

Comparison of *PRKAG3* and *RYR1* gene effect on carcass traits and meat quality in Slovenian commercial pigs

M. ŠKRLEP, T. KAVAR, M. ČANDEK-POTOKAR

Agricultural Institute of Slovenia, Ljubljana, Slovenia

ABSTRACT: The effect of polymorphisms at *PRKAG3* (*R200Q* and *I199V*) and *RYR1* (*R615C*) genes on carcass traits and meat quality was examined in a sample of 257 commercial pigs, crosses of Landrace × Large White as maternal line and Pietrain ($N = 96$), Pietrain × Landrace ($N = 42$) or Pietrain × Hampshire ($N = 119$) as paternal line. Pigs were genotyped (PCR-RFLP) and traits of interest were measured (which included carcass and ham weight, measurements of fatness, meatiness, ultimate pH, colour parameters and drip loss). The observed genotype frequencies at *PRKAG3* gene were 9.7%, 38.9%, 32.7%, 6.2% and 12.5% for *R/R-I/I*, *R/R-I/V*, *R/R-V/V*, *Q/R-I/V* and *Q/R-V/V* genotype, respectively. *RYR1* genotype frequencies were 57.2% for *N/N* and 42.8% for *N/n* genotype. Studied polymorphisms exhibited a significant effect on meat quality, but mainly an insignificant effect on carcass traits. No significant interaction between *PRKAG3* and *RYR1* was found. Carriers of *RYR1* mutant allele “*n*” had less intense *longissimus dorsi* muscle colour (subjective score, Minolta L^* and b^*) and higher drip loss. Regarding *PRKAG3*, the ultimate pH decreased and Minolta L^* , a^* , b^* and drip loss increased in the following order: *R/R-I/I*, *R/R-I/V*, *R/R-V/V*, *Q/R-I/V* and *Q/R-V/V*, according to the presence of *199I* and absence of *200Q* alleles. The study shows that the *I199V* polymorphism is an important source of variation in pigs free of *200Q*. In particular the *199I* proves beneficial for meat quality. The results of combining the *RYR1* and *PRKAG3* genotypes indicate that *R/R-I/I* genotype could be used in counterbalancing the negative effects of “*n*” allele on meat quality.

Keywords: *PRKAG3*; *RYR1*; genotype frequencies; carcass properties; meat quality; gene interaction

Genetic improvement of meat quality by selective breeding is difficult therefore marker-assisted selection has been suggested as a promising strategy for genetic improvement (Meuwissen and Goddard, 1996). Much focus has lately been given to mapping individual loci (QTL) for economically important traits which can supplement conventional breeding procedures (Příbyl, 1995) and enable researchers to identify gene(s) responsible for the observed phenotypic variations (Bidanel and Rothschild, 2002). In pigs, two major genes have mainly been

studied, i.e. the so called “*hal*” or *RYR1* gene (particularly *R615C* substitution or the “*n*” allele) and *RN* gene (*R200Q* substitution in *PRKAG3* gene). Lately, *RYR1* gene has also been referred to as *CRC* (calcium release channel) gene. The recessive “*n*” allele at *RYR1* gene influences the rate of pH fall by favouring the calcium release in muscle cells (Gueblez et al., 1995; De Smet et al., 1996; Larzul et al., 1997; Monin et al., 1999; Fisher et al., 2000). On the other hand, dominant allele *RN*⁻ induces high glycogen levels in α W muscle fibres (Marinova

Supported by the Slovenian Research Agency (Program No. P4-0072 and Project No. L4- 9468) by the Ministry of Agriculture, Forestry and Food of Slovenia.

et al., 1992; Čandek-Potokar et al., 1999) and consequently low ultimate pH (Le Roy et al., 1990; Sellier and Monin, 1994; Le Roy et al., 2000). In many breeding schemes these causative mutations were preserved due to their beneficial impact on leanness (Pommier et al., 1992; Leach et al., 1996; Hamilton et al., 2000; Le Roy et al., 2000). In addition to RN^- mutation, evidence for new alleles affecting meat quality in *PRKAG3* gene (Ciobanu et al., 2001) has been demonstrated. *PRKAG3* gene encodes a muscle specific isoform of the regulatory γ -subunit of the adenosine monophosphate-activated protein kinase (AMPK), an enzyme that has a key role in regulating the energy metabolism. Five nonsynonymous substitutions (*T30N*, *G52S*, *L53P*, *I199V* and *R200Q*) have been found in the *PRKAG3* gene (Milan et al., 2000; Ciobanu et al., 2001). Besides the *R200Q* substitution (also known as RN^-), the *I199V* substitution showed the most significant effect on meat quality (i.e. muscle pH and colour) with the allele *199I* considered as more favourable (Ciobanu et al., 2001; Lindahl et al., 2004 a,b). Milan et al. (2000) showed that due to the absence of recombination between two neighbouring codons (199 and 200) only three combinations were found in domestic pig. Haplotypes *199I-200R* and *199V-200R* are considered as ancestral, and were identified in most pig breeds, including wild boar. Haplotype *199V-200Q* is considered as the most recent since it was identified only in Hampshire breed. The effect of RN^- mutation on meat quality has been well documented. However, there is a need for more information regarding the effect of other *PRKAG3* polymorphisms on meat quality. Moreover, as genes act in mutual interactions (Szyda et al., 2006; Matějčíček et al., 2008) we were interested in a comparison of the impact of two genes proved important for meat quality. Thus the aim of the present study was to associate *PRKAG3* genotypes (*Q/R-I/V*, *Q/R-V/V*, *R/R-I/I*, *R/R-I/V* and *R/R-V/V*) with meat quality traits and to relate their impact to one of the *RYR1* genotypes (*N/N* or *N/n*).

