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The physicochemical characteristics of the *Longissimus lumborum* muscle of crossbred pigs in relation to the *CLPS* and *RYR1* genes polymorphism

Cechy fizyko-chemiczne mięśnia *Longissimus lumborum* mieszańców świń w zależności od polimorfizmu genów *CLPS* i *RYR1*

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Abstract

The experiment was conducted on 109 F_1 crossbred fatteners [(Polish Large White x Polish Landrace) x Pietrain]. The animals were slaughtered at about 105 kg of live body weight. The detection of the gene polymorphism was conducted with the PCR-RFLP procedure (CLPS/DdeI and RYR1/HinP1). The meat quality of the *Longissimus lumborum* muscle in respect to colipase (CLPS) and stress susceptibility (RYR1) genotypes was evaluated. Such traits as the pH of meat, drip loss, water holding capacity and basic chemical component were analyzed. Assessed from a sample of three-breed crossbred porkers, the existence of two alleles CLPS (A and B) candidate gene and three of its genotypes AA, AB and BB as well as two alleles of the RYR1 gene (C and T) and three of its genotypes (CC, CT, TT) has been confirmed. No significant differences were showed in subgroups (CLPS genotype x RYR1 genotype) among the meat quality traits. Moreover, the interactions between the CLPS and RYR1 genes were statistically not significant for the performed values. The candidate gene (colipase) did not differ the analysed traits connected with the quality of meat. However, a significant influence of the major gene (RYR1) on meat quality traits was confirmed. Meat obtained from three-breed crossbred pigs was usually of good quality.

Keywords: colipase gene, pigs, pork, stress susceptibility gene

Streszczenie

Doświadczenie przeprowadzono na 109 tucznikach mieszańcach F1 [(wielka biała polska x polska biała zwisłoucha) x pietrain]. Zwierzęta poddano ubojowi przy masie ciała około 105 kg. Polimorfizm genów oznaczono metodą PCR-RFLP (CLPS/DdeI i RYR1/HinP1). Jakość mięsa oznaczono na mięśniu Longissimus lumborum w zależności od genotypów kolipazy (CLPS) oraz podatności świń na stres (RYR1). Oznaczono następujące cechy mięsa: pH, swobodny wyciek soku, wodochłonność oraz podstawowy skład chemiczny. W badanej próbie mieszańców trójrasowych stwierdzono występowanie dwóch alleli genu kandydujacego CLPS (A i B), oraz trzech jego genotypów AA, AB i BB oraz dwóch alleli genu RYR1 (C i T) i trzech jego genotypów (CC, CT, TT). Nie stwierdzono istotnych zależności w podgrupach (CLPS genotyp x RYR1 genotyp) względem cech jakości mięsa. Interakcje pomiędzy genami CLPS i RYR1 były nieistotne statystycznie względem ocenianych cech. Gen kandydujący (kolipaza) nie zróżnicował analizowanych cech związanych z jakością mięsa. Potwierdzono jednocześnie istotny wpływ genu głównego (RYR1) na cechy jakości mięsa. Pozyskane mięso od mieszańców trójrasowych cechowało się na ogół dobrą jakością.

Słowa kluczowe: gen kolipazy, gen podatności na stres, świnie, wieprzowina

Introduction

The search for the QTLs (quantitative traits of loci) affecting the carcass and the meat quality of pigs indicated that the region of chromosome 7 contains the genes for the Major Histocompatibility Complex (MHC) which are genes affecting the backfat thickness and marbling of meat (Rothschild et al., 1995). In this region the colipase gene (CLPS) (Baskin and Pomp, 1998) was identified, which is considered as a candidate gene of the QTL for the carcass fat content in pigs (Demeure et al., 2003). Many studies indicate that genes of the pig Major Histocompatibility Complex are significantly associated with different production traits, among others: body weight at birth and at weaning, growth rate, backfat thickness and carcass traits (Rothschild et al., 1986; Warner and Rothschild, 1991). The CLPS porcine gene has been identified on chromosome 7 (Baskin and Pomp, 1998), and is near the main locus of the histocompatibility pig complex. Besides, Demeure et al. (2003) indicate the QTL region for growth, backfat thickness and carcass quality of pigs is near the Major Histocompatibility Complex, especially in the region located between the SLA and the marker S0102 on chromosome 7. In this region only the colipase gene was mapped, suggesting that the CLPS gene can significantly affect the fat content in pigs (Genêt et al., 2001). Thus confirmed the earlier study by Wang et al. (1998), who pointed out that the candidate CLPS gene is responsible for the fat content in the carcass of pigs.

