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THE EFFECT OF *PRKAG3* AND *RYR1* GENES ON MEAT QUALITY TRAITS IN THE LOCAL KRŠKOPOLJE PIG BREED

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Abstract: Polymorphisms in *PRKAG3* (Ile199Val and Gly52Ser) or *RYR1* (Arg615Cys) genes have been reported to exert a major effect on pig meat quality. In the present study, the effects of these polymorphisms were investigated in 234 Krškopolje pigs genotyped with the Choice Genetics' custom SNP chip. The observed genotype frequencies for *RYR1* N/N were 66.2% and for N/n 33.8%. The frequencies of *PRKAG3* Ile199Val were 4.7%, 33.8% and 61.5% for Ile/Ile, Ile/Val and Val/Val, respectively, and Gly52Ser frequencies were 28.6%, 42.3% and 29.1% for Gly/Gly, Gly/Ser and Ser/Ser, respectively. Pigs carrying recessive "n" allele on *RYR1* exhibited paler colour with higher drip loss ($P<0.05$). As for *PRKAG3* polymorphisms, the Ile/Ile genotype (Ile199Val) was associated with lower muscle thickness, whereas Val/Val genotype was associated with higher monounsaturated fatty acids content ($P<0.05$). Gly/Ser heterozygotes (Gly52Ser) exhibited lower muscle pH 24h *post-mortem* ($P<0.05$) and brighter meat colour compared to Ser/Ser genotypes ($P<0.05$). By combining different genotypes of *RYR1* and *PRKAG3*, we could not confirm the hypothesis that the negative effects of the *RYR1* mutation could be counterbalanced by certain favourable *PRKAG3* alleles (like 199Ile) due to their low frequency.

Key words: local pig breed, SNPs, meat quality, breeding program

Introduction

Published research has identified a number of polymorphisms that affect carcass properties and meat quality in cosmopolitan pig breeds. However, in many local pig breeds, including the Slovenian Krškopolje pig, the effects of some major

polymorphisms known to affect meat quality have not yet been studied. The exception is Arg615Cys mutation on *RYR1* gene, which has undesirable effect on meat quality *(Salmi et al., 2010)* and has been found in relatively high frequency (i.e. 0.20) in Krškopolje pig population *(Tomažin et al., 2021).* An interesting polymorphism (Ile199Val substitution in *PRKAG3* gene; *Milan et al., 2000*) has been reported to have beneficial effect on meat quality and could counterbalance the negative effect of *RYR1* allele in cosmopolitan breeds *(Škrlep et al., 2010)*. The variant 199Ile has been associated with lower glycolytic potential and consequently higher pH24, while another polymorphism, the Gly52Ser substitution, has been shown to affect ham pH24 and loin muscle lightness *(Ciobanu et al., 2001)*. In Krškopolje pig, the frequency of 199Ile has been reported to be 0.22, thus having the potential for application in the breeding program *(Muñoz et al. 2018)*. Therefore, the main objective of the present study was to associate *PRKAG3* genotypes (Ile199Val and Gly52Ser) with meat quality traits in local Krškopolje pig breed and to relate their impact to *RYR1*genotype.

Materials and Methods

The samples of Krškopolje pig breed (n=237) were collected for DNA extraction and meat quality analysis. At the age of 400.5 ± 7.8 (mean ± SE) days, pigs were slaughtered, eviscerated and carcasses were weighed. Measurements of muscle thickness and backfat thickness above GM muscle were taken on split carcasses. The value of pH after 45 minutes *post-mortem* (pH45) was measured in *longissimus dorsi* muscle using MP120 Mettler Toledo pH meter (Mettler-Toledo, GmbH, Schwarzenbach, Switzerland). One day after slaughter, the samples of *longissimus dorsi* muscle were taken for meat quality. Briefly, pH (after 24 hours) was measured using pH meter Mettler-Toledo MP120 (Mettler-Toledo, GmbH, Schwarzenbach, Switzerland) and objective colour (i.e. CIE L*, a*, and b* colour parameters) using Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan). Water holding capacity was determined by measuring drip loss (after 24 hours of storage) according to the EZ method *(Christensen, 2003).* Warner-Bratzler (WB) shear force was measured on three to four cooked cylindrical cores excised perpendicular to the direction of fibres using a TA Plus texture analyser (Ametek, Lloyd Instruments Ltd., Fareham, UK). Fatty acid composition of backfat (i.e. saturated, monounsaturated and polyunsaturated fatty acid content) and intramuscular fat content were determined using the near-infrared spectral analysis (NIR Systems 6500, Foss NIR System, Silver Spring, MD, USA) using in-house calibrations.