MATERIAL AND METHODS

Animals

Phenotypic data were collected on 257 commercial pigs. At the end of the slaughter line small pieces of ear lops were taken for the determination

of *RYR1* (*R615C* amino acid substitution according to Brenig and Brem, 1992 i.e. mutated allele '*n*') and *PRKAG3* genotypes (*I199V* and *R200Q* substitutions according to Milan et al., 2000). Pigs ($N = 257$) were approximately the six months old progeny of commercial crosses of Landrace \times Large White crossbred dams sired by Pietrain (Pi) ($N = 96$), Pietrain \times Landrace (Pi \times Ln) ($N = 42$) or Pietrain \times Hampshire (Pi \times Ha) ($N = 119$). Pigs were of both sexes and from one herd. Animals were slaughtered in eight batches according to the routine abattoir procedure, i.e. CO_2 stunning, vertical exsanguination, vapour scalding, dehairing and evisceration followed by the veterinary inspection and carcass classification. The cooling of carcasses was performed first by passing them through a blast chilling chamber for 2 h at -15 to $-8^\circ C$ and followed by storage at $0-2^\circ C$ (8–12 h) until the internal carcass temperature dropped below to $7^\circ C$.

Carcass and meat quality measurements

Carcass properties were measured 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 the third last rib 7 cm laterally from the carcass split line. One day after the slaughter, the remaining carcass and meat quality measurements were performed. The hind leg was cut off the carcass between the 6th and 7th lumbar vertebra and the shank was removed. The weight of leg (ham) was recorded before and after the removal of the skin and subcutaneous fat and ham leanness (%) was assessed as the ratio between muscle with bones and whole ham weight. A cross-section of 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). The *longissimus dorsi* muscle (*LD*) area and corresponding fat area as well as the ratio between muscle and fat area were determined on images by the LUCIA.NET 1.16.5 computer software (Laboratory Imaging s.r.o., Prague, Czech Republic). The measurements of colour and pH were taken on the freshly cut surface of *LD*. Colour of *LD* was assessed using a 6-point Japanese colour scale (Nakai et al., 1975). Colour 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 (pH_{LD}) 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 the last rib for drip loss determination according to the method (EZ drip loss) published by Christensen (2003). Drip loss was determined after 24- and 48-hour storage at 4°C and expressed as a percentage of the initial weight. The intramuscular fat content of *LD* was estimated in minced samples using NIRS (NIR System Model 6500 Spectrometer, Silver Spring, MD, USA) as described in Prevornik et al. (2005).

Statistical analysis

Analysis of variance was performed using statistical package SAS (SAS Inst., Inc., Cary, NC, USA) and GLM procedure. The model included fixed effects of *PRKAG3* genotypes (*Q/R-I/V*, *Q/R-V/V*, *R/R-I/I*, *R/R-I/V* and *R/R-V/V*), *RYR1* genotypes (*N/N*, *N/n*), sex, crossbreed, slaughter batch and two-way interaction (*PRKAG3* × *RYR1*). Carcass weight was included as a covariate. Significant differences between the least square means (LSM) were evaluated using the PDIFF option. No signifi-

cant effect of the interaction of *RYR1* and *PRKAG3* genotypes was found.

RESULTS AND DISCUSSION

Genotype frequencies

The observed frequencies of *RYR1* and *PRKAG3* genotypes in the studied sample ($N = 257$) of commercial pigs are presented in Table 1. Almost a half of the animals (42.8%, $n = 110$) were carriers of *RYR1* “*n*” allele (*N/n*) and 18.7% ($n = 48$) of *RN⁻* mutation (*Q* at the *PRKAG3* 200 codon). The frequencies of *PRKAG3* genotypes were 9.7% (*R/R-I/I*), 38.9% (*R/R-I/V*), 32.7% (*R/R-V/V*), 6.2% (*Q/R-I/V*) and 12.5% (*Q/R-V/V*). While pigs carrying the “*n*” allele were present in all crossbreeds, the carriers of *200Q* were present only in Pi × Ha crosses. The frequencies observed in the present study do not necessarily reflect the actual allele frequencies in the population since such screening demands pedigree information, which was not available in the present study. However, the relatively high frequency of *RYR1* “*n*” allele observed in the present study can be related to the fact that experimental pigs had at least 25% of Pietrain blood. The incidence of *PRKAG3* gene *Q200* allele was lower and proportional to the share of Hampshire breed in the investigated sample. Regarding codon 199 of *PRKAG3* gene, the observed low incidence

Table 1. Frequency of pigs according to crossbreed, *RYR1* and *PRKAG3* genotype in the studied sample of Slovenian commercial pigs

	<i>RYR1</i>						Total			
	<i>N/N</i>			<i>N/n</i>			<i>N/N</i>	<i>N/n</i>	total	(%)
	Pi	Ln × Pi	Pi × Ha	Pi	Ln × Pi	Pi × Ha				
<i>PRKAG3</i>										
<i>R/R-I/I</i>	5	3	8	4	2	3	16	9	25	9.7
<i>R/R-I/V</i>	23	10	29	12	13	13	62	38	100	38.9
<i>R/R-V/V</i>	19	7	14	33	7	4	40	44	84	32.7
<i>Q/R-I/V</i>			8			8	8	8	16	6.2
<i>Q/R-V/V</i>			21			11	21	11	32	12.5
Total	47	20	80	49	22	39	147	110	257	100.0
(%)	18.3	7.8	31.1	19.1	8.6	15.2	57.2	42.8	100.0	

Pi = Pietrain; Ln = Landrace; Ha = Hampshire

Table 2. LSM (SE) for carcass traits as affected by *RYR1* and *PRKAG3* genotypes

