https://doi.org/10.17221/31/2018-CJAS

Association of *miR-208b* Polymorphism with Meat Quality Traits and Texture Parameters in Pigs

Daniel Polasik[1], Mirosław Tyra[2], Magdalena Szyndler-Nędza[2], Grzegorz Żak[2], Barry Lambert[3], Arkadiusz Terman[1]*

[1]Department of Genetics and Animal Breeding, West Pomeranian University of Technology Szczecin, Szczecin, Poland

[2]Department of Animal Genetics and Breeding, National Research Institute of Animal Production, Balice, Poland

[3]Department of Animal Science and Veterinary Technology, Tarleton State University, Stephenville, USA

**Corresponding author: daniel.polasik@zut.edu.pl*

ABSTRACT

Polasik D., Tyra M., Szyndler-Nędza M., Żak G., Lambert B., Terman A. (2018): **Association of *miR-208b* polymorphism with meat quality traits and texture parameters in pigs**. Czech J. Anim. Sci., 63, 435–442.

Previous studies have shown that single nucleotide polymorphism (SNP) (rs328743478) located downstream of *pre-miR-208b* in *MYH7* gene is correlated with fibre number composition and drip loss in pigs. Because fibre characteristics are linked to meat quality, the aim of this study was to analyse the relationships between rs328743478 polymorphism and meat quality and texture parameters. The study utilised 578 pigs belonging to Polish Landrace, Polish Large White, Puławska, Pietrain, Duroc, and Hampshire breeds. Association study was performed for the first three breeds separately and for all six breeds joined together. Obtained results showed a significant influence ($P \leq 0.05$) of *miR-208b* genotypes on water holding capacity (WHC) in the whole population and individual breeds and on intramuscular fat content (IMF) and luminosity in the whole population as well as in Landrace and Puławska breeds, respectively. By analysing texture parameters, we found association ($P \leq 0.05$ or $P \leq 0.01$) between rs328743478 polymorphism and toughness, firmness, and chewiness measured in *m. semimembranosus* and *m. longissimus lumborum*. The highest values of these traits in *m. semimembranosus* were noticed for *AA* genotype, however in *m. longissimus lumborum* for *GG* in the whole population and some individual breeds. It was found that *miR-208b* genotypes were also associated with cohesiveness ($P \leq 0.01$), springiness, and hardness ($P \leq 0.05$) but obtained results were not consistent among breeds and the whole population. The obtained results confirm that *miR-208b* polymorphism is associated with some economically important traits in pigs.

Keywords: miRNAs; SNP; performance traits; *Sus scrofa domestica*

Meat quality characteristics are quantitative traits whose values result from the simultaneous influence of a gene or many genes and the environment. Genes vary in their contribution to production traits. The influence of some genes on a given trait can be particularly prominent compared to other genes and the environmental impact (Zak and Pieszka 2009). It is therefore important and relevant to investigate the relationship between different genes and the quality of meat.

MicroRNAs (miRNAs or miRs) are non-coding RNA molecules, approximately 22 nucleotides long, which repress gene expression, mainly at the posttranscriptional level. They act through

https://doi.org/10.17221/31/2018-CJAS

binding to the 3' untranslated regions of protein-coding transcripts and lead to degradation of target mRNA or inhibition of translation (Bartel 2004). MicroRNAs, which are specific for muscle, are called myomiRs. Only a few types of myomiRs have been identified so far: *miR-1*, *miR-133a*, *miR-133b*, *miR-206*, *miR-208a*, *miR-208b*, *miR-486*, and *miR-499*. They play an important role in myogenesis, embryonic muscle growth, and cardiac function including hypertrophy. Most of genes that encode myomiRs are located inside the host genes, except *miR-133b* and *miR-206*, which are located in intergenic regions (McCarthy 2008; Horak et al. 2016). In the swine transcriptome 382 precursors and 411 mature types of miRNAs have been described in miRBase (release 21.0) (Kozomara and Griffiths-Jones 2014). Among them ssc-*miR-378*, *ssc-miR-1,* and *ssc-miR-206* were abundantly expressed in skeletal muscles (Hou et al. 2012). Besides earlier mentioned roles of miRNAs, they can also control the fibre type of skeletal muscles as shown during experiments with *miR-208b* and *miR-499* double knockout (dKO) mice. These genes show high homology and are co-expressed in *m. soleus*. Analysis demonstrated that dKO mutants were characterized by substantial loss of type I myofibres in the soleus and increase in the expression of fast type IIx/d and type IIb myosin isoforms (van Rooij et al. 2009). In pigs, *miR-208b* is expressed from myosin heavy chain 7 gene (*MYH7*), located on SSC7, however *miR-499* from myosin heavy chain 7B gene (*MYH7B*) on SSC17. Both genes encode closely related isoforms of slow myosins that are present in cardiac, as well as in slow skeletal muscle (van Rooij et al. 2009; Aken et al. 2016). Porcine *MYH7* gene consists of 40 exons separated by 39 introns and spans 22 kb compared to 39 exons over 21.5 kb in human *MYH7* (NCBI build 37.3) because of an additional untranslated 5'-exon in pigs (Murgiano et al. 2012).

