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MEAT QUALITY OF KRŠKOPOLJE PIGS AS AFFECTED BY *RYR1* GENOTYPE

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Abstract: The effect of *RYR1* genotype on carcass and meat quality traits was investigated in the only Slovenian local pig breed Krškopolje. Thirty-six castrates originating from 12 litters were genotyped for c. C1843T (p. Arg615Cys) at *RYR1* locus (recessive allele further denoted as "n" and wild as "N"). Pigs with mutated recessive allele (N/n) had lower growth rate and leaner carcasses (exhibited greater lean meat content, muscle thickness and loin eye area, thinner backfat and smaller fat area over LD muscle). A pronounced effect of *RYR1* on meat quality was observed. The rate of pH fall in *longissimus dorsi* (LD) muscle of N/n pigs was faster (lower pH at 45 min after slaughter) but there was no effect of *RYR1* on ultimate pH. Lower water holding capacity (higher drip loss and higher thawing and cooking loss) of meat from N/n was noted. Meat of N/n pigs exhibited also higher shear force resistance (indicative of lower tenderness).

Key words: Krškopolje pig; RYR1 gene; growth; carcass; meat quality

Introduction

Krškopolje pig is the only Slovenian local breed. In the recent years, the census of this black pig breed with white belt has been steadily increasing. Among the breeders, these pigs are reputed for having good meat quality, especially suitable for processing into high quality dry-cured products. However, only a few studies objectively evaluating meat quality of Krškopolje pigs exist. In pigs, *RYR1* gene encoding the ryanodine receptor in the calcium channel of the sarcoplasmic reticulum is one of the major genes affecting meat quality. The recessive mutation c. C1843T (p. Arg615Cys) at *RYR1* locus, further denoted as "n" allele, is responsible for susceptibility to stress and appears to be incompletely recessive regarding meat quality. It has marked positive effects on muscle development and

carcass lean content *(Monin, 2004).* The "n" allele at *RYR1* is associated with the loss of control of free calcium and is associated with a very fast post-mortem pH fall (below 6.0 within 30 minutes to 1 hour) which together with high body temperature causes muscle protein denaturation resulting in decreased water holding capacity and discoloration of meat *(Monin, 2004).* Relatively high frequencies of "n" allele at *RYR1* were reported for Krškopolje pigs; i.e. on a sample of 17 pigs; *Čandek-Potokar et al. (2003)* reported 70% of heterozygous (N/n) and 18% of homozygous (n/n) carriers, whereas earlier study on 10 pigs *(Kastelic, 2001),* showed 40% of N/n and 10% of n/n pigs, denoting allele "n" frequency of 0.53 and 0.30, respectively. In an earlier study, allele "n" frequency of 0.432 was reported for Krškopolje pigs by *Dovč et al. (1996).* All studies highlighted the problem of high incidence of the *RYR1* mutation to be taken into account in the breeding programme. The current situation with regard to *RYR1* gene and the effect on meat quality has thus been one of the objectives of the TREASURE project.

Materials and Methods

The subject of the present study are Krškopolje castrates (n=36) originating from 12 different litters reared at the same farm and fed complete feed mixtures adapted to the stage of growth (for more details see *Batorek et al., 2016*). At the average age (±SD) of 228±6 days and weight of 121±14 kg the pigs were slaughtered in a commercial abattoir according to the routine procedure. The samples of ears were collected for DNA extraction. Carcasses were weighed and measurements of fat and muscle thickness taken (by official classification body) for estimation of lean meat content according to the method approved for Slovenia (OJ EU L56/28, 2008). Back fat thickness at the level of last rib was additionally measured. Measurement of pH was taken in *longissimus dorsi* muscle (LD) at the level of last rib 45 minutes (pH45) post mortem using MP120 Mettler Toledo pH meter (Mettler-Toledo, GmbH, Schwarzenbach, Switzerland). A day after slaughter the pH (pH24) was measured in LD muscle and a sample of LD with overlying subcutaneous fat taken for further assessments of marbling (1-7 scale), area of LD and corresponding fat, objective colour (CIE L*, a*, and b* colour parameters measured with Minolta Chroma Meter CR-300, Minolta Co. Ltd, Osaka, Japan). For chemical analysis, the samples were minced, and protein, water and intramuscular fat (IMF) content determined by near-infrared spectral analysis (NIR Systems 6500, Foss NIR System, Silver Spring, MD, USA) using internal calibrations *(Prevolnik et al., 2005).* Water holding capacity was determined as drip loss (after 24, 48 and 96 hours of storage) according to the EZ method *(Christensen, 2003),* thawing and cooking loss. For thawing loss, a LD chop