Variable	<i>RYR1</i>		<i>PRKAG3</i>						<i>P</i>
	N/N	N/n	R/R-I/I	R/R-I/V	R/R-V/V	Q/R-I/V	Q/R-V/V		
Number of pigs	147	110	25	100	84	16	32		
Carcass weight (kg)	91.6 (2.4)	93.1 (2.4)	93.7 (3.0)	93.6 (2.2)	93.6 (2.3)	88.3 (3.5)	92.7 (2.7)	NS	
^a HGP fat (mm)	14.2 (0.6)	13.5 (0.6)	14.2 (0.9)	14.3 (0.6)	13.9 (0.6)	13.6 (1.0)	13.2 (0.8)	NS	
^a HGP muscle (mm)	64.1 (1.4)	65.0 (1.4)	63.2 (2.0)	63.9 (1.3)	63.9 (1.4)	65.8 (2.1)	65.9 (1.7)	NS	
HGP meat (%) ^a	60.7 (0.8)	61.6 (0.8)	61.2 (1.2)	60.6 (0.7)	60.9 (0.8)	61.3 (1.2)	61.9 (1.0)	NS	
^b LD muscle area (cm ²)	50.9 (1.4)	51.4 (1.4)	50.3 (1.8)	50.5 (1.3)	51.1 (1.4)	51.5 (2.1)	52.5 (1.6)	NS	
^b Fat area over LD (cm ²)	15.3 (0.7)	14.5 (0.7)	14.7 (0.9)	15.2 (0.7)	15.0 (0.7)	15.2 (1.1)	14.4 (0.9)	NS	
LD meat/fat ratio	3.57 (0.22)	3.72 (0.22)	3.67 (0.28)	3.55 (0.20)	3.52 (0.21)	3.64 (0.32)	3.86 (0.25)	NS	
Ham (kg)	11.9 (0.14)	12.1 (0.14)	11.8 (0.18)	11.9 (0.12)	11.9 (0.13)	12.1 (0.23)	12.3 (0.19)	NS	
Ham (meat + bones) (kg)	9.8 (0.2)	10.1 (0.2)	9.7 (0.2)	9.8 (0.2)	9.8 (0.2)	10.1 (0.3)	10.4 (0.2)	a	
Ham leanness (%)	81.9 (0.9)	83.9 (0.9)	82.2 (1.1)	82.3 (0.8)	82.2 (0.9)	83.4 (1.5)	84.4 (1.2)	NS	

P > 0.1 insignificant (NS); *P* < 0.1 = tendency (a); **P* < 0.05; ***P* < 0.01; ****P* < 0.001

^ameasured with the Hennessy grading probe between the 2nd and 3rd last rib

^bmeasured at the cross-section of *Longissimus dorsi* muscle at the level of the last rib

of *I/I* genotype (9.4%, 11.9%, 10.6% for *Pi*, *Pi* × *Ln* and *Pi* × *Ha* crosses, respectively) agrees with the results generally reported in the available literature for different modern breeds or crosses (Ciobanu et al. 2001; Josell et al., 2003a; Huang et al., 2004; Lindahl et al., 2004a,b; Stalder et al., 2005; Otto et al., 2007; Ramos et al., 2008) with the exception of Berkshire breed where the frequency of *I/I* genotype is reported as high as 74% (Ciobanu et al., 2001). In the present study, *I/I* genotype was always associated with *200R* allele in agreement with the results reported in the previously mentioned literature. Milan et al. (2000) demonstrated that due to the absence of the recombination between neighbouring codons 199 and 200 only three haplotypes exist in domestic pig; *199I-200R* and *199V-200R* haplotypes are considered as ancestral since they were identified in most breeds including wild boar, whereas *199V-200Q* haplotype was identified only in Hampshire breed and thus it is considered as the most recent.

Effect of *RYR1* genotype

The effect of *RYR1* genotype on carcass properties (Table 2) was significant ($P < 0.05$) only in the case of ham weight (muscles with bones) and ham leanness (%). Pigs that were carriers of the mutant allele (*N/n*) had leaner and heavier hams than *N/N* pigs. It is worth mentioning that, although not significantly, *N/n* animals exhibited higher muscularity and lower fatness, which is in agreement with the recognized opinion that the “*n*” allele affects carcass leanness positively (Krenková et al., 1999). However, according to the literature, the difference in carcass leanness between carriers (*N/n*) and mutation-free pigs (*N/N*) is not so clear. Similarly to our results, Leach et al. (1996) found no significant difference in backfat thickness between *N/n* and *N/N* pigs, but they demonstrated certain superiority of *N/n* pigs in carcass yields. Fisher et al. (2000) found larger LD muscle thickness and/or area in *N/n* compared to *N/N* pigs, while other studies reported no significant differences in LD muscle between these two genotypes (De Smet et al., 1996; Leach et al., 1996; Hamilton et al., 2000). Like in the present study, Fisher et al. (2000) also observed heavier and leaner hams in *N/n* pigs; however in their case the differences were not significant. According to Larzul et al. (1997) *N/n* pigs were closer to *N/N* than to *n/n* pigs for carcass composition traits, still giving 6% better

carcass yield than *N/n* pigs. Our results confirm a certain advantage of the presence of *RYR1* “*n*” allele for carcass quality and explain why this allele is conserved in some breeding schemes.

Regarding meat quality traits the effect of *RYR1* genotype (Table 3) was important for the colour (subjective colour score as well as Minolta L^* value) and drip loss, but not for the pH_u . In our study, pigs carriers of the mutant “*n*” allele (*N/n*) had 0.2 point less intense colour than *N/N* pigs, in agreement with the 1.7 point higher Minolta L^* value. The *N/n* pigs also had 2.4% points higher drip loss. Insignificantly, however in agreement with lower backfat thickness, *N/n* pigs had less intramuscular fat than *N/N* pigs. Regarding the intramuscular fat content, our results agree with those of De Smet et al. (1996) reporting slightly inferior values for *N/n* compared to *N/N* pigs, while Monin et al. (1999) reported significantly higher intramuscular fat content for *N/N* than *N/n* pigs. No difference in ultimate pH between *N/N* and *N/n* pigs is in agreement with many studies (Casteels et al., 1995; De Smet et al., 1996; Larzul et al., 1997; Monin et al., 1999; Fisher et al., 2000; Fernandez et al., 2002; de Oliveira Band et al., 2005; Otto et al., 2007). The presence of “*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; Krenková et al., 2001) but is not related to the ultimate pH, in accordance with similar glycolytic potential observed for *N/n* and *N/N* pigs (Larzul et al., 1997). Nevertheless, some authors (Pommier and Houde, 1993; Leach et al., 1996; Hamilton et al., 2000) reported lower pH_u of *N/n* compared to *N/N* pigs. Significantly lighter colour of *N/n* compared to *N/N* pigs in the present study corroborates the results of Pommier and Houde (1993) and Fisher et al. (2000). This result could be explained by a higher rate of pH fall in *N/n* pigs, however it is difficult to confirm that since we did not measure it. Contrary to us, some other studies found no differences between *N/N* and *N/n* pigs for colour (De Smet et al., 1996; Larzul et al., 1997; Fernandez et al., 2002; de Oliveira Band et al., 2005). Regarding the water holding capacity of LD muscle, significantly higher drip loss obtained in our study for *N/n* than *N/N* pigs agrees well with numerous results for various measurement methods (Sather et al., 1991; Pommier and Houde, 1993; Casteels et al., 1995; De Smet et al., 1996; Leach et al., 1996; Fisher et al., 2000; Hamilton et al., 2000; de Oliveira Band et al., 2005).