Kim et al. (2015) detected a single nucleotide polymorphism (SNP) (rs328743478, NC_010449.4:g.17104G>A) in intron 30 of porcine *MYH7* gene, located in *pri-miR-208b* sequence that has an impact on the secondary structure of *pri-miR-208b*. Analysis showed that it influenced mature *miR-208b* expression, which correlates with target (transcription factor SOX-6) and host (*MYH7*) genes expression. Moreover, the SNP was associated with proportions of types I and IIb fibre numbers ($P < 0.010$), as well as drip loss ($P = 0.012$) in three pig breeds (Berkshire, Landrace, and Yorkshire). Because skeletal muscles consist mainly of muscle fibres, the quality of meat depends largely on their characteristics. Muscle fibre characteristics influence appearance quality traits (AQT) as well as eating quality traits (EQT) (Joo et al. 2013). The knowledge about their relationship with genetic background may improve meat quality of pigs. Therefore, the aim of this study was to analyse polymorphism rs328743478 and its influence on meat quality traits and texture parameters in Polish pigs.

MATERIAL AND METHODS

Animals. The study utilised 578 sows that belong to six breeds: Polish Landrace ($n = 269$), Polish Large White ($n = 189$), Puławska ($n = 68$), Pietrain ($n = 31$), Duroc ($n = 14$), and Hampshire ($n = 7$). Polish Landrace pigs were the offspring of 57 boars and 158 sows, Polish Large White – 34 and 123, however Puławska – 18 and 42, respectively. For the whole population the number of sows was 357, however of boars – 127. Animals were maintained in Pig Tests Stations (National Research Institute of Animal Production) located in Pawłowice, Chorzelów, and Mełno. Feeding and housing conditions were equal for all animals. Pigs were introduced into stations at 12 weeks of age and fed *ad libitum* from 30 up to 100 (± 2.5) kg of weight. At the conclusion of the feeding period, pigs were slaughtered by stunning with high-voltage electric tongs followed by exsanguination. The mean age of slaughtered pigs was 176.55 ± 4.04 days. For further analysis, samples from *m. longissimus lumborum* and *m. semimembranosus* were collected.

Meat quality and texture. The following meat quality traits were measured ($n = 509$): intramuscular fat content (IMF), meat colour, pH, and water holding capacity (WHC). IMF content was determined in thawed *m. longissimus dorsi* homogenates by a fat analyser SOX 406 (Gerhardt, Germany). The pH was estimated 45 min (pH45) and 24 h (pH24) after slaughter in *m. longissimus dorsi* and *m. semimembranosus* by pH-Star (Matthäus, Germany). Meat colour parameters of loin (L* – lightness, a* – redness, b* – yellowness) were determined using a CR-310 chromameter (Minolta, Japan). To estimate WHC, the Grau–Hamm method was applied (Hamm 1986).

https://doi.org/10.17221/31/2018-CJAS

Firmness and toughness were determined in 481 pigs by using the Warner–Bratzler shear force (WBS) analyses on raw (r) and cooked samples of *m. longissimus lumborum* and *m. semimembranosus*. Slices of muscle with a diameter of 3.5 cm and weight of approximately 200 g were placed into polyethylene bag and heated in water bath to reach 80°C. Samples were then chilled at 4°C for 24 h. Two cores 15-mm in diameter, representing each sample, were taken from the muscle by slicing parallel to the direction of muscle and sheared by using a WB triangular blade (4.5 mm/s) on a Texture Analyser TA.XT plus (Stable Micro Systems, UK).

The following texture profile parameters (TPA) were determined for both muscles: hardness, springiness, cohesiveness, chewiness, and resilience. For TPA estimation, the double compression procedure was applied (cylinder SMS P/25, base diameter 50 mm). All parameters were calculated according to the procedure given by Ropka-Molik et al. (2014).