(8×5×3 cm) was weighed, vacuum packed and frozen at -20°C. After thawing (overnight at 4°C), the sample was gently drained with a paper towel and reweighed. The same sample was afterwards used for determination of cooking loss and shear force. For cooking loss, the samples were cooked in a thermostatic water bath (ONE 7-45, Memmert GmbH, Schwabach, Germany) until the internal temperature reached 72°C, cooled and reweighed. After cooling, four 1.27-cm-diameter cores were excised and shear force was measured perpendicular to muscle fibres using a TA Plus texture analyser (Ametek Lloyd Instruments Ltd., Fareham, UK) equipped with a 60° V-shaped rectangular-edged blade and a crosshead speed set at 3.3 mm/s. For the determination of total collagen, hydroxyproline was determined according to ISO 3496 standard (1994). For insoluble collagen fraction, LD sample was heated (to 77 °C for 90 min) in Ringer's solution and centrifuged. The supernatant was discarded and the pellet was then further processed as in the case of total collagen. Soluble collagen was determined from the difference in total and insoluble collagen content. Lipid oxidation was evaluated by measuring thiobarbituric acid reactive substances (TBARS) according to the method described by *Lynch and Frei (1993).* Briefly, LD samples were homogenised with 0.15 M KCl (with the addition of BHT), and incubated with 1% (w/v) 2-thiobarbituric acid in 50 mM NaOH and 2.8% (w/v) trichloroacetic acid in a thermostatic heating block (100 °C) for 10 min. After cooling to room temperature, the pink chromogen was extracted into n-butanol and its absorbance was measured at 535 nm (BioSpectrometer Fluorescence, Eppendorf, Hamburg, Germany). Protein oxidation of LD muscle was measured spectrophotometrically (BioSpectrometer Fluorescence, Eppendorf, Hamburg, Germany) according to the method of Oliver et al. (1987) as modified by *Mercier et al. (1998)* in myofibril isolates prepared according to the method of *Pietrzak et al. (1997).* Concentration of carbonyl groups was expressed in nmol/mg proteins.

Genomic DNA was extracted from pig ear tissue using Isolate II Genomic DNA kit (BIO-52067, Bioline), according to the manufacturer's instructions. Polymerase chain reaction (PCR) was performed to screen for C/T SNP (C1843T) in the amplified 134 bp fragment of RYR1 gene, using the following primer pair; forward: 5'-GTGCTGGATGTCCTGTGTTCCCT-3' and reverse: 5'-ACCTCATCAACTATGTCACCAG-3' *(Brenig & Brem, 1992).* Thermocycler programme was as follows: 5 min at 95 °C, 30 cycles at 95 °C for 30 s, 62 °C for 30 s, and 72 °C for 20s, followed by final elongation step at 72 °C for 5 minutes. The reaction volume was 20 µl and contained 1 x PCR buffer, 1 µM primers, 150 µM dNTPs, 1.2 mM MgCl2, 0.5 U DNA Taq polymerase (Thermo Fisher Scientific), and approximately 50-200 ng template DNA.

The fragment was digested with restriction endonuclease HhaI (ER1851, Thermo Fisher Scientific) to obtain fragments of 50 and 84 bp in case of wild type allele. The restriction reaction consisted of 10 µl PCR product, 1.5 µl restriction buffer, 4.2 µl H2O, 0.3 µl (3U) of restriction enzyme, and was incubated for 3 h at 37 °C. Fragments after restriction were analyzed on 2.5 % agarose gel stained with ethidium bromide.

Data was submitted to analysis of variance using the General Linear Models (GLM) procedure of the SAS/STAT module (SAS 8e, 2000; SAS Inc., Cary, NC, USA). The model included the fixed effect of *RYR1* genotype (the pig of n/n genotype was excluded from the analysis). In the case of carcass traits, final live weight was included as covariate in the model. Differences between groups were considered significant if $P<0.05$. The results in the tables are presented as least square means (LS-means) with root-mean-square errors (RMSE). Effect size is presented as Hedges' *g* (difference between means of N/n and N/N divided by pooled SD).

Results and Discussion

The frequencies of *RYR1* genotypes were 1, 15 and 20 for n/n, N/n and N/N, respectively. Based on the present experiment, the incidence of recessive "n" allele in Krškopolje breed is estimated at 0.24, which is relatively high. The incidence of *RYR1* mutation is generally reported low in local southern European pig breeds *(Pugliese and Sirtori, 2012).* However, a large variation (from 0 to 44%) was reported for French native breeds *(Labroue et al., 2001).* The presence of *RYR1* mutation has also been attested for Portuguese Bísaro pig *(Santos e Silva et al., 2000)*. Even higher incidence was previously reported for Krškopolje pig *(Dovč et al., 1996; Čandek-Potokar et al., 2003).* This indicates the introgression of this alelle from the modern breeds into the native breeds (in the case of Krškopolje pig probably from German Landrace or Pietrain) that most probably happened during the period of the severe reduction of the census of less performing local pig breeds *(Pugliese and Sirtori, 2012).*

