

THE EFFECT OF CLPS AND RYR1 GENE POLYMORPHISM ON MEAT QUALITY OF ŻŁOTNICKA SPOTTED PIGS

WPLÝW POLIMORFIZMU GENU CLPS I RYR1 NA JAKOŚĆ MIĘSA ŚWIŃ RASY ŻŁOTNICKIEJ PSTREJ

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ABSTRACT

The aim of the study was to relate the polymorphisms at CLPS and RYR1 genes to pork quality in case of Żłotnicka Spotted breed. The experiment was carried out on 30 purebred Żłotnicka Spotted pigs, which were reared in standard conditions and slaughtered at approximately 100 to 110 kg body weight. Genetic polymorphisms were determined with the PCR-RFLP procedure. Polymorphism at the colipase gene (CLPS) was identified with DdeI restriction enzyme and stress sensibility gene (RYR1) with HinPI. In the studied pig sample three genotypes at locus CLPS/DdeI and two genotypes at locus RYR1/HinPI were found. The genotype frequencies at CLPS gene were 0.07 for AA, 0.40 for AB and 0.53 for BB. Colipase gene affected intramuscular fat content in RYR1/CT pigs. Meat from pigs with genotype BB had higher intramuscular fat content (2.50%) compared to AA group (1.59%); $P \leq 0.05$. Moreover, significant influence of gene RYR1^T on pH₁ trait was shown. Values of all analyzed traits were within ranges characterizing good meat quality of the native Polish Żłotnicka Spotted pig.

KEYWORDS: pigs, meat quality, CLPS gene, RYR1 gene

STRESZCZENIE

Celem pracy było określenie polimorfizmu genu CLPS oraz RYR1 wśród świń rasy żłotnickiej pstrej oraz analiza zależności pomiędzy genotypami obu genów a cechami jakości mięsa. Doświadczenie przeprowadzono na 30 osobnikach czystej rasy żłotnickiej pstrej. Tuczniaki utrzymywano w ujednoliconych warunkach i poddano ubojowi przy masie ciała 100 – 110 kg. Identyfikacji polimorfizmu genów dokonano metodą PCR-RFLP. Polimorfizm genu kolipazy (CLPS) identyfikowano przy użyciu enzymu restrykcyjnego DdeI, natomiast genu podatności świń na stres (RYR1) przy użyciu HinPI. W analizowanej populacji świń stwierdzono występowanie trzech genotypów w locus CLPS/DdeI oraz dwóch w locus RYR1/HinPI. Frekwencja genotypów CLPS wynosiła 0,07 dla AA, 0,40 dla AB oraz 0,53 dla BB. Gen kolipazy różnicował zawartość tłuszczu śródmięśniowego wśród świń RYR1/CT. Mięso świń o genotypie BB (2,50%) cechowało się wyższą zawartością tłuszczu śródmięśniowego wobec grupy AA (1,59%); $P \leq 0,05$. Jednocześnie, stwierdzono wpływ genu głównego (RYR1^T) na cechę pH₁. Wartości wszystkich analizowanych cech charakteryzowały dobrą jakość mięsa świń polskiej rodzimej rasy żłotnickiej pstrej.