Analysis of polymorphism. Genomic DNA was isolated from muscle tissue using the ReliaPrep™ gDNA Tissue Miniprep System (Promega, USA). For SNP in *miR-208b* gene (rs328743478) analysis, the following primer sequences were picked using Primer3 software (Untergasser et al. 2012): forward – TCC ATG TCT GCA GCC TAG AG, reverse – TCA GAA GCA TTG TTG GCG AA. Primers were designed based on NC_010449.5, sequence (position of *MYH7* 75650841–75672908), which is reverse and complementary to the reported sequence (Kim et al. 2015). Polymerase chain reaction (PCR) cycling conditions were as follows: initial denaturation at 95°C for 5 min, 32 cycles of 95°C for 45 s, 55°C for 45 s, 72°C for 45 s, and final synthesis at 72°C for 5 min. PCRs were performed in total volume of 10 µl containing: 30–60 ng of DNA, 2xPCR Mix (A&A Biotechnology, Poland), 7 pmol of each primer, and PCR grade water. Obtained amplicons were digested by *Hin*fI (Thermo Fisher Scientific, USA) enzyme at 37°C overnight and separated in 2% agarose gels. Individual genotypes were determined based on the following restriction fragments lengths: *AA* – 130, 30 bp, *AG* – 160, 130, 30 bp, *GG* – 160 bp.

Statistical analysis. All analysed traits were assessed in a fixed model using the least squares method of the General Linear Model procedure in the SAS software (Statistical Analysis System, Version 8.02). The following model was applied:

$$Y_{ijk} = \mu + b_i + g_j + (bg)_{ij} + e_{ijk}$$

where:

Y_{ijk} = estimated trait
μ = overall mean of the trait
b_i = fixed effect of *i* breed
g_j = fixed effect of *j* genotype group of *miR-208b* gene
$(bg)_{ij}$ = interaction between *gj* genotype group and breed (when significant)
e_{ijk} = random residual error

Due to very low frequency in Polish Landrace breed, *AA* genotype was excluded from the association analysis. Because in Duroc and Hampshire breeds one genotype (*GG*) was present, the association analysis was not performed for them. Genotype and allele frequencies, gene diversity (expected heterozygosity), and Hardy–Weinberg equilibrium were estimated using PowerMarker v. 3.25 software (Liu and Muse 2005). All pigs were free of *RYR1 T* allele.

RESULTS AND DISCUSSION

In this study, we analysed SNP in intron 30 of porcine *MYH7* gene where the locus for *miR-208b* is located. This polymorphism leads to significant changes in the levels of mature *miR-208b*, which is positively associated with the expression levels of *MYH7* gene. It also influences fibre number composition (%) and drip loss (%) in pigs (Kim et al. 2015).

The present study shows some differences in the frequency of rs328743478 genotypes and alleles between the investigated pig populations (Table 1). In all breeds *GG* genotype was the most frequent except Pietrain where *AG* genotype was the most abundant. Frequency of the *G* allele in Polish Landrace (0.89) was somewhat higher than observed in Landrace (0.81) (Kim et al. 2015). Gene diversity, however in Pietrain, was more than twice as in Polish Landrace. The distribution of the genotypes was in Hardy–Weinberg equilibrium in all analyzed breeds.

Analyses between rs328743478 variants and meat quality traits are presented in Table 2.

In our study, we found that intramuscular fat content was the highest for *AA* genotype ($P \leq 0.05$) in relation to others for the whole population analysed. It has been shown that IMF is positively correlated with red muscle fibre content but negatively with white muscle fibre content (Hwang et al.

https://doi.org/10.17221/31/2018-CJAS

Table 1. Genotype and allele frequencies with some population indexes calculated for the *miR-208b* single nucleotide polymorphism (rs328743478)

Breed	*n*	Genotype			Allele		Gene diversity	χ^2	*P*
		AA (*n*)	*AG* (*n*)	*GG* (*n*)	*A*	*G*			
Polish Landrace	269	0.01 (2)	0.21 (57)	0.78 (210)	0.11	0.89	0.201	0.782	0.377
Polish Large White	189	0.06 (12)	0.36 (68)	0.58 (109)	0.24	0.76	0.368	0.101	0.751
Puławska	68	0.09 (6)	0.41 (28)	0.50 (34)	0.29	0.71	0.415	0.005	0.545
Pietrain	31	0.06 (2)	0.58 (18)	0.36 (11)	0.35	0.65	0.458	2.230	0.135
Duroc	14	–	–	1.00 (14)	–	1.00	–	–	–
Hampshire	7	–	–	1.00 (7)	–	1.00	–	–	–

2010). It is consistent with earlier results of Kim et al. (2015), where the *AA* genotype was correlated with type I fibre percentage in pigs. In the Polish Landrace breed, where 2 genotypes were analysed, *AG* animals had higher IMF ($P \leq 0.05$) than those with *GG* genotype. Another parameter correlated with *miR-208b* genotypes was meat luminosity (L*). Values of this trait were statistically lower ($P \leq 0.05$)

Table 2. Association between *miR-208b* polymorphism (rs328743478) and meat quality traits in different pig breeds and in whole population analysed (Least Squares Mean ± standard error)