DNA was extracted from ear tissue using Qiamp DNA Mini kit (Qiagen, (Qiagen GmbH, Hilden, Germany) following manufacturer instructions. The DNA

samples were genotyped using Choice Genetics' custom SNP-array containing polymorphisms on *RYR1* (Arg615Cys) and *PRKAG3* (Ile199Val, Gly52Ser, Leu53Pro, Arg200Gln) genes. These genotypes were extracted using PLINK 1.9 software *(Purcel et al., 2007)*.

Analysis of variance was performed using sasLM package in R software (version 4.3.1). The model included fixed effects of *RYR1* ("NN" and "Nn"), *PRKAG3* Ile199Val (Ile/Ile, Ile/Val, Val/Val) or Gly52Ser (Gly/Gly, Gly/Ser, Ser/Ser), farm (n = 9) and two-way interaction (*PRKAG3* x *RYR1*). Carcass weight was included as a covariate. Significant differences between the estimated marginal means were evaluated using the PDIFF function.

Results and Discussion

The observed frequencies of *RYR1*and *PRKAG3* genotypes are presented in Table 1. Almost 34% (n = 79) of Krškopolje pigs were carriers of *RYR1* recessive allele (Nn). The genotypic frequencies of *PRKAG3* Ile199Val SNP were 4.7, 33.8 and 61.5% for Ile/Ile, Ile/Val and Val/Val genotypes, respectively, while for *PRKAG3* Gly52Ser the genotypic frequencies were 28.6, 42.3 and 29.1% for Gly/Gly, Gly/Ser and Ser/Ser, respectively. The *PRKAG3* Leu53 and Arg200 were fixed in Krškopolje pig. In line with previous studies *(Muñoz et al., 2018; Tomažin et al., 2021)*, a relatively high frequency of the *RYR1* recessive ("n") allele has been confirmed. It is a consequence of the uncontrolled introgression of the mutation from the modern breeds in the past and a lack of systematic elimination of this allele from the present population of Krškopolje pigs *(Kastelic and Čandek-Potokar, 2013)*. Regarding *PRKAG3* genotypes, similar frequencies of 199Ile (i.e. 0.22) and 200Arg (i.e. 1.00) have been reported for Krškopolje pig by *Muñoz et al. (2018)*, while frequencies for Gly52Ser and Leu53Pro are reported here for the first time.

The effect of *RYR1* genotype (Table 2) was significant for several meat quality traits. For example, lower water-holding capacity (higher drip loss) in "Nn" animals ($P < 0.05$) is consistent with results of our previous studies on Krškopolje pig *(Tomažin et al., 2021)* and on numerous other cosmopolitan breeds using different measurement methods *(Salmi et al., 2010)*. The reduced water holding capacity in animals carrying recessive "n" allele is the result of the loss in regulation of calcium release from the sarcoplasmic reticulum which accelerates the rate of pH decline *(Monin et al., 2004)*. In the present study, a tendency for decreased pH45 ($P = 0.06$), lighter, more red and more yellow colour of meat, larger drip loss ($P < 0.05$) and higher WB shear force ($P = 0.05$) were observed in

"Nn" animals which is consistent with many studies on cosmopolitan breeds *(see meta-analysis of Salmi et al., 2010; Oliván et al., 2018; McPhee and Trout, 1994).*

Table 1. Frequency of *RYR1* and *PRKAG3* genotype in Krškopolje pigs

	RYR1		**Total**	**%**
	NN	**Nn**		
PRKAG3				
Ile199Val - Ile/Ile	5	6	11	4.7
Ile199Val - Ile/Val	50	29	79	33.8
Ile199Val - Val/Val	100	44	144	61.5
Gly52Ser - Gly/Gly	39	28	67	28.6
Gly52Ser - Gly/Ser	65	34	99	42.3
Gly52Ser - Ser/Ser	51	17	68	29.1
Leu53Pro - Leu/Leu	155	79	234	100
Arg200Gln - Arg/Arg	155	79	234	100
Total	155	79	234	
(%)	66.2	33.8		

We did not detect any significant differences between "NN" and "Nn" genotypes for the backfat and muscle thickness, although "Nn" pigs usually exhibit higher carcass leanness or lower fatness *(Salmi et al., 2010).* There was no difference in backfat fatty acid composition, which could be due to different diets that pigs received.