Table 3. LSM (SE) for meat quality traits as affected by *RYRI* and *PRKAG3* genotypes

Variable	<i>RYRI</i>					<i>PRKAG3</i>					<i>P</i>
	<i>N/N</i>	<i>N/n</i>	<i>P</i>	<i>R/R-II</i>	<i>R/R-IV</i>	<i>R/R-V/V</i>	<i>Q/R-IV</i>	<i>Q/R-V/V</i>	<i>P</i>		
Number of pigs	147	110		25	100	84	16	32			
^e IM fat (%)	1.34 (0.05)	1.27 (0.05)	NS	1.18 (0.08)	1.37 (0.04)	1.33 (0.05)	1.39 (0.11)	1.25 (0.07)			NS
^f H _u	5.50 (0.01)	5.48 (0.01)	NS	5.56 ^c (0.02)	5.52 ^{bc} (0.01)	5.50 ^b (0.01)	5.46 ^{ab} (0.03)	5.42 ^a (0.02)			***
^f <i>LD</i> colour (1–6)	3.5 (0.1)	3.3 (0.1)	*	3.6 (0.1)	3.5 (0.1)	3.5 (0.1)	3.3 (0.2)	3.3 (0.2)			NS
Minolta <i>L</i> *	50.1 (0.4)	51.8 (0.5)	**	49.7 ^a (0.7)	50.8 ^{ab} (0.4)	51.5 ^b (0.5)	50.8 ^{ab} (1.0)	52.0 ^b (0.6)			*
Minolta <i>a</i> *	7.4 (0.2)	7.6 (0.2)	NS	6.6 ^a (0.3)	7.2 ^{ab} (0.2)	7.4 ^b (0.2)	8.1 ^{bc} (0.5)	8.4 ^c (0.3)			**
Minolta <i>b</i> *	3.3 (0.1)	3.6 (0.2)	a	2.8 ^a (0.2)	3.3 ^b (0.1)	3.5 ^{bc} (0.2)	3.6 ^{bc} (0.4)	4.0 ^c (0.2)			**
Drip loss after 24 h (%)	3.7 (0.3)	6.1 (0.4)	***	3.5 ^a (0.6)	4.6 ^a (0.3)	4.6 ^a (0.4)	5.1 ^{ab} (0.8)	6.6 ^b (0.5)			**
Drip loss after 48 h (%)	6.1 (0.4)	8.5 (0.4)	***	5.6 ^a (0.6)	6.8 ^a (0.3)	7.0 ^a (0.4)	7.7 ^{ab} (0.9)	9.4 ^b (0.6)			***

P > 0.1 insignificant (NS); *P* < 0.1 = tendency (a); **P* < 0.05; ***P* < 0.01; ****P* < 0.001

^{a,b,c,d}LSM values within a row followed by different superscript letters are significantly (*P* < 0.05) different

^eintramuscular fat content determined by NIRS

^f*Longissimus dorsi* muscle colour evaluated according to a 6-point Japanese colour scale

Effect of *PRKAG3*

In the present study, the *PRKAG3* genotype showed no significant effect on any of the measured carcass traits (Table 2). It only tended ($P < 0.10$) to affect ham weight (ham muscles with bones); the carriers of *RN* mutation (200Q) had slightly heavier hams. Although the carcass properties were not significantly affected by *PRKAG3*, the carriers of *RN* mutation were on the whole slightly leaner than non-carriers. Many literature reports indicate the connection of higher lean meat content with *RN* genotype (Enfält et al., 1997a, 2006; Le Roy et al., 2000; Miller et al., 2000a). On the other hand, studies where no effect of *RN* was reported are

also numerous (Enfält et al., 1997b; Hamilton et al., 2000; Miller et al., 2000b; Moeller et al., 2003). An interesting observation can be drawn regarding *I/V* pigs which corroborates our previous study on *PRKAG3* codon 199 (Škrlep et al., 2009). The heterozygotes on codon 199 (*R/R-I/V* pigs) would be expected to take intermediate position between *R/R-I/I* and *R/R-V/V* pigs. However, they exhibited the thickest backfat and the largest fat area over LD. The *199I* allele seems to be less favourable for the muscularity opposed to the *199V* and *200Q* alleles. There is not much literature data about the association of *PRKAG3* genotypes with carcass traits. In particular, the information on *I/I* genotype is limited. Available studies of Lindahl et al. (2004a,b)