Trait	Genotype	Landrace (*n* = 229)	Large White (*n* = 182)	Puławska (*n* = 67)	Whole population (*n* = 509)
IMF (%)	*AA*	–	1.191 ± 0.109	1.190 ± 0.055	1.189 ± 0.057^{ab}
	AG	1.173 ± 0.046^{a}	1.180 ± 0.055	1.225 ± 0.027	1.123 ± 0.032^{a}
	GG	1.146 ± 0.041^{a}	1.210 ± 0.057	1.205 ± 0.025	1.145 ± 0.030^{b}
Meat colour (L*)	*AA*	–	51.1 ± 0.635	52.2 ± 0.738^{ab}	52.5 ± 0.599^{ab}
	AG	54.8 ± 0.850	52.9 ± 0.348	53.7 ± 0.358^{b}	53.8 ± 0.354^{b}
	GG	54.4 ± 0.757	53.10 ± 0.347	54.2 ± 0.337^{a}	54.2 ± 0.327^{a}
Meat colour (a*)	*AA*	–	18.30 ± 0.433	16.51 ± 0.413	18.29 ± 0.394
	AG	16.34 ± 0.582	17.87 ± 0.237	16.77 ± 0.200	17.95 ± 0.233
	GG	17.44 ± 0.517	17.95 ± 0.236	16.87 ± 0.188	18.06 ± 0.215
Meat colour (b*)	*AA*	–	5.185 ± 0.368	2.147 ± 0.244	5.07 ± 0.325
	AG	3.258 ± 0.477	5.227 ± 0.202	2.322 ± 0.118	5.11 ± 0.192
	GG	3.477 ± 0.425	5.340 ± 0.201	2.301 ± 0.111	5.21 ± 0.177
pH45 (*m. longissimus dorsi*)	*AA*	–	6.315 ± 0.067	6.400 ± 0.060	6.32 ± 0.049
	AG	6.274 ± 0.062	6.219 ± 0.036	6.265 ± 0.029	6.26 ± 0.028
	GG	6.225 ± 0.055	6.284 ± 0.036	6.277 ± 0.027	6.27 ± 0.026
pH24 (*m. longissimus dorsi*)	*AA*	–	5.503 ± 0.030	5.672 ± 0.026	5.56 ± 0.024
	AG	5.649 ± 0.033	5.620 ± 0.017	5.616 ± 0.013	5.56 ± 0.014
	GG	5.628 ± 0.029	5.619 ± 0.017	5.630 ± 0.012	5.55 ± 0.013
pH45 (*m. semimembranosus*)	*AA*	–	6.368 ± 0.061	6.360 ± 0.052	6.37 ± 0.045
	AG	6.257 ± 0.052	6.349 ± 0.034	6.260 ± 0.025	6.37 ± 0.027
	GG	6.285 ± 0.046	6.331 ± 0.034	6.252 ± 0.024	6.38 ± 0.025
pH24 (*m. semimembranosus*)	*AA*	–	5.670 ± 0.038	5.678 ± 0.054	5.63 ± 0.027
	AG	5.652 ± 0.032	5.643 ± 0.021	5.628 ± 0.026	5.59 ± 0.016
	GG	5.656 ± 0.029	5.636 ± 0.021	5.654 ± 0.024	5.59 ± 0.015
WHC (%)	*AA*	–	31.2 ± 2.006^{a}	30.7 ± 2.544^{ab}	30.2 ± 1.507^{ab}
	AG	33.2 ± 1.881^{a}	32.1 ± 0.105	33.1 ± 1.233^{b}	32.4 ± 0.895^{b}
	GG	34.3 ± 1.683^{a}	33.4 ± 1.149^{a}	35.6 ± 1.160^{a}	32.5 ± 0.843^{a}

IMF (%) = intramuscular fat content, L* = luminosity, a* = redness, b* = yellowness, pH45, pH24 = pH measured 45 min and 24 h after slaughter, respectively, WHC (%) = water holding capacity, *n* = number of animals analysed in given group

^{a,b}values in the same row marked with different superscripts differ statistically at $P \leq 0.05$

https://doi.org/10.17221/31/2018-CJAS

for *AA* genotype than for *AG* and *GG* genotypes in Puławska breed and the whole population. It has been previously shown that *miR-208b* together with *miR-499* promote a fast-to-slow myofibre-type switch and thus are positively associated with red fibres (Quiat et al. 2011; Ma et al. 2015). It is consistent with results of Kim et al. (2015) again, because *AG* and *GG* genotypes were associated with lower type I fibres content in muscle, however *AA* genotype was linked with the highest level of *pre-miR-208b* ($P = 0.053$) and mature *miR-208b* ($P = 0.009$). Previous studies were also focused on luminosity of pork in relation to DNA polymorphism and a significant correlation was found only for alpha-ketoglutarate dependent dioxygenase (*FTO*) and calpastatin (*CAST*) genes (Fu et al. 2013; Ropka-Molik et al. 2014).