SŁOWA KLUCZOWE: świnię, jakość mięsa, gen CLPS, gen RYR1

STRESZCZENIE SZCZEGÓŁOWE

Celem pracy było określenie zależności pomiędzy genotypami CLPS i RYR1 a jakością mięsa świń rasy złotnickiej pstrej. Doświadczeniem objęto 30 tuczników rasy złotnickiej pstrej. Zwierzęta poddano ubojowi przy masie ciała od 100 do 110 kg. W czasie uboju od zwierząt pobrano krew do próbek zawierających antykoagulant K_3EDTA w celu określenia genotypu kolipazy oraz genu podatności świń na stres. Identyfikacji polimorfizmu obu genów dokonano metodą PCR-RFLP. W przypadku genu CLPS zastosowano endonukleazę DdeI według metody Baskina i Pompa [2], natomiast mutację genu RYR1 przy użyciu enzymu restrykcyjnego HinPI [11]. Ocenę jakości mięsa przeprowadzono na próbach mięsa pobranych z części lędźwiowej mięśnia najdłuższego grzbietu. W 45 minut po uboju oznaczono stopień zakwaszenia tkanki mięśniowej (pH_1) przy użyciu pH-metru niemieckiej firmy Matthaüs. Końcowe zakwaszenie tkanki mięśniowej (pH_k) określono 48 godzin po uboju w wodnych ekstraktach mięsa. Swobodny wyciek soku z mięsa oszacowano na podstawie próby mięsa o masie 150 g zgodnie z metodą Honikela [12]. Ocenę barwy mięsa przeprowadzono przy użyciu fotokolorymetru Minolta CR 310 [13] oraz dodatkowo jasność barwy przy wykorzystaniu spektrofotometru Spekol 11 [24]. Skład chemiczny mięsa tj. zawartość wody, białka ogólnego, popiołu oraz tłuszczu śródmięśniowego oznaczono metodą AOAC [1]. Uzyskane wyniki opracowano statystycznie wykorzystując program komputerowy STATISTICA 8.0 PL [27]. W badanej populacji świń rasy złotnickiej pstrej stwierdzono występowanie wszystkich trzech genotypów kolipazy. Najliczniej reprezentowany był genotyp homozygotyczny BB (0,53) wobec pozostałych dwóch grup genotypowych (AB 0,40 oraz AA 0,07). Badana populacja tuczników czysto rasowych okazała się być mniej zróżnicowana pod względem drugiego analizowanego genu (RYR1). Wystąpiły dwie jego formy (RYR1/CC oraz RYR1/CT). Rozkład liczebny obu grup był identyczny (RYR1/CC $n=15$ oraz RYR1/CT $n=15$) (tabela 1). Analiza wyników zestawionych w podgrupach (genotyp CLPS x genotyp RYR1) nie wykazała istotnych zależności w przypadku większości cech jakości mięsa badanych zwierząt. Uzyskane wartości w tym zakresie były wyrównane we wszystkich analizowanych grupach genotypowych. Jednocześnie można zauważyć, że mięso świń rasy złotnickiej pstrej charakteryzowało się niewielkimi stratami soku mięśniowego (AA 2,84%, AB 2,31% oraz BB 2,61%), oraz korzystną, ciemną barwą (AA 21,16%, AB 19,03% oraz BB 19,46%) (tabela 2). Stwierdzono natomiast istotne różnice w badanych podgrupach (genotyp CLPS x genotyp RYR1) w zakresie składu chemicznego mięsa. Większą zawartością

tłuszczu śródmięśniowego cechowało się mięso zwierząt o genotypie BB (2,50%) względem AA (1,59%) w grupie zwierząt nosicieli genu podatności świń na stres (RYR1/CT); $P \leq 0,05$ (tabela 3). Gen główny (RYR1) istotnie wpłynął na stopień zakwaszenia tkanki mięśniowej oceniany w 45 minut po uboju (RYR1/CC 6,39 wobec RYR1/CT 6,05); $P \leq 0,05$ (tabela 2). Badany polimorfizm genu kandydującego CLPS jak i genu głównego RYR1 nie wpłynął w sposób istotny na jakość mięsa świń rasy złotnickiej pstrej. Ponadto, nie wykazano istotnych interakcji pomiędzy badanymi genami. Uzyskane mięso charakteryzowało się dobrą jakością, a na szczególną uwagę zasługuje optymalna zawartość tłuszczu śródmięśniowego oraz pożądana, ciemna barwa mięsa.

INTRODUCTION

Złotnicka Spotted pigs is the only indigenous Polish breed of pigs which was not improved by any other breed. Pigs of Złotnicka Spotted breed are characterized by specific quantitative and qualitative productive traits [17, 23]. The use of Złotnicka Spotted breed for meat production is still on satisfactory level. Pig meat quality is strongly affected by genetic factors. The influence of certain major genes like RYR1 has been well documented [20, 10, 26], and the presence of mutant allele (C→T) is known to decrease pig meat quality. Much less is known about the effect of colipase gene (CLPS), but it can be of interest because colipase enzyme demonstrates important effects on appetite and lipid metabolism [9, 8, 21].