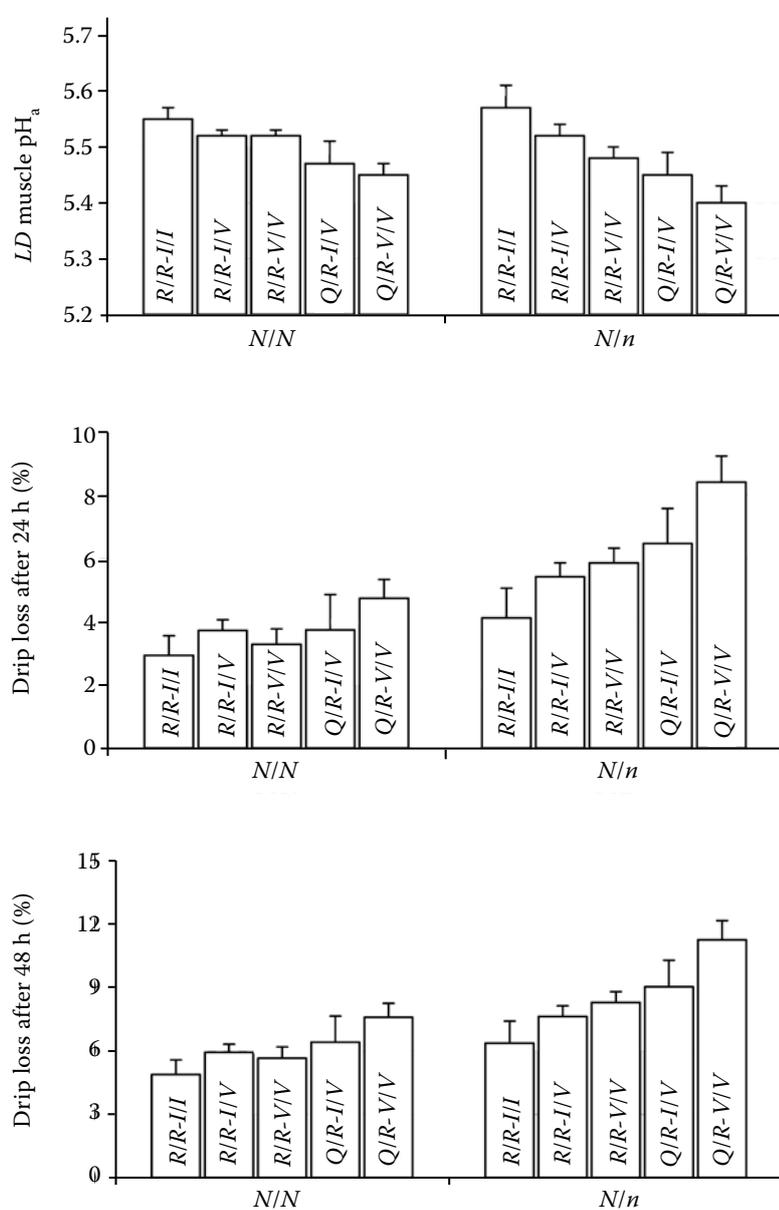


Figure 1. Ultimate pH and drip loss of *Longissimus dorsi* muscle (LSM ± SE) according to *PRKAG3* (*R/R-I/I*, *R/R-I/V*, *R/R-V/V*, *Q/R-I/I*, *Q/R-I/V*, *Q/R-V/V*) and *RYRI* (*N/N*, *N/n*) genotypes

and Enfält et al. (2006) pooled the results of *I/I* and *I/V* genotype into one genotype group due to very low frequencies of *I/I* genotype and the absence of important differences between *I/I* and *I/V* pigs. Despite that Enfält et al. (2006) indicated in their conclusion that the presence of the *199I* allele decreased lean meat content compared to other alleles (*199V* and *200Q*), in agreement with our results.

The effect of *PRKAG3* gene on meat quality traits proved to be very important (Table 3). Significant effects were observed for pH_{U} , Minolta L^* , a^* and b^* values and especially drip loss, while no significant effect was observed for intramuscular fat and *LD* colour score. The pH_{U} of *LD* muscle evolved according to the absence of *200Q* and the presence of *199I* allele sorted from the lowest to the highest in the following order: *Q/R-V/V* < *Q/R-I/V* < *R/R-V/V* < *R/R-I/V* < *R/R-I/I* (Figure 1). Among the genotypes free of *200Q* allele we observed a significant difference for pH_{U} between *R/R-I/I* and *R/R-V/V* pigs. It is worth noting that no significant difference was observed for pH_{U} of *LD* muscle between *R/R-V/V* pigs and *Q/R-I/V* pigs whereas the difference was significant between *R/R-V/V* and *Q/R-V/V* pigs indicating the opposite effect of *200Q* and *199I* allele on pH_{U} . In agreement with a decreasing pH_{U} of *LD* muscle, we observed an increase in Minolta L^* , a^* , b^* and drip loss giving consequently the same but reversed order of the genotypes. Here again, for the genotypes free of *200Q* mutation, we observed notable differences ($P < 0.10$) between *R/R-I/I* and *R/R-I/V* or *R/R-V/V* pigs for *LD* muscle Minolta measurements and drip loss. Likewise, no differences between *R/R-V/V* pigs and *Q/R-I/V* pigs and significant differences between *R/R-V/V* and *Q/R-V/V* pigs were detected.

Considering the effect of the *200Q* allele or the so called *RN* phenotype it has indubitably been proved that its presence induces lower ultimate pH value and water holding capacity (Andersson and Hansson, 1996; Enfält et al., 1997b; Lundström et al., 1998; Lebret et al., 1999; Hamilton et al., 2000; Le Roy et al., 2000; Miller et al., 2000a; Josell et al., 2003b; Lindahl et al., 2004a,b), and our study only confirms these evidences. According to Ciobanu et al. (2001), the *RN* phenotype is considered to be a combined effect of haplotype *199V-200Q* rather than a mere result of *R200Q* substitution. There is also substantial data about the probable effect of other polymorphisms in *PRKAG3* on meat quality, in particular codon 199. Namely the allele *199I* was associated with higher pH and lower drip loss of *LD*

muscle (Ciobanu et al., 2001; Lindahl et al., 2004a,b; Otto et al., 2007; Škrlep et al., 2009) Indeed, our study supports a favourable effect of allele *199I* and an adverse additive effect of alleles *200Q* and *199V* on meat quality traits. However, we should not overlook possible limitations of our results since they were obtained with a simple association study which can give false-positive results due to confounding effects of population stratification (Hernandez-Sanchez et al., 2003).