The third meat quality trait associated with rs328743478 genotypes was water holding capacity. In the Polish Large White, Puławska breeds, and in the whole population we found that values were higher for *GG* genotype compared to *AA* ($P \leq 0.05$) or *AG* (also in Polish Landrace). Associations between *miR-208b* variants with IMF and L* are connected indirectly with former results (Kim et al. 2015) through the influence on muscle fibre percentage, WHC is connected directly through the association with drip loss (%). The genetic correlation between WHC and drip loss is naturally very high (Jennen et al. 2007), therefore we observed the same relation between these two traits and *miR-208b* genotypes as Kim et al. (2015). These results also agree with those reporting that drip loss in *m. longissimus dorsi* muscle from Duroc ×

Table 3. Association between *miR-208b* polymorphism (rs328743478) and *m. longissimus lumborum* texture parameters in different pig breeds and in whole population analysed (Least Squares Mean ± standard error)

Trait	Genotype	Landrace ($n = 218$)	Large White ($n = 158$)	Puławska ($n = 67$)	Whole population ($n = 481$)
Firmness (r)	*AA*	–	33.2 ± 3.331	21.3 ± 3.510	28.9 ± 2.767
	AG	26.0 ± 3.523	30.4 ± 1.751	18.90 ± 1.763	26.2 ± 1.525
	GG	29.4 ± 3.080	34.5 ± 1.818	18.95 ± 1.713	28.9 ± 1.414
Toughness (r)	*AA*	–	93.3 ± 9.702^{ab}	63.4 ± 11.17	83.4 ± 8.086^{ab}
	AG	75.1 ± 9.789	82.6 ± 5.099^{a}	54.4 ± 5.609	84.2 ± 4.458^{bc}
	GG	84.8 ± 8.556	95.1 ± 5.294^{b}	49.4 ± 5.449	89.8 ± 4.132^{ac}
Firmness	*AA*	–	92.5 ± 7.324^{Ab}	73.9 ± 6.853	78.7 ± 5.408^{Ab}
	AG	94.7 ± 6.457^{a}	82.1 ± 3.850^{Ac}	74.6 ± 3.442	83.8 ± 2.982^{bc}
	GG	84.5 ± 5.644^{a}	85.1 ± 3.997^{bc}	73.9 ± 3.345	89.0 ± 2.764^{Ac}
Toughness	*AA*	–	190 ± 20.50	183 ± 18.18	185 ± 14.19^{AB}
	AG	221 ± 16.04^{a}	224 ± 10.77	171 ± 9.130	198 ± 7.825^{BC}
	GG	193 ± 14.02^{a}	228 ± 11.18	175 ± 8.871	214 ± 7.254^{AC}
Hardness	*AA*	–	9.335 ± 1.317	6.768 ± 1.387	9.523 ± 0.984
	AG	8.946 ± 1.129	11.99 ± 0.692	6.068 ± 0.697	10.69 ± 0.542
	GG	9.461 ± 0.987	10.85 ± 0.719	7.339 ± 0.677	10.65 ± 0.503
Springiness	*AA*	–	0.709 ± 0.022	0.687 ± 0.025	0.710 ± 0.016^{Ac}
	AG	0.695 ± 0.019^{a}	0.729 ± 0.012	0.664 ± 0.012	0.717 ± 0.009^{bc}
	GG	0.675 ± 0.017^{a}	0.734 ± 0.012	0.695 ± 0.012	0.736 ± 0.008^{Ab}
Cohesiveness	*AA*	–	0.675 ± 0.020	0.631 ± 0.022^{B}	0.682 ± 0.015
	AG	0.631 ± 0.018	0.678 ± 0.010	0.605 ± 0.011^{AB}	0.675 ± 0.008
	GG	0.654 ± 0.016	0.676 ± 0.011	0.642 ± 0.011^{A}	0.690 ± 0.008
Chewiness	*AA*	–	4.615 ± 0.626	2.911 ± 0.702^{b}	4.402 ± 0.484^{ab}
	AG	4.891 ± 0.556^{a}	5.988 ± 0.329	2.617 ± 0.352^{ab}	5.360 ± 0.267^{b}
	GG	4.094 ± 0.486^{a}	5.532 ± 0.341	3.453 ± 0.342^{a}	5.518 ± 0.247^{a}
Resilience	*AA*	–	0.302 ± 0.012	0.266 ± 0.014	0.302 ± 0.009
	AG	0.271 ± 0.011	0.299 ± 0.006	0.255 ± 0.007	0.297 ± 0.005
	GG	0.286 ± 0.009	0.303 ± 0.006	0.276 ± 0.007	0.308 ± 0.005

r = raw tissue, n = number of animals analysed in given group

values marked with different superscripts differ statistically at $^{a–c}P \leq 0.05$ or $^{A–C}P \leq 0.01$

https://doi.org/10.17221/31/2018-CJAS

(Yorkshire × Landrace) pigs is positively related to type IIb percentage (Ryu and Kim 2005). Recent studies have indicated the associations between SNPs and other miRNAs and production traits in pigs. They included meat quality (*miR-206* and *miR-133b*), lean meat production (*miR-206*) as well as muscle fibre characteristics (*miR-206*, *miR-133b*, and *miR-1*) (Hong et al. 2012; Lee et al. 2013).