The aim of the study was to associate polymorphisms at CLPS and RYR1 genes with quality of meat in Złotnicka Spotted pigs.

MATERIAL AND METHODS

The experiment was carried out on 30 purebred Złotnicka Spotted pigs (12 gilts and 18 castrated males from 5 litters). Maintenance and feeding were similar for all animals and in accordance with standard procedures. Fatteners were slaughtered at about 100 to 110 kg live body weight according to the meat industry standards.

Blood samples were collected from pigs and put into test tubes containing K_3EDTA . They were kept at $-25^\circ C$ until analysed. Genomic DNA was isolated using standard molecular biology methods. PCR-RFLP polymorphism of CLPS/DdeI gene was determined according to Baskin and Pomp [2]. RYR1/HinPI genotypes were identified with the method of Fujii et al. [11].

Meat quality traits were determined in the lumbar part of Longissimus dorsi (LL) muscle. Value of pH_1 was measured 45 minutes after the slaughter using the pH-

Table 1. Frequency of genotypes at *locus CLPS* and *RYR1*
Tabela 1 Frekwencja genotypów w *locus CLPS* oraz *RYR1*

<i>RYR1</i> genotype		<i>CLPS</i> genotype			Total
		<i>AA</i>	<i>AB</i>	<i>BB</i>	
<i>CC</i>	number	-	7	8	15
	frequency	-	0.47	0.53	1.00
<i>CT</i>	number	2	5	8	15
	frequency	0.13	0.34	0.53	1.00
<i>TT</i>	number	-	-	-	-
	frequency	-	-	-	-
Total	number	2	12	16	30
	frequency	0.07	0.40	0.53	1.00

Table 2. Major meat quality traits as related to genotype at *locus CLPS* and *RYR1*
Tabela 2 Cechy jakości mięsa w zależności od genotypów w *locus CLPS* oraz *RYR1*

Trait		<i>RYR1</i> genotype	<i>CLPS</i> genotype			Average
			<i>AA</i>	<i>AB</i>	<i>BB</i>	
pH ₁		<i>CC</i>	-	6.36 ± 0.38	6.41 ± 0.47	6.39 ^x ± 0.42
		<i>CT</i>	6.46 ± 0.64	5.85 ± 0.29	6.07 ± 0.49	6.05 ^y ± 0.46
Average			6.46 ± 0.64	6.15 ± 0.42	6.24 ± 0.50	-
pH _u		<i>CC</i>	-	5.54 ± 0.10	5.49 ± 0.06	5.51 ± 0.08
		<i>CT</i>	5.50 ± 0.04	5.52 ± 0.10	5.48 ± 0.12	5.50 ± 0.10
Average			5.50 ± 0.04	5.53 ± 0.09	5.49 ± 0.09	-
Drip loss, %		<i>CC</i>	-	2.16 ± 1.73	2.67 ± 0.90	2.44 ± 1.33
		<i>CT</i>	2.84 ± 1.12	2.51 ± 1.29	2.55 ± 1.56	2.58 ± 1.34
Average			2.84 ± 1.12	2.31 ± 1.51	2.61 ± 1.23	-
Colour lightness, %		<i>CC</i>	-	19.42 ± 3.99	20.02 ± 2.75	19.74 ± 3.27
		<i>CT</i>	21.16 ± 2.57	18.49 ± 4.51	18.89 ± 4.01	19.06 ± 3.88
Average			21.16 ± 2.57	19.03 ± 4.04	19.46 ± 3.37	-
Minolta L*		<i>CC</i>	-	48.11 ± 2.68	48.92 ± 2.76	48.54 ± 2.66
		<i>CT</i>	47.13 ± 0.84	47.87 ± 2.56	48.53 ± 3.56	48.12 ± 2.92
Average			47.13 ± 0.84	48.01 ± 2.51	48.73 ± 3.08	-
a*		<i>CC</i>	-	17.06 ± 0.58	17.10 ± 0.62	17.08 ± 0.58
		<i>CT</i>	16.95 ± 0.24	16.64 ± 0.48	16.90 ± 0.61	16.82 ± 0.53
Average			16.95 ± 0.24	16.89 ± 0.56	17.00 ± 0.61	-
b*		<i>CC</i>	-	2.36 ± 1.02	2.60 ± 1.15	2.49 ± 1.06
		<i>CT</i>	1.71 ± 0.37	2.35 ± 1.18	2.96 ± 1.63	2.59 ± 1.39
Average			1.71 ± 0.37	2.36 ± 1.04	2.78 ± 1.37	-