Comparison of *PRKAG3* and *RYR1* effects on meat quality

In view of the important effect of both genes (*RYR1* and *PRKAG3*) on meat quality observed in our study (and relatively feeble effect on carcass traits) and given that the variation of meat quality encountered in practice is considerable, it is of interest to relate and/or associate the impact of the *RYR1* “*n*” allele to the impact of *PRKAG3* polymorphisms (*I199V* and *R200Q*) on meat quality.

Comparing the differences among the genotypes of the individual gene on drip loss, it seems that the impact of the *RYR1* “*n*” allele was lower than that of *PRKAG3* genotypes (for *N/N* vs. *N/n* the difference being about 2.4% points and for *R/R-I/I* vs. *Q/R-V/V* being 3.1% and 3.8% points for drip loss after 24 and 48 hours, respectively). However, based on the pooled differences in drip loss between *200Q* carriers and homozygous *200R* pigs (1.6 and 2.1% for drip loss after 24 and 48 h, respectively) the impact of *200Q* mutation seems a bit lower than that of the allele “*n*”. The observed important impact of *PRKAG3* genotype on drip loss is thus a combined (additive) effect of codons 199 and 200. Indeed, in the absence of *200Q* mutation, the difference between *R/R-I/I* and *R/R-V/V* is smaller (1.1 and 1.4% for drip loss 24 and 48 h, respectively). These differences tended towards significance ($P < 0.10$), and can be expected to represent an important source of variation to take into consideration when undesired mutations (“*n*” and *200Q*) are eliminated from the population. Using the same method as in the present study (EZ drip loss 48 h), Otto et al. (2007) reported a similar difference to ours between the homozygous *PRKAG3 I199V* genotypes (0.96% points) and *N/N* and *N/n* genotypes (1.15% point).

Like in our case, the comparable study of Hamilton et al. (2000), using *rn⁺/rn⁺* and *RN/rn⁺*, *N/N* and

N/n pigs, found no significant interactions between these genes either for carcass or for meat quality measurements (ultimate pH, Minolta L*, a* and b* and drip loss). Similarly Otto et al. (2007), studying the associations of multiple DNA markers with meat quality traits, reported no interaction between *PRKAG3* codon 199 and *RYR1* gene for drip loss. Based on our results, some interesting assumptions could be made, which, if properly used in breeding, could help in search of the compromise between carcass yield and meat quality and contribute to lower variability in meat quality. When comparing the differences between the *PRKAG3* genotypes (Figure 1) within the *RYR1* homozygous *N/N* and heterozygous *N/n* pigs, similar values of pH_U, drip loss 24 and 48 h were observed in the case of *N/N-R/R-V/V* and *N/n-R/R-I/I*. These results indicate that by using the “*n*” allele in pig breeding schemes to improve carcass yields the negative impact of “*n*” allele on meat quality could be counterbalanced by the presence of *I/I* genotype. The poorest meat quality is expected to come from the co-appearance of “*n*” allele on *RYR1* gene and *200Q* alleles on *PRKAG3*, which should be avoided. Based on the results of the present study, the meat industry should consider if the advantage in carcass yields (≈1% point) when using “*n*” or *200Q* allele can justify the loss in meat quality translated to the considerably higher drip loss (2 to 4% points).

CONCLUSIONS

No major effect of *RYR1* and *PRKAG3* genotypes was observed on carcass traits, while an important effect was observed on meat quality traits. The presence of *RYR1* “*n*” or *PRKAG3* *200Q* allele (*RN⁻* phenotype) had an adverse effect, while allele *199I* showed a favourable effect on meat quality. Concerning the effect of *PRKAG3* codon 199 polymorphisms, the differences between *I/I* and *V/V* genotypes tended towards significance, and can be expected to become an important source of variation in pigs free of *200Q* mutation. Combining the *RYR1* and *PRKAG3* genotypes indicates that the poorest meat quality is to be expected in the presence of both “*n*” and *200Q*, which should be avoided. Small improvement of carcass yields observed in carriers of *RYR1* (“*n*”) and *PRKAG3* (*200Q*) mutations is not justified in view of the significant increase in drip loss which they cause. The important result of the present study is that the negative impact of “*n*” allele

on meat quality could be counterbalanced by the presence of *I/I* genotype on *PRKAG3* gene.

Acknowledgements

Sincere thanks to anonymous reviewers for their comments and suggestions which helped us improve this article.

REFERENCES

- Bidanel J.P., Rothschild M.F. (2002): Current status of quantitative trait loci mapping in pigs. *Pigs News Information*, 23, N39–N54.
- Brenig B., Brem G. (1992): Genomic organization and analysis of the 5' end of the porcine ryanodine receptor gene (*RYR1*). *FEBS Letters*, 298, 277–279.
- Brewer M.S., Jensen J., Sosnicki A.A., Fields B., Wilson E., McKeith F.K. (2002): The effect of pig genetics on palatability, color and physical characteristics of fresh pork loin chops. *Meat Science*, 61, 249–256.
- Casteels M., Van Oeckel M.J., Boschaerts L., Spincemaille G., Boucque C.V. (1995): The relationship between carcass, meat and eating quality of three pig genotypes. *Meat Science*, 40, 253–269.
- Christensen L.B. (2003): Drip loss sampling in porcine *m. longissimus dorsi*. *Meat Science*, 63, 469–477.
- Ciobanu D., Bastiaansen J., Malek M., Helm J., Woollard J., Plastow G.S., Rothschild M. (2001): Evidence for new alleles in the protein kinase adenosine monophosphate activated γ_3 subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. *Genetics*, 159, 1151–1162.
- Čandek-Potokar M., Lefaucheur L., Ecolan P. (1999): Determination of glycogen in single skeletal muscle fibres by computerized image analysis. Application to the quantification of glycogen according to fibre type in two muscles and three pig genotypes. *Research Reports of the Veterinary Faculty University of Ljubljana*, 36, 57–66.
- de Oliveira Band G., Guimareães S.E.F., Lopes P.S., Schierholt A.S., Silv, K.M., Pires A.V., Júnior A.A.B., de Miranda Gomide L.A. (2005): Relationship between the porcine stress syndrome gene and pork quality traits of F2 pigs resulting from divergent crosses. *Genetics and Molecular Biology*, 28, 88–91.
- De Smet S.M., Pauvels H., De Bie S., Demeyer D.I., Callewier J., Eeckhout W. (1996): Effect of halothane genotype, breed, feed withdrawal and lairage on pork quality of Belgian slaughter pigs. *Journal of Animal Science*, 74, 1854–1863.