Because fresh meat quality is strongly related to fibre type composition in muscle (Joo et al. 2013) we also analysed the rs328743478 polymorphism according to shear-force traits and texture profile parameters of two muscles – *m. longissimus lumborum* (Table 3) and *m. semimembranosus* (Table 4). The obtained results showed that *miR-208b* polymorphism is associated with shear-force traits. In the whole population, the highest values ($P \leq 0.05$) for firmness and toughness were observed for the *AA* genotype in raw and cooked *m. semimembranosus* muscle. This trend was also present for Large White breed. In the case of *m. longissimus lumborum* we observed the reverse tendency, because the *GG* genotype was associated with the highest values of shear-force traits, except firmness of raw meat where values for *AA* and *GG* variants were the same. These differences may reflect *m. longissimus lumborum* and *m. semimembranosus* fibre properties. Both are white skeletal muscle, but are characterised by different proportions of fibre types. Gentry et al. (2004) showed that in pigs born and reared either indoors or outdoors, the content of types I and IIb fibres in *m. longissimus lumborum* is higher, however type IIa is lower if

Table 4. Association between *miR-208b* polymorphism (rs328743478) and *m. semimembranosus* texture parameters in different pig breeds and in whole population analysed (Least Squares Mean ± standard error)

Traits	Genotype	Landrace (n = 218)	Large White (n = 158)	Puławska (n = 67)	Whole population (n = 481)
Firmness (r)	*AA*	–	28.4 ± 2.525	26.5 ± 2.080	27.2 ± 1.750^{ab}
	AG	25.1 ± 2.099	27.2 ± 1.531	21.8 ± 1.009	25.4 ± 1.021^{b}
	GG	24.8 ± 1.830	26.5 ± 1.540	24.0 ± 0.957	25.2 ± 0.946^{a}
Toughness (r)	*AA*	–	89.4 ± 8.716^{a}	74.8 ± 6.635	86.2 ± 5.870^{ab}
	AG	77.3 ± 7.124	78.6 ± 5.286	63.0 ± 3.217	77.1 ± 3.426^{a}
	GG	77.1 ± 6.212	78.7 ± 5.317^{a}	68.2 ± 3.053	78.3 ± 3.175^{b}
Firmness	*AA*	–	97.3 ± 9.332^{a}	86.6 ± 9.043	96.6 ± 6.114^{ab}
	AG	86.1 ± 6.917	92.7 ± 5.660	81.6 ± 4.384	87.1 ± 3.568^{a}
	GG	88.5 ± 6.031	93.2 ± 5.693^{a}	84.5 ± 4.162	89.2 ± 3.307^{b}
Toughness	*AA*	–	239 ± 24.6	202 ± 19.03	235 ± 14.54^{ab}
	AG	217 ± 16.01^{a}	236 ± 14.94	195 ± 9.226	218 ± 8.484^{a}
	GG	232 ± 13.96^{a}	230 ± 15.02	197 ± 8.758	223 ± 7.862^{b}
Hardness	*AA*	–	11.42 ± 1.659	13.06 ± 1.483^{a}	38.3 ± 20.45
	AG	18.70 ± 2.40^{a}	11.32 ± 1.006	8.596 ± 0.719^{a}	35.6 ± 11.94
	GG	24.0 ± 1.54^{a}	11.22 ± 1.012	10.70 ± 0.682	39.0 ± 11.06
Springiness	*AA*	–	0.745 ± 0.036	0.702 ± 0.031	0.747 ± 0.023
	AG	0.757 ± 0.028	0.785 ± 0.022	0.715 ± 0.015	0.767 ± 0.014
	GG	0.773 ± 0.024	0.756 ± 0.020	0.724 ± 0.014	0.763 ± 0.013
Cohesiveness	*AA*	–	0.659 ± 0.181	0.624 ± 0.032	0.693 ± 0.075
	AG	0.681 ± 0.023	0.816 ± 0.110	0.608 ± 0.015	0.765 ± 0.044
	GG	0.681 ± 0.020	0.716 ± 0.110	0.632 ± 0.015	0.727 ± 0.041
Chewiness	*AA*	–	5.284 ± 0.854^{ab}	5.929 ± 0.847	5.797 ± 0.617^{a}
	AG	4.619 ± 0.761	5.807 ± 0.518^{a}	4.850 ± 0.411	5.140 ± 0.360^{a}
	GG	4.873 ± 0.663	5.823 ± 0.521^{b}	5.020 ± 0.390	5.427 ± 0.334
Resilience	*AA*	–	0.280 ± 0.014	0.252 ± 0.016	0.287 ± 0.010
	AG	0.299 ± 0.013	0.302 ± 0.008	0.244 ± 0.008	0.295 ± 0.006
	GG	0.298 ± 0.011	0.291 ± 0.008	0.265 ± 0.008	0.295 ± 0.006