The RYR1 gene is a gene with proven significant effects on carcass traits and meat quality of pigs. This gene was considered as a major gene used in the selection aimed at improving the quality of meat and carcass conformation. Many studies showed that burdening an animal with the $RYR1^T$ gene remained in essential relation with the physicochemical characteristics of meat, and more importantly with the indicators of technological suitability (Kapelański et al., 1999; Sellier and Monin, 1994). The greatest severity and the reduced meat quality most often occurs in individuals of the homozygous recessive TT, than in the heterozygotes CT, and little or not at all in individuals of the homozygous dominant CC (Kapelański et al., 1999).

The aim of the presented study was to examine the polymorphism of CLPS and RYR1 in three-breed crossbred pigs and the relevance of the relation between the analyzed genotypes with the quality characteristics of meat.

Material and methods

The experimental material consisted of 109 porkers, the offspring of crossbred sows (Polish Landrace x Polish Large White) mated to the Pietrain sires. Fatteners were kept in the same environmental conditions in the individual farm near Toruń in Poland. The feeding of animals was standardized with a feed ration balanced in respect to energy (12.7 MJ*kg^{-1} metabolizable energy) and protein (180 g crude protein) according to Polish Pig Feeding Standards (1993). The animals were slaughtered after reaching 105 kg (± 5 kg) of live body weight and 170 days (±) of age, according to the meat industry standards (Council Regulation, 2009).

Approximately 45 minutes after slaughter, the meat acidification (pH_1) was measured using the R. Matthaüs pH-meter with a combined glass electrode. This measurement was performed in the *Longissimus lumborum* muscle at the last rib of the right half-carcass. For the meat quality estimations of the muscle samples were taken about 24 – hours post mortem from the *Longissimus lumborum* muscle (first three lumbar vertebrae). The water holding capacity (WHC) was determined according to the filter paper press method with the Grau and Hamm (1952) method and expressed as a percentage of free water. Drip loss was recorded on about 2 cm of thick slice (approx. 150 g) of meat (Honikel, 1987). Additionally, basic meat chemical components, i.e. protein, fat, ash and dry matter was assayed according to the AOAC (2003). Moreover, the ultimate muscle acidification (pH_u) was measured approximately 48 h after slaughter in meat-water slurry.

The samples of blood from pigs were taken during exsanguinations at slaughter into the test tubes containing EDTA*K_3 and were stored in -25 ºC until required. The genomic DNA was isolated using standard molecular biology methods, from leukocytes by the method according to Kawasaki (1990). The PCR amplification of the CLPS gene was performed according to the Baskin and Pomp (1998) methods. The polymorphism in the ryanodine receptor gene (RYR1) was determined using the HinP1 restriction enzyme according to the Fujii et al. (1991) method.

The statistical analysis was evaluated to compare the quality traits of meat and the basic chemical components between pigs of different CLPS and RYR1 genotypes. The least squares method of the GLM procedure in the STATISTICA 8.0 PL (2008) package was used for its analysis. The interaction effects of CLPS and RYR1 genotypes were included in the linear model according to the following formula:

$Y_{ijkl} = \mu + a_i + b_j + ab_k + e_{ijkl}$

where:

Y_{ijkl} – trait measured

μ – overall mean
a_i – effect of CLPS genotype (i = AA, AB, BB)
b_j – effect of RYR1 genotype (j = CC, CT, TT)
ab_k – interaction CLPS x RYR1 genotype
e_{ijkl} – random error

Duncan’s test was used for a detailed comparison of the last squares means for the analyzed CLPS and RYR1 genotypes.

Results

The number of animals and the frequency of genotypes at the CLPS and RYR1 loci are shown in Table 1. Within the analysed sample of three-breed crossbreds porkers, the existence of three genotypes of the colipase gene (AA, AB, BB), as well as three genotypes of the major gene RYR1 (CC, CT, TT), have been confirmed. The animals of the AB and CT subgroups were more numerous (n=28) than animals from others subgroups. The animals of BB genotype at CLPS loci and TT genotype at RYR1 loci were absent in that pig population.

The two-factor analysis of variance did not show any significant interactions between CLPS/DdeI and RYR1/HinP1 genotypes in the analysed pig population.