- Enfält A.-C., Lundström K., Hansson I., Johansen S., Nyström P.-E. (1997a): Comparison of non-carriers and heterozygous carriers of RN- allele for carcass composition, muscle distribution and technological meat quality in Hampshire-sired pigs. *Livestock Production Science*, 47, 221–229.
- Enfält A.-C., Lundström K., Karlsson A., Hansson I. (1997b): Estimated frequency of the RN- allele in Swedish Hampshire pigs and comparison of glycolytic potential, carcass composition, and technological meat quality among Swedish Hampshire, Landrace, and Yorkshire pigs. *Journal of Animal Science*, 75, 2924–2935.
- Enfält A.-C., von Seth G., Josell L., Lindahl G., Hedebro-Velander I., Braunschweig M., Andersson L., Lundström K. (2006): Effects of a second mutant allele (*VI99I*) at *PRKAG3* (RN) locus on carcass composition in pigs. *Livestock Science*, 99, 131–139.
- Fernandez X., Neyraud E., Astruc T., Santé V. (2002): Effects of halothane genotype and pre-slaughter treatment on pig meat quality. Part 1. *Post mortem* metabolism, meat quality indicators and sensory traits of m. *Longissimus lumborum*. *Meat Science*, 62, 429–437.
- Fisher P., Mellett F.D., Hoffman L.C. (2000): Halothane genotype and pork quality. 1. Carcass and meat quality characteristics of three halothane genotypes. *Meat Science*, 54, 97–105.
- Gueblez R., Paboeuf F., Sellier P., Bouffaud M., Boulard J., Brault D., le Tiran M.-H., Petit G. (1995): Effet du génotype halothane sur les performances d'engraissement de carcasse et de qualité de la viande du porc charcutier. *Journées Recherche Porcine en France*, 27, 155–164.
- Hamilton D.N., Ellis M., Miller K.D., McKeith F.K., Parrett D.F. (2000): The effect of halothane and rendement napole genes on carcass and meat quality characteristics of pigs. *Journal of Animal Science*, 78, 2862–2867.
- Hernández-Sánchez J., Visscher P., Plastow G., Haley C. (2003). Candidate gene analysis for quantitative traits using the transmission disequilibrium test: the example of the Melanocortin 4-Receptor in pigs. *Genetics*, 164, 637–644.
- Huang L.-S., Ma J.-W., Ren J., Ding N.-S., Guo Y.-M., Ai H.-S., Li L., Zhou L.-H., Chen C.-Y. (2004): Genetic variation of the porcine *PRKAG3* gene in Chinese indigenous pig breed. *Genetics Selection Evolution*, 36, 481–486.
- Josell L., Enfält A.-C., von Seth G., Lindahl G., Hedebro-Velander I., Andersson L., Lundström K. (2003a): The influence of RN genotype, including the new *VI99I* allele, on the eating quality of pork loin. *Meat Science*, 65, 1341–1351.
- Josell L., von Seth G., Tornberg E. (2003b): Sensory quality and the incidence of PSE of pork in relation to crossbreed and RN phenotype. *Meat Science*, 65, 651–660.
- Krenková L., Kuciel J., Urban, T. (1999): Association of the *RYR1*, *GH*, *LEP* and *TF* genes with carcass and meat quality traits in pigs. *Czech Journal of Animal Science*, 44, 481–486.
- Krenková L., Urban T., Kuciel J. (2001): Usefulness of pH_1 and EC_{50} values, glycogen, glucose and lactic acid content in biopsy samples taken from pigs of different *RYR1* genotypes for meat quality prediction. *Czech Journal of Animal Science*, 46, 41–46.
- Larzul C., Le Roy P., Gueblez R., Talmant A., Gouge J., Sellier P., Monin G. (1997): Effect of halothane (*N/N*, *N/n*, *nn*) on growth, carcass and meat quality traits of pigs slaughtered at 95 kg or 125 kg live weight. *Journal of Animal Breeding and Genetics*, 114, 309–320.
- Leach L.M., Ellis M., Sutton D.S., McKeith F.K., Wilson E.R. (1996): The growth performance, carcass characteristics, and meat quality of halothane carrier and negative pigs. *Journal of Animal Science*, 74, 934–943.
- Lebret B., Le Roy P., Monin G., Lefaucheur L., Caritez J. C., Talmant A., Elsen J.M., Sellier P. (1999): Influence of the three RN genotypes on chemical composition, enzyme activities, and myofiber characteristics of porcine skeletal muscle. *Journal of Animal Science*, 77, 1482–1489.
- Le Roy P., Naveau J., Elsen J.M., Sellier P. (1990): Evidence of a new major gene influencing meat quality in pigs. *Genetic Research*, 55, 33–40.
- Le Roy P., Elsen J.M., Caritez J.C., Talmant A., Juin H., Sellier P., Monin G. (2000): Comparison between the three porcine RN genotypes for growth, carcass composition and meat quality traits. *Genetics Selection Evolution*, 32, 165–186.
- Lindahl G., Enfält A.-C., von Seth G., Josell L., Hedebro-Velander I., Andersen H.J., Braunschweig M., Andersson L., Lundström K. (2004a): A second mutant allele (*VI99I*) at the *PRKAG3* (RN) locus – I. Effect on technological meat quality of pork loin. *Meat Science*, 66, 609–619.
- Lindahl G., Enfält A.-C., von Seth G., Josell L., Hedebro-Velander I., Andersen H.J., Braunschweig M., Andersson L., Lundström K. (2004b): A second mutant allele (*VI99I*) at the *PRKAG3* (RN) locus – II. Effect on colour characteristics of pork loin. *Meat Science*, 66, 621–627.
- Lundström K.A., Andersson A., Hansson I. (1996): Effect of the RN gene on technological and sensory meat quality in crossbred pigs with Hampshire as terminal sire. *Meat Science*, 42, 145–153.
- Lundström K.A., Enfält A.-C., Tornberg E., Agerhem H. (1998): Sensory and technological meat quality in carriers and non-carriers of the RN- allele in Hampshire

