capacity, while it only tended to be significant in case of ultimate pH (pHu). On the other hand, no significant effect was observed on the LD color. In agreement with other studies (Josell *et al.* 2003; Lindahl *et al.* 2004a; Otto *et al.* 2007), no significant effect was observed for intramuscular fat content, LD muscle ultimate pH was significantly higher in I/I homozygous pigs, compared to V/V genotype, with I/V pigs being closer to V/V pigs, which corroborates the results of Ciobanu *et al.* (2001) and Otto *et al.* (2007). Regarding the color, in the present study, PRKAG3 I199V genotypes were not significantly different in color measurements (Minolta L^*, a^* and b^* color note), although a slight tendency could be detected in the Minolta L value and color note (I/I versus V/V). On the contrary, Ciobanu *et al.* (2001) reported

TABLE 3.
LEAST SQUARES MEANS (SE) OF I199V GENOTYPES FOR MEAT QUALITY TRAITS

	PRKAG3 codon 199			*P*
	I/I	I/V	V/V	
Number of pigs	33	158	83	
Imf (%)†	1.45 (0.08)	1.39 (0.06)	1.42 (0.06)	0.62
pHu	5.58[a] (0.03)	5.52[b] (0.03)	5.51[b] (0.03)	0.07
LD color (1–6)‡	3.6 (0.1)	3.5 (0.1)	3.4 (0.1)	0.28
Minolta *L**	49.7 (0.6)	50.5 (0.5)	51.0 (0.5)	0.15
Minolta *a**	6.5 (0.3)	6.9 (0.3)	6.9 (0.3)	0.23
Minolta *b**	2.9 (0.2)	3.0 (0.2)	3.0 (0.2)	0.93
*a**/*b**	2.5 (0.3)	2.7 (0.2)	2.7 (0.2)	0.70
Drip loss 24 h (%)	2.6[a] (0.5)	3.3[ab] (0.4)	3.9[b] (0.5)	0.05
Drip loss 48 h (%)	4.6[a] (0.7)	5.6[ab] (0.6)	6.2[b] (0.6)	0.04

a,b Least squares means within a row and followed by a different letter are significantly ($P < 0.05$) different.
† Intramuscular fat content.
‡ *Longissimus dorsi* muscle color evaluated according to 6-point Japanese color scale.

significantly lower *L** value for I/I and I/V pigs compared to V/V pigs, while Otto *et al.* (2007) observed significantly lower *L** values for I/I pigs, but no differences between I/V and V/V pigs. Lindahl *et al.* (2004b) reported a lower *L** value (not significant) and lower *a** and *b** values (significant) in the presence of the I allele (for the pooled I/I/ I/V group). Water-holding capacity, assessed as the measurement of drip loss (24 h and 48 h), was significantly affected by the I199V PRKAG3 polymorphism. The lowest drip loss was observed for I/I pigs, but the difference was significant only in contrast to V/V pigs (1.2–1.6%, $P < 0.05$). The heterozygous I/V pigs were situated in between; however, the differences to I/I (0.7–1.0%) or V/V (0.5–0.6%) tended toward significance ($P < 0.15$). Contrary to our observation of I/V position being more or les intermediate, Otto *et al.* (2007), using four different methods for drip loss evaluation, found significantly lower drip loss values only for I/I genotype; thus, their study positioned genotype I/V closer to V/V genotype. In contrast to the favorable effect of the 199I allele demonstrated in the present study, Lindahl *et al.* (2004a) observed no significant differences for 24 and 96 h drip loss between V/V and pooled result for I/V and I/I genotypes. Our study confirms the positive effect of allele I on water-holding capacity. The differences in results regarding the genotypes, in particular the position of heterozygous I/V pigs, can be related to the fact that other studies did not control for the 200Q allele, which is always connected to 199V and is known to strongly affect the ultimate pH and drip loss.

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