Table 1. Number and frequency of genotypes CLPS and RYR1 in three-breed crossbred pigs

Tabela 1. Liczebność i częstotliwość występowania genotypu CLPS i RYR1 wśród mieszańców trójrasowych

RYR1 genotype		CLPS genotype			Total
		AA	AB	BB	
CC	number	10	20	3	33
	frequency	0.30	0.61	0.09	1.00
CT	number	22	28	6	56
	frequency	0.39	0.50	0.11	1.00
TT	number	6	14	-	20
	frequency	0.30	0.70	-	1.00
Total	number	38	62	9	109
	frequency	0.34	0.56	0.10	1.00

The performed analysis of variance did not show any statistically significant differences between acidification and wateriness of the muscle tissue with respect to various genotypes in terms of the CLPS gene (Table 2). In all subgroups (CLPS x

RYR1 genotype) the juice loss and loose water were low and typical for the normal meat of good culinary and technological usefulness. Simultaneously, the most pronounced effects of the genotype at the RYR1 locus on the meat quality was demonstrated. The data showed a significant unfavourably effect of the stress susceptibility TT genotype on meat pH_1 ($P \le 0.01$). At the same time individuals of the CC/RYR1 genotype had better water holding capacity of meat ($P \le 0.01$) and a lower drip loss ($P \le 0.01$) than the CT and TT ones.

Table 2. Acidification and wateriness of meat as related to polymorphism of CLPS and RYR1 genotypes

Tabela 2. Zakwaszenie i wodnistość mięsa w zależności od genotypów CLPS i RYR1

Trait	RYR1 genotype	CLPS genotype			Average
		AA	AB	BB	
pH_1	CC	6.68 ± 0,30	6.41 ±0.33	6.60 ±0.47	6.51^{A} ±0.34
	CT	6.27 ±0.36	6.35 ±0.39	6.36 ±0.30	6.32^{A} ±0.36
	TT	6.00 ±0.57	5.78 ±0.46	-	5.85^{B} ±0.49
Average		6.33 ±0.44	6.24 ±0.46	6.44 ±0.35	NS
pH_u	CC	5.48 ±0.57	5.49 ±0.93	5.49 ±0.87	5.49^{b} ±0.08
	CT	5.50 ±0.12	5.50 ±0.08	5.58 ±0.21	5.51^{ab} ±0.12
	TT	5.62 ±019	5.55 ±0.19	-	5.57^{a} ±0.19
Average		5.52 ±0.13	5.51 ±0.12	5.55 ±0.18	NS
WHC, (Loose water), %	CC	19.40 ±1.66	19.31 ±1.87	17.73 ±1.75	19.20^{Bb} ±1.81
	CT	20.26 ±2.48	19.82 ±2.79	20.47 ±1.91	20.06^{b} ±2.56
	TT	19.42 ±5.03	22.51 ±3.19	-	21.59^{Aa} ±3.96
Average		19.90 ±2.79	20.26 ±2.87	19.56 ±2.22	NS
Drip loss, %	CC	2.05 ±1.75	3.25 ±1.84	2.07 ±1.04	2.78^{Bb} ±1.81
	CT	4.53 ±2.88	4.01 ±2.51	3.26 ±1.65	4.13^{ABc} ±2.58
	TT	3.88 ±2.67	6.26 ±2.74	-	5.55^{Aa} ±2.88
Average		3.77 ±2.75	4.27 ±2.60	2.86 ±1.53	NS

Significance of differences: A, B – $P \le 0.01$; a, b – $P \le 0.05$

NS - interaction not statistically significant

Table 3 presents basic data of chemical meat composition. The meat of the examined animals had similar chemical content with regards to water, crude protein, intramuscular fat and ash in each subgroup. There has been no influence of the colipase gene polymorphism identified in this scope. However, in respect to meat intramuscular fat content the CC/RYR1 genotype pigs had a significantly higher level than the TT/RYR1 one ($P \leq 0.05$).

Table 3. The physicochemical traits of meat as related to polymorphism of CLPS and RYR1 genotypes