- crosses and in purebred Yorkshire pigs. *Meat Science*, 48, 115–124.
- Marinova P., Lefaucheur L., Fernandez X., Monin G. (1992): Relationship between metabolic type and glycogen content in skeletal muscle fibres of large white and hampshire crossbred pigs. *Journal of Muscle Foods*, 3, 91–97.
- Matějčík A., Matějčíková J., Štípková M., Hanuš O., Genčurová V., Kyselová J., Němcová E., Kott T., Šefrová J., Krejčová M., Melčová S., Hölzelová I., Bouška J., Frelich J. (2008): Joint effects of *CSN3* and *LGB* genes on milk quality and coagulation properties in Czech Fleckvieh. *Czech Journal of Animal Science*, 53, 246–252.
- Meuwissen T.H.E., Goddard M.E. (1996): The use of marker haplotypes in animal breeding schemes. *Genetics Selection Evolution*, 28, 161–176.
- Milan D., Jeon J.-T., Looft C., Anarger V., Robic A., Thelander M., Rogel-Gaillard C., Paul S., Iannucelli N., Rask L., Ronne H., Lundström K., Reinsch N., Gellin J., Kalm E., Le Roy P., Chardon P., Andersson L. (2000): A mutation in *PRKAG3* associated with excess glycogen content in pig skeletal muscle. *Science*, 288, 1248–1251.
- Miller K.D., Ellis M., Bidner B., McKeith F.K., Wilson E.R. (2000a), Porcine Longissimus glycolytic potential level effects on growth performance, carcass, and meat quality characteristics. *Journal of Muscle Foods*, 11, 169–181.
- Miller K.D., Ellis M., McKeith F.K., Bidner B.S., Meisinger D.J. (2000b): Frequency of the Rendement Napole RN-allele in a population of American Hampshire pigs. *Journal of Animal Science*, 78, 1811–1815.
- Moeller S.J., Baas T.J., Leeds T.D., Emmett R.S., Irvin K.M. (2003): Rendement Napole gene effects and a comparison of glycolytic potential and DNA genotyping for classification of Rendement Napole status in Hampshire-sired pigs. *Journal of Animal Science*, 81, 402–410.
- Monin G., Larzul C., Le Roy P., Culioli J., Mourot J., Rousset-Akrim S., Talmant A., Touraille C., Sellier P. (1999): Effects of halothane genotype and slaughter weight on texture of pork. *Journal of Animal Science*, 77, 408–415.
- Nakai H., Saito F., Ikeda T., Ando S., Komatsu A. (1975): Standard models of pork colour. *Bulletin of National Institute of Animal Industry (Japan)*, 29, 69–74.
- Otto G., Roehe R., Looft H., Thoelking L., Knap P.W., Rothschild M.F., Plastow G.S., Kalm E. (2007): Associations of DNA markers with meat quality traits in pigs with emphasis on drip loss. *Meat Science*, 75, 185–195.
- Pommier S., Houde A. (1993): Effect of the genotype for malignant hyperthermia as determined by restriction endonuclease assay on the quality characteristics of commercial pork loins. *Journal of Animal Science*, 71, 420–425.
- Pommier S.A., Houde A., Rousseau F., Savoie Y. (1992): The effect of malignant hyperthermia genotype as determined by restriction endonuclease assay on carcass characteristics of commercial crossbred pigs. *Canadian Journal of Animal Science*, 72, 973–976.
- Prevolnik M., Čandek-Potokar M., Škorjanc D., Velikonja-Bolta Š., Škrlep M., Žnidaršič T., Babnik D. (2005): Predicting intramuscular fat content in pork and beef by near infrared spectroscopy. *Journal of Near Infrared Spectroscopy*, 13, 77–85.
- Příbyl J. (1995): A way of using markers for farm and animal selection. *Czech Journal of Animal science*, 40, 375–382.
- Ramos A.M., Glenn K.L., Serenius T.V., Stalder K.J., Rothschild M.F. (2008): Genetic markers for the production of US country ham. *Journal of Animal Breeding and Genetics*, 125, 248–257.
- Sather A.P., Murray A.C., Zadawski S.M., Johnson P. (1991): The effect of halothane gene on pork production and meat quality of pigs reared under commercial conditions. *Canadian Journal of Animal Science*, 71, 956–967.
- Sellier P., Monin G. (1994): Genetics of pig meat quality: a review. *Journal of Muscle Foods*, 5, 187–219.
- Stalder K.J., Rothschild M.F., Lonergan S.M. (2005): Associations between two gene markers and indicator traits affecting fresh and dry-cured ham processing quality. *Meat Science*, 69, 451–457.
- Szyda J., Grindflek E., Wideroe I.S., Lien S. (2006): Search for linked QTL and genetic epistasis in swine with application of the false discovery rate. In: 8th World Congress on Genetics Applied to Livestock Production, August 13–18, 2006. Belo Horizonte, MG, Brasil.
- Škrlep M., Kavari T., Santé-Lhoutellier V., Čandek-Potokar M. (2009): Effect of I199V polymorphism at *PRKAG3* gene on carcass and meat quality traits in Slovenian commercial pigs. *Journal of Muscle Foods*, 20, 367–376.

Received: 2009–01–09

Accepted after corrections: 2009–09–30

Corresponding Author

Dr. Marjeta Čandek-Potokar, Agricultural Institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana, Slovenia
Tel. +386 1 2805 124, fax +386 1 2805 255, e-mail: meta.candek-potokar@kis.si