In the present study, 10.4 kg difference in live weight at slaughter between N/N and N/n pigs resulted in higher average daily gain in N/N than N/n pigs (Table 1). Majority of published studies on modern white breeds reported no differences in daily gain between N/N and N/n pigs *(Sather et al., 1991; Leach et al., 1996; Larzul et al., 1997; Tor et al., 2001).* However, similarly as in the present study, lower daily gain of N/n vs. N/N genotype was reported for the lean line of Landrace and Large White pigs *(McPhee et al., 1992).* For N/n also lower feed

intake was reported *(McPhee et al., 1992; Leach et al., 1996)* which corroborates with lower growth rate reported for N/n pigs.

A strong effect of *RYR1* genotype was noted for carcass traits (Table 1). Carcasses of N/n pigs exhibited better muscularity than N/N pigs demonstrated as greater lean meat content (P=0.005) thicker muscle (P=0.002) and bigger loin eye area (P<0.001). N/n pigs had also thinner backfat than N/N pigs and smaller fat area measured over LD (P=0.087). The literature results are not consistent with regard to the differences between N/n and N/N pigs in muscle and fat tissue development. *Leach et al. (1996)* found no significant difference in backfat thickness between N/n and N/N pigs, but demonstrated superiority of N/n pigs in carcass yields. *Fisher et al. (2000)* found larger LD muscle thickness and area in N/n compared to N/N pigs, while other studies reported no significant differences in size of LD muscle between the two genotypes *(De Smet et al., 1996; Leach et al., 1996; Hamilton et al., 2000)*. There were no differences in chemically determined IMF or marbling scores (Table 2). N/n pigs exhibited higher protein content (P=0.018), which is in agreement with *Pommier et al. (1998)* who reported higher protein % (but also smaller fat %) in LD muscle of N/n compared to N/N pigs.

Table 1. Effect of *RYR1* genotype on carcass traits of Krškopolje pigs

	N/N (n=20)	N/n (n=15)	RMSE	P value	Hedges' *g*
Average daily gain (kg/day)	0.543	0.501	0.059	0.0476	-0.7
Warm carcass weight, kg	96.3	97.6	2.2	0.1211	-0.6
Dressing %	79.7	80.2	1.8	0.1011	0.7
Lean meat content, %	40.3	45.0	4.2	0.0050	1.2
Muscle thickness, mm	64.6	70.0	4.4	0.0021	0.3
Fat thickness over *gluteus medius*, mm	38.8	33.4	5.4	0.0096	-1.2
Fat thickness at last rib, mm	38.9	36.1	5.0	0.1278	-0.6
Carcass length a, cm	100.9	99.7	2.5	0.2190	-0.9
Carcass length b, cm	85.4	84.3	2.2	0.1604	-1.0
Loin eye area, cm^2	33.1	39.3	4.5	0.0008	0.8
Loin eye fat area, cm^2	28.8	26.5	3.5	0.0872	-0.9

RMSE – root-mean-square error

Regarding meat quality traits (Table 2) the results on *RYR1* gene effect are consistent with what has been previously demonstrated for modern white breeds. N/n pigs exhibited lower pH45 (pH measured 45 min post mortem) and no

differences in pH24 (ultimate pH) which is consistent with many studies *(De Smet et al., 1996; Larzul et al., 1997; Monin et al., 1999; Fisher et al., 2000)*. It is known that "n" allele influences the rate of pH fall by favouring calcium release in muscle cells *(Monin, 2004)* thus stimulating the activity of ATPase, while similar glycolytic potential, responsible for the amplitude of pH fall, is reported for N/n and N/N pigs *(Larzul et al., 1997).* No differences between genotypes on CIE colour parameters also corroborate with results of *Larzul et al. (1997)* who reported that the "n" allele appears to be almost completely recessive for meat colour. Water holding capacity of LD muscle was strongly affected by *RYR1* genotype, confirmed also by large values of effect size. Lower water holding capacity in N/n pigs is in agreement with numerous results using various measurement methods *(Sather et al., 1991; De Smet et al., 1996; Leach et al., 1996; Fisher et al., 2000; Hamilton et al., 2000; Škrlep et al., 2010).* Higher thawing and cooking losses of N/n pigs additionally confirm lower water holding capacity of N/n genotype. *RYR1* genotype did not show any effect on oxidation of lipids (TBARS) or proteins (carbonyl groups). Higher shear force (measured on cooked LD samples) in N/n than N/N pigs agrees with the literature showing either higher shear force values or lower sensory tenderness panel scores for stress-susceptible pigs *(Boles et al., 1991; McPhee and Trout, 1995).* The results of *Monin et al. (1999)* also indicated that the "n" allele of the *RYR1* was associated with pork texture and could be considered as detrimental to sensory acceptance. With regard to the effect on connective tissue, assessed as collagen content (total, insoluble and soluble) in LD muscle and its solubility, there was no effect of *RYR1* genotype noted. Although hydroxyproline content was shown as one of the main discriminant characteristics between Large White and highly muscular Pietrain pigs *(Sellier et al., 1971; Baland and Monin, 1987)*, it was also suggested, that no clear relationship existed between muscular development and collagen content in pigs *(Baland and Monin, 1987).*