Tabela 3. Cechy fizyko-chemiczne mięsa w zależności od genotypów CLPS i RYR1

Trait	RYR1 genotype	CLPS genotype			Average
		AA	AB	BB	
Water, %	CC	74.16 ±0.80	74.18 ±0.87	74.67 ±0.56	74.22 ±0.82
	CT	74.44 ±0.86	74.37 ±0.63	74.19 ±0.77	74.37 ±0.73
	TT	74.64 ±0.74	74.59 ±1.11	-	74.61 ±0.99
Average		74.40 ±0.82	74.36 ±0.83	74.35 ±0.71	NS
Crude protein, %	CC	23.54 ±0.66	23.30 ±0.70	22.98 ±0.48	23.34 ±0.67
	CT	23.40 ±0.51	23.59 ±0.63	23.29 ±0.58	23.48 ±0.58
	TT	23.36 ±0.49	23.29 ±0.77	-	23.31 ±0.69
Average		23.43 ±0.54	23.43 ±0.69	23.19 ±0.54	NS
Intramuscular fat, %	CC	1.18 ±0.54	1.17 ±0.56	0.94 ±0.21	1.15^{a} ±0.53
	CT	1.00 ±0.42	1.02 ±0.37	0.85 ±0.37	0.99^{ab} ±0.39
	TT	0.88 ±0.13	0.89 ±0.32	-	0.89^{b} ±0.27
Average		1.03 ±0.43	1.04 ±0.44	0.88 ±0.31	NS
Ash, %	CC	1.26 ±0.11	1.26 ±0.08	1.33 ±0.05	1.27 ±0.09
	CT	1.26 ±0.07	1.27 ±0.09	1.23 ±0.08	1.26 ±0.08
	TT	1.23 ±0.13	1.25 ±0.07	-	1.25 ±0.09
Average		1.25 ±0.09	1.27 ±0.08	1.26 ±0.09	NS

Significance of differences: a, b – $P \leq 0.05$

NS – interaction not statistically significant

Discussion

The meat quality values are controlled by an unknown number of genes mapping in the quantitative trait loci – QTL – regions. Some of these genes may have very important influence on production traits. The colipase gene is one of many genes defined as being the candidate gene. The polymorphism of candidate genes and their connections with important traits of breeding pigs is still very significant. The CLPS gene is viewed as one of the genes which may have an influence on the quantitative traits and especially with the fatness of carcass in pigs (Demeure et al., 2003). It was previously suggested by Wang et al. (1998) and also by Genêt et al. (2001), that the CLPS gene can significantly affect the fatness of pigs. So far, it was not indicated that the colipase gene may influence on the meat quality. For now, two major genes affecting pork meat quality are known: RYR1 (ryanodine receptor) that regulates Ca^{++} transport across the muscle cell membranes and RN (Rendement Napole) gene affecting the glycogen content of the muscle (Davoli and Braglia, 2008). The influence of certain major genes like RYR1 has been well documented (Fisher et al., 2000; Sellier and Monin, 1994). The recessive homozygous (TT) are marked with higher carcass meatiness, which at the same time are of worse quality.

In the experiment conducted by Kurył et al. (2001) conducted on countless population of pigs, the heterozygous (AB/CLPS) individuals were the most numerous. Different frequency of colipase genotypes was obtained by Blicharski et al. (2004) on the Polish Large White gilts, where the homozygous AA/CLPS animals were most numerous. In researches performed by Jankowiak (2007) on the Polish Large White pigs the AB/CLPS it was most frequent but on crossbreeds (Polish Large White x Polish Landrace) the AA/CLPS genotype was more numerous.

The meat quality depends on many parameters. In assessing the value and the meat quality, particular attention is paid to these indicators, which leads to a large reduction in measurable losses. According to Koćwin-Podsiadła et al. (2004), the pork quality is determined not only by the chemical composition and nutritional value, but also by the animal health status, sensory and technological traits. The main parameters serving to determine the pork quality are as follows: the level of meat acidification, the colour and its uniformity and stability, the water holding capacity, natural juice loose and the chemical composition of meat. The determination of the muscular tissue acidification degree in the first hour after the slaughter is assumed to be an indicator of the PSE meat occurrence. Czech researches (Pulkrábek et al., 2004) suggest that the pH value measured from 45 to 60 minutes after slaughter exceeding 5.8 are considered normal while those equal to or lower than 5.8 indicate a reduced pork quality. In their own experiment conducted on the Złotnicka Spotted pigs (Jankowiak et al., 2010b) the value of pH_1 and pH_u were not different between the analyzed genotypes of the CLPS gene. Among the meat characteristics affecting its processing value, the following may be distinguished: the wateriness of meat, which is the ability to maintain a specific quantity of water. Similar dependence of the impact of the main gene polymorphism (RYR1) on drip loss were obtain by Kortz et al. (2003). Significantly lower values of loose water in meat from the CC group (2.50%) than the CT (4.50%) were observed. The same authors pointed on better the bound water content in meat from the CC/RYR1 fatteners than the CT/RYR1 and TT/RYR1 (respectively: 73.10%, 70.73% and 67.44%); ($P \leq