Table 2. Estimated marginal means for carcass and meat quality traits as affected by *RYR1* genotypes

	RYR1		RMSE	P-value
	NN	Nn		
Carcass traits				
Backfat above GM muscle, mm	42.9	41.6	7.38	0.292
Muscle thickness, mm	71.8	73.5	6.66	0.131
Meat quality traits				
pH45	6.32	6.23	0.250	0.063
pH24	5.46	5.45	0.078	0.719
CIE L*	53.1	55.4	3.99	< 0.001
CIE a*	10.2	10.8	1.78	< 0.05
CIE b*	6.3	7.1	1.53	< 0.01
IMF, %	5.03	4.70	1.532	0.183
Drip loss, %	4.4	5.3	1.98	< 0.05
Shear force, N	45.9	49.1	9.49	0.054
Backfat fatty acid composition				
SFA*	43.0	42.6	1.88	0.153
MUFA*	46.8	46.6	1.55	0.983
PUFA*	10.9	10.4	1.68	0.109

IMF = intramuscular fat content, GM = *gluteus medius*, SFA = saturated fatty acid content, MUFA = polyunsaturated fatty acid content, PUFA = polyunsaturated fatty acid content,
Results on fatty acid composition are presented in g/100 g of total fatty acids
[a, b] Values within a row with different a and b superscripts differ significantly at $P < 0.05$.

As for the *PRKAG3* Ile199Val (Table 3), animals carrying Ile/Ile genotype exhibited lower muscle thickness compared to Val/Val and Ile/Val genotypes ($P < 0.05$). In addition, a tendency for greater backfat thickness was observed in animals carrying Ile/Ile compared to heterozygous Ile/Val genotype ($P = 0.06$). The Ile/Ile compared to Val/Val genotype was associated with lower monounsaturated fatty acid content compared to Val/Val, with the Ile/Val closer to Val/Val ($P < 0.05$). In agreement with previous reports on cosmopolitan breeds, the 199Ile allele was at least favourable for muscularity *(Enfält et al., 2006; Škrlep et al., 2010)*. However, no differences in meat quality traits were observed between *PRKAG3* alleles on codon 199 which is contrary to literature reports. In cosmopolitan pig breeds it was previously shown that Ile199 genotype affected colour, pH and water holding

capacity *(Ciobanu et al., 2001; Škrlep et al., 2010; Otto et al., 2007).* Possible reason for discrepancies could be low number of Ile/Ile pigs or that genotype effect can be also breed-dependent *(Ryan et al., 2012).*

Table 3. Estimated marginal means for carcass and meat quality traits as affected by *PRKAG3* Ile199Val genotype

	***PRKAG3* Ile199Val**			**RMSE**	**P-value**
	Ile/Ile	**Ile/Val**	**Val/Val**		
Carcass traits					
Backfat above GM muscle, mm	46.5	40.9	43.1	7.30	0.062
Muscle thickness, mm	66.8[a]	71.9[b]	72.7[b]	6.61	< 0.05
Meat quality traits					
pH45	6.13	6.31	6.29	0.252	0.436
pH24	5.48	5.44	5.46	0.078	0.213
L*	54.9	54.1	53.6	4.12	0.543
a*	10.6	10.5	10.3	1.82	0.763
b*	7.1	6.6	6.6	1.58	0.599
IMF, %	5.0	4.9	5.0	1.54	0.892
Drip loss, %	4.4	4.8	4.6	2.02	0.717
Shear force, N	40.9	46.2	47.9	9.50	0.115
Fatty acid composition					
SFA	42.9	42.9	42.8	1.89	0.915
MUFA	45.9[a]	46.6[a]	47.1[b]	1.53	< 0.05
PUFA	10.9	10.4	10.0	1.68	0.191

GM = *gluteus medius*, IMF = intramuscular fat content, SFA = saturated fatty acid content, MUFA = polyunsaturated fatty acid content, PUFA = polyunsaturated fatty acid content,
Results on fatty acid composition are presented in g/100 g of total fatty acids
[a, b] Values within a row with different a and b superscripts differ significantly at $P < 0.05$.

Considering the *PRKAG3* Gly52Ser polymorphism (Table 4), animals with Ser/Ser genotype exhibited higher pH24 values and darker colour of *longissimus* muscle compared to Gly/Ser genotype ($P < 0.05$). Water-holding capacity tended to be increased in Gly/Ser genotypes ($P = 0.09$). The study of *Ciobanu et al. (2001)* showed that this polymorphism affected ham pH (higher value in Ser/Ser compared to Gly/Gly genotype) and loin CIE L* (lightness) parameter (lighter meat in Ser/Ser than in Gly/Ser and Gly/Gly genotypes).