Table 2. Effect of *RYR1* genotype on meat quality (*longissimus dorsi* muscle)

	N/N (n=20)	N/n (n=15)	RMSE	P value	Hedges' *g*
pH45	6.60	6.41	0.21	0.0116	-0.9
pH24	5.63	5.55	0.17	0.2268	-0.4
Subjective colour (1-6)	4.38	4.30	0.64	0.7320	-0.1
Marbling (1-7)	4.05	3.97	1.03	0.8134	-0.1
Colour parameters CIE L*	51.2	52.8	3.2	0.1424	0.5
CIE a*	7.1	7.6	1.3	0.2543	0.4
CIE b*	1.5	2.0	1.2	0.2122	0.4
C*	7.3	8.0	1.5	0.1867	0.5
h°	11.3	13.9	7.5	0.3268	0.3
Chemical analysis					
Intramuscular fat, %	2.92	2.85	0.85	0.8113	-0.1
Protein, %	23.2	23.6	0.5	0.0176	0.8
Water, %	72.3	72.6	0.8	0.4147	0.3
Water to protein ratio	3.38	3.37	0.15	0.9743	-0.0
Water holding capacity					
Drip loss after 24 h, %	3.2	7.0	1.7	<0.0001	2.2
Drip loss after 48 h, %	4.8	9.0	2.0	<0.0001	2.1
Drip loss after 96 h, %	7.4	11.5	2.2	<0.0001	1.8
Thawing loss, %	10.4	14.8	3.0	0.0001	1.5
Cooking loss, %	27.2	29.6	3.1	0.0241	0.8
Hardness (WBSF, N)	53.1	59.7	8.2	0.0241	0.8
Collagen (mg/g)					
Total collagen	2.77	2.67	0.20	0.1924	-0.4
Insoluble collagen	2.25	2.18	0.16	0.1764	-0.5
Soluble collagen	0.52	0.50	0.09	0.6100	-0.2
Collagen solubility, %	18.6	18.6	2.7	0.9970	-0.0
Oxidation parameters					
TBARS, µg/kg	28.1	27.8	2.2	0.7480	-0.1
Carbonyl groups, nmol/g protein	1.43	1.38	0.29	0.5721	-0.2

RMSE – root-mean-square error, WBSF – Warner-Bratzler shear force, TBARS – thiobarbituric acid reactive substances

Conclusion

The incidence of mutated *RYR1* allele in the population of Krškopolje pig remains relatively high. In agreement with the results on modern white pig breeds, the presence of mutated allele at *RYR1* locus in the case of local pig breed Krškopolje negatively affects meat quality demonstrated as lower water holding capacity, and lower tenderness. An intensive breeding effort is needed to eliminate this mutation from the Krškopolje pig population in order to produce meat of better quality suitable for processing into high quality products.

Kvalitet mesa krškopoljskih prasadi u odnosu na *RYR1* genotip

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Rezime

Uticaj genotipa *RYR1* na kvalitet trupova i kvaliteta mesa istraživani su na grlima krškopoljske rase, jedine slovenačke lokalne rase svinja. Trideset šest kastrata poreklom iz 12 legala genotipizovano je za c. C1843T (str Arg615Cys) u *RYR1* lokusu (recesivni alel dalje označen kao "n" a wild-type alel kao "N"). Svinje sa mutiranim recesivnim alelom (N/n) imale su nižu stopu rasta i manje masne trupove (pokazali su veći sadržaj mesnatosti, debljinu mišića i područje slabine, tanju leđnu slaninu i manju prekrivnost LD mišića masnim tkivom). Izražen efekat *RYR1* na kvalitet mesa je primećen. Stopa opadanja pH u *longissimus dorsi* (LD) mišiću N/n svinja je bila brža (niži pH 45 min posle klanja), ali nije postojao efekat *RYR1* na krajnji pH. Zabeležen je manji kapacitet zadržavanja vode (veći kalo otapanje i kuvanja) mesa od N/n. Meso N/n svinja pokazalo je i veću otpornost na sile rasecanja (što ukazuje na slabiju mekoću mesa).

Ključne reči: krškopoljska svinja, RYR1 gen, porast, trup, kvalitet mesa

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