r = raw tissue, n = number of animals analysed in given group

^{a,b}values marked with the same letter differ statistically at $P \leq 0.05$

https://doi.org/10.17221/31/2018-CJAS

compared to *m. semimembranosus* muscle. In our study, an opposite tendency for both muscles was also observed for one of the TPA – chewiness. The highest value of this parameter in *m. semimembranosus* was found for animals with *AA* genotype, however in *m. longissimus lumborum* for *GG* ($P \leq 0.05$). The opposite trend between these muscles was observed in the Polish Large White breed for hardness, springiness, and chewiness during the analysis of microsomal triglyceride transfer protein gene (*MTP*) (Ropka-Molik et al. 2016). The reason of the association disagreement between analysed groups may be associated with the influence of breed. It was proved during analysis of performance traits in Polish Large White, Polish Landrace, Puławska, and Duroc breeds. The effect of the breed ($P \leq 0.001$–$P \leq 0.05$) was observed for firmness, toughness (cooked and raw), and chewiness in both *m. longissimus lumborum* and *m. semimembranosus* (Ropka-Molik et al. 2014). The breeds under our study are also characterised by differentiated performance traits values. For instance, Puławska is a conservative breed that belongs to fat-meat type, however the rest of analysed breeds are meat types. The study of Polish Landrace and Puławska pigs showed some differences regarding growth, carcass and texture traits. Taking into consideration texture parameters, Polish Landrace breed was characterised by higher firmness and toughness in *m. longissimus lumborum* ($P < 0.01$) and firmness in *m. semimembranosus* ($P < 0.05$) in relation to Puławska (Piorkowska et al. 2018). We also found similar tendency in *m. longissimus lumborum* (raw and cooked), however in *m. semimembranosus* these values were similar, except toughness (cooked).

miR-208b genotypes were also associated with other TPA – hardness, springiness ($P \leq 0.05$), and cohesiveness ($P \leq 0.01$) in individual breeds or the whole population, but results were not consistent among them. In the case of hardness measured in *m. semimembranosus* we observed inverted association again. Its higher value was noticed in Landrace pigs for *GG* genotype, however in Puławska pigs for *AA* genotype.

CONCLUSION

In summary, the SNP in *miR-208b* (rs328743478) through the influence on *miR-208b* expression, fibre number composition, and drip loss affect other meat quality traits such as intramuscular fat content, luminosity, and water holding capacity ($P \leq 0.05$). Moreover, it is strongly ($P \leq 0.01$) associated with shear-force traits (firmness and toughness) and one of the texture parameters in ham and loin muscles. It indicates that the *miR-208b* polymorphism can be applied as a good marker for texture parameters as well as can be considered as a marker for some meat quality traits.

REFERENCES

Aken B.L., Ayling S., Barrell D., Clarke L., Curwen V., Fairley S., Fernandez Banet J., Billis K., Garcia Giron C., Hourlier T., Howe K., Kahari A., Kokocinski F., Martin F.J., Murphy D.N., Nag R., Ruffier M., Schuster M., Tang Y.A., Vogel J.H., White S., Zadissa A., Flicek P., Searle S.M.J. (2016): The Ensembl gene annotation system. Database: The Journal of Biological Databases and Curation, 2016: baw093.

Bartel D.P. (2004): MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, 116, 281–297.

Fu Y., Li L., Ren S. (2013): Effect of FTO expression and polymorphism on fat deposition in Suzhong pigs. Asian-Australasian Journal of Animal Sciences, 26, 1365–1373.

Gentry J.G., McGlone J.J., Miller M.F., Blanton Jr. J.R. (2004): Environmental effects on pig performance, meat quality, and muscle characteristics. Journal of Animal Science, 82, 209–217.

Hamm R. (1986): Functional properties of the myofibrilar system and their measurement. In: Bechtel P.J. (ed.): Muscle as Food. Academic Press Inc., London, UK, 135–199.

Hong J.S., Noh S.H., Lee J.S., Kim J.M., Hong K.C., Lee Y.S. (2012): Effects of polymorphisms in the porcine microRNA miR-1 locus on muscle fiber type composition and miR-1 expression. Gene, 506, 211–216.