Table 4. Meat quality of *longissimus dorsi* muscle according to PRKAG3 Gly52Ser genotype

	PRKAG3 Gly52Ser			RMSE	P-value
	Gly/Gly	Gly/Ser	Ser/Ser		
Carcass traits					
Backfat above GM muscle, mm	42.3	41.6	44.2	7.33	0.122
Muscle thickness, mm	71.3	73.1	71.4	6.67	0.260
Meat quality traits					
pH45	6.24	6.32	6.28	0.252	0.459
pH24	5.46^{ab}	5.44^{a}	5.47^{b}	0.077	< 0.05
L*	53.3^{ab}	54.8^{b}	52.7^{a}	4.02	< 0.01
a*	10.8	10.3	10.0	1.80	0.112
b*	6.5	6.8	6.3	1.56	0.116
IMF, %	5.1	5.0	4.6	1.53	0.216
Drip loss, %	4.4	5.1	4.4	1.99	0.088
Shear force, N	45.8	46.8	48.6	9.57	0.367
Fatty acid composition					
SFA	43.0	42.8	42.8	1.89	0.781
MUFA	46.6	46.8	47.1	1.54	0.228
PUFA	10.3	10.4	9.9	1.68	0.243

GM = *gluteus medius*, IMF = intramuscular fat content, SFA = saturated fatty acid content, MUFA = polyunsaturated fatty acid content, PUFA = polyunsaturated fatty acid content,
Results on fatty acid composition are presented in g/100 g of total fatty acids
[a, b] Values within a row with different a and b superscripts differ significantly at $P < 0.05$.

In order to assess potential counterbalancing of negative effects of recessive "n" allele, genotype *RYR1* was combined with *PRKAG3* (Ile199Val) and effects on carcass and meat quality traits were assessed (Figure 1). Despite several differential effects observed for carcass and meat quality traits in regard to the combination of *RYR1* and *PRKAG3* Ile199Val, we could not confirm our hypothesis on counterbalancing effect between the genotypes. Namely, in our study we detected just 11 animals carrying *PRKAG3* Ile/Ile allele (see Table 1) originating from 4 different farms. Still, the combination of genotype "Nn" at *RYR1* with Ile/Val at *PRKAG3* (Figure 1) indicated smaller backfat thickness, the combination of N/n at *RYR1* with Ile/Ile the most tender meat and the lowest polyunsaturated fatty acids content ($P < 0.05$).

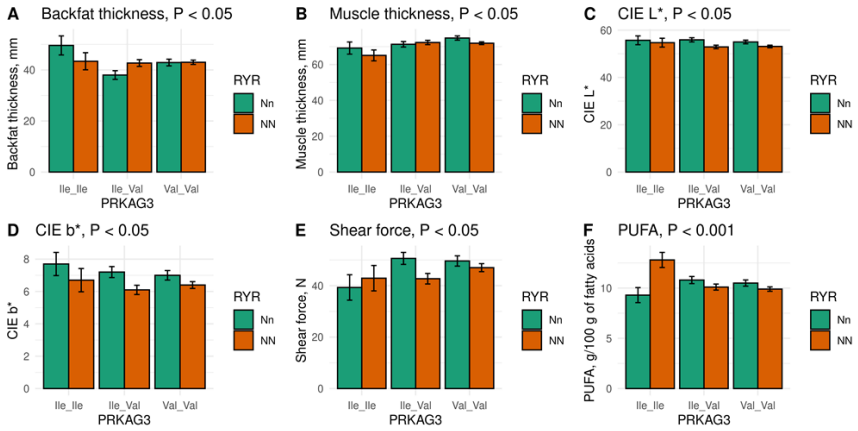

Figure 1. Carcass and meat quality traits affected by the combination of *RYR1* (NN and Nn) and *PRKAG3* (Ile199Val) polymorphisms

Conclusions

In the present study, the *RYR1* genotype in Krškopolje pig affected meat quality traits (i.e. objective colour parameters, drip loss and pH45), while the *PRKAG3* Ile199Val genotype was associated with muscle thickness and backfat monounsaturated fatty acid content. The combination of both alleles (i.e. *RYR1* "Nn" and *PRKAG3* Ile/Ile) could not confirm the hypothesis on counterbalancing effect on meat quality traits due to low number of detected *PRKAG3* Ile/Ile animals of Krškopolje pig. Therefore, additional animals carrying *PRKAG3* Ile/Ile genotype should be included in the analysis.

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