Within column means bearing different are related to *RYR1* genotypes; x, y at P ≤ 0.05

meter (Matthaus company), and ultimate pH_u was determined in minced meat-distilled water slurry 48h after slaughter. Meat colour was determined on minced meat samples using Spekol 11 spectrophotometer with a reflectance attachment and regression equations [24] to estimate lightness and also with colorimeter Minolta CR 310 to obtain L*, a* and b* values [10]. Drip loss was estimated on approximately 150 g slice of meat as described by Honikel [12]. Basic chemical composition (water, crude protein, ash, intramuscular fat content)

was assayed according to AOAC [1]. Arithmetic means and their standard deviations were calculated. Two-way analysis of variance was performed using Duncan's test. The analysed factors were CLPS gene and RYR1 gene effects. Interactions between gene effects were not significant. Statistical evaluation and estimation of significant differences were carried out using the computer programme STATISTICA 8.0 PL [27].

Table 3. Basic chemical composition of meat as related to genotype at *locus CLPS* and *RYR1*
Tabela 3 Podstawowy skład chemiczny mięsa w zależności od genotypów w *locus CLPS* oraz *RYR1*

Trait	<i>RYR1</i> genotype	<i>CLPS</i> genotype			Average
		<i>AA</i>	<i>AB</i>	<i>BB</i>	
		mean ± s.d.	mean ± s.d.	mean ± s.d.	
Water, %	<i>CC</i>	-	74.27 ± 0.59	73.59 ± 0.73	73.91 ± 0.73
	<i>CT</i>	73.87 ± 0.33	73.62 ± 0.44	73.60 ± 0.57	73.64 ± 0.49
Average		73.87 ± 0.33	74.00 ± 0.61	73.60 ± 0.64	-
Crude protein, %	<i>CC</i>	-	22.70 ± 0.52	22.88 ± 0.35	22.79 ± 0.43
	<i>CT</i>	23.42 ± 0.59	22.96 ± 0.77	22.77 ± 0.54	22.92 ± 0.62
Average		23.42 ± 0.59	22.81 ± 0.62	22.82 ± 0.45	-
Ash, %	<i>CC</i>	-	1.13 ± 0.03	1.12 ± 0.02	1.12 ± 0.02
	<i>CT</i>	1.14 ± 0.01	1.12 ± 0.04	1.12 ± 0.04	1.12 ± 0.03
Average		1.14 ± 0.01	1.13 ± 0.03	1.12 ± 0.03	-
Intramuscular fat, %	<i>CC</i>	-	1.90 ± 0.36	2.41 ± 0.75	2.17 ± 0.64
	<i>CT</i>	1.59 ^b ± 0.25	2.29 ^{ab} ± 0.49	2.50 ^a ± 0.45	2.31 ± 0.52
Average		1.59 ^b ± 0.25	2.06 ^{ab} ± 0.45	2.46 ^a ± 0.60	-

Within rows means bearing different letter are related to *CLPS* genotypes; a, b at $P \leq 0.05$

RESULTS AND DISCUSSION

Table 1 shows the number of pigs and the frequency of particular genotypes at *CLPS* and *RYR1* genes. In the analyzed sample of pigs of the Złotnicka Spotted breed we detected three genotypes at *CLPS/DdeI* gene. Genotype *BB* was more frequent (0.53) than genotype *AB* (0.40) or genotype *AA* (0.07). These results were different from the ones obtained by Kurył et al. [13] on a population of pigs covering Złotnicka Spotted breed and from the results of Jankowiak [14] on crossbreeds fatteners [Pietrain x (Polish Large White x Polish Landrace)], where the *AB/CLPS* individuals were the most numerous. The experiment conducted on Polish Large White gilts by Blicharski et al. [4] proved homozygous *AA/CLPS* pigs as the most numerous.