Horak M., Novak J., Bienertova-Vasku J. (2016): Muscle-specific microRNAs in skeletal muscle development. Developmental Biology, 410, 1–13.

Hou X., Tang Z., Liu H., Wang N., Ju H., Li K. (2012): Discovery of microRNAs associated with myogenesis by deep sequencing of serial developmental skeletal muscles in pigs. PLoS ONE, 7, e52123.

Hwang Y.H., Kim G.D., Jeong J.Y., Hur S.J., Joo S.T. (2010): The relationship between muscle fiber characteristics and meat quality traits of highly marbled Hanwoo (Korean native cattle) steers. Meat Science, 86, 456–461.

Jennen D.G., Brings A.D., Liu G., Jungst H., Tholen E., Jonas E., Tesfaye D., Schellander K., Phatsara C. (2007): Genetic aspects concerning drip loss and water-holding

https://doi.org/10.17221/31/2018-CJAS

capacity of porcine meat. Journal of Animal Breeding and Genetics, 124, 2–11.

Joo S.T., Kim G.D., Hwang Y.H., Ryu Y.C. (2013): Control of fresh meat quality through manipulation of muscle fiber characteristics. Meat Science, 95, 828–836.

Kim J.M., Lim K.S., Hong J.S., Kang J.H., Lee Y.S., Hong K.C. (2015): A polymorphism in the porcine miR-208b is associated with microRNA biogenesis and expressions of SOX-6 and MYH7 with effects on muscle fibre characteristics and meat quality. Animal Genetics, 46, 73–77.

Kozomara A., Griffiths-Jones S. (2014): miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Research, 42, D68–D73.

Lee J.S., Kim J.M., Lim K.S., Hong J.S., Hong K.C., Lee Y.S. (2013): Effects of polymorphisms in the porcine microRNA MIR206/MIR133B cluster on muscle fiber and meat quality traits. Animal Genetics, 44, 101–106.

Liu K., Muse S.V. (2005): PowerMarker: An integrated analysis environment for genetic marker analysis. Bioinformatics, 21, 2128–2129.

Ma J., Wang H., Liu R., Jin L., Tang Q., Wang X., Jiang A., Hu Y., Li Z., Zhu L., Li R., Li M., Li X. (2015): The miRNA transcriptome directly reflects the physiological and biochemical differences between red, white, and intermediate muscle fiber types. International Journal of Molecular Sciences, 16, 9635–9653.

McCarthy J.J. (2008): MicroRNA-206: the skeletal muscle-specific myomiR. Biochimica et Biophysica Acta, 1779, 682–691.

Murgiano L., Tammen I., Harlizius B., Drogemuller C. (2012): A de novo germline mutation in MYH7 causes a progressive dominant myopathy in pigs. BMC Genetics, 13: 99.

Piorkowska K., Zukowski K., Ropka-Molik K., Tyra M. (2018): Detection of genetic variants between different Polish Landrace and Puławska pigs by means of RNA-seq analysis. Animal Genetics, 49, 215–225.

Quiat D., Voelker K.A., Pei J., Grishin N.V., Grange R.W., Bassel-Duby R., Olson E.N. (2011): Concerted regulation of myofiber-specific gene expression and muscle performance by the transcriptional repressor Sox6. Proceedings of the National Academy of Sciences of the United States of America, 108, 10196–10201.

Ropka-Molik K., Bereta A., Tyra M., Rozycki M., Piorkowska K., Szyndler-Nedza M., Szmatola T. (2014): Association of calpastatin gene polymorphisms and meat quality traits in pig. Meat Science, 97, 143–150.

Ropka-Molik K., Podstawski P., Piorkowska K., Tyra M. (2016): Association of gene coding for microsomal triglyceride transfer protein (MTP) and meat texture characteristic in pig. Annals of Animal Science, 16, 721–729.

Ryu Y.C., Kim B.C. (2005): The relationship between muscle fiber characteristics, postmortem metabolic rate, and meat quality of pig longissimus dorsi muscle. Meat Science, 71, 351–357.

Untergasser A., Cutcutache I., Koressaar T., Ye J., Faircloth B.C., Remm M., Rozen S.G. (2012): Primer3 – new capabilities and interfaces. Nucleic Acids Research, 40, e115.

van Rooij E., Quiat D., Johnson B.A., Sutherland L.B., Qi X., Richardson J.A., Kelm Jr. R.J., Olson E.N. (2009): A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance. Developmental Cell, 17, 662–673.

Zak G., Pieszka M. (2009): Improving pork quality through genetics and nutrition. Annals of Animal Science, 9, 327–338.

Received: 2018–02–15
Accepted after corrections: 2018–08–08