The polymorphisms at *RYR1* gene were also determined. On the basis of the PCR-RFLP tests conducted, it was ascertained that within the analyzed population there were two genotypes *CC/RYR1* and *CT/RYR1* (and no *TT/RYR1*). The number of pigs with different *RYR1* gene status were identical in both genotype groups ($n=15$ *CC* and $n=15$ *CT*). Bogucka and Kapelański [5] and Buczyński et al. [6] obtained comparable results in the population of Złotnicka Spotted pigs, they also obtained very low proportion of homozygous *TT/RYR1* animals.

The basic parameters of meat quality are presented in Table 2. No significant differences were identified between subgroups (*CLPS* genotype x *RYR1* genotype) for the analysed traits, presumably due to the very low number of pigs in some of the subgroups. The value of pH is one of the most important physicochemical traits determining meat quality. The pH_1 or pH_u values were

not different between the analyzed genotypes of *CLPS* gene. In the present experiment pH_1 values were situated between 6.46 (*AA/CLPS*) and 6.15 (*AB/CLPS*). The values of pH_1 exceeding 5.8 obtained 45 to 60 minutes after slaughter are considered as normal while those equal to or lower than 5.8 indicate a reduced quality of pork [22]. In all Złotnicka Spotted pigs the pH_u values were between 5.53 (*AB/CLPS*) to 5.49 (*BB/CLPS*).

On the other hand a significant influence of *RYR1* polymorphism on pH_1 value was observed. Lower ($P \leq 0.05$) pH_1 was noted for genotype *CC/RYR1* (6.39) in relation to *CT/RYR1* (6.05), in accordance with research carried out by other authors [7, 19].

Drip loss characterizes the loss of meat weight during storage. No significant effect of *CLPS* or *RYR1* gene was observed for that trait. In all subgroups (*CLPS* genotype x *RYR1* genotype) the juice loss was low and typical for the normal meat of good culinary and technological usefulness.

Meat colour was determined twice. The first measurement was carried out with the aid of the Spekol 11 spectrophotometer. The results obtained were statistically similar in all respective genotypes at *CLPS* (from 19.03% for *AB* to 21.16% for *AA*) and *RYR1* (19.06 for *CT* and 19.74 for *CC*) genes. Earlier Kapelański et al. [17, 18] showed similar value of lightness for the Złotnicka Spotted meat. The values obtained in the study presented herein describe a very good quality of the meat. The results collected for colour lightness with Minolta CR 310 colorimeter confirmed the results of Spekol 11 spectrophotometer.

The basic chemical composition of the meat is important

for the nutritional value of food products. One of the most important traits influencing the sensory properties of meat, positively correlated with its tenderness, juiciness and taste is the intramuscular fat content (IMF). Bejerholm and Barton-Gade [3] proved that the IMF between 2% and 3% is to be considered as optimal level. Moreover, Schwörer et al. [25] showed that a decrease in intramuscular fat content below 1% can be considered unacceptable. Rak et al. [23] reported low IMF in case of the Żłotnicka Spotted pigs. The results of the present study (Table 3) showed higher level ($P \leq 0.05$) and more advantageous IMF for animals of CLPS/BB genotype (2.50%) compared to animals CLPS/AB (1.59%) only in case of pigs in RYR1/CT group. The colipase gene polymorphism also differentiated significantly the intramuscular fat content. Pigs from CLPS/BB group (2.46%) were characterized by larger content of IMF in the meat than pigs from CLPS/AA group (1.59%), which is in accordance with our previous research [15]. An overall lower intramuscular fat content has been observed in the study by Blicharski et al. [4] for Polish Large White gilts. In the cited experiment the level of IMF varied from 1.07% (BB/CLPS) to 1.18% (AA/CLPS) [4].

Summarizing the results presented here, one may state that no significant association was identified between polymorphisms at CLPS and RYR1 genes and traits of meat quality. Significant effect of CLPS gene was found only for intramuscular fat content, while in case of RYR1 gene, the only significant effect was obtained for pH_1 . It is worth noting, that low number of pigs in certain subgroups could be responsible for the lack of significance. However, the attention should be paid on the desirable, dark meat color and optimal level of IMF. The meat of the Żłotnicka Spotted breed of pigs proved to be of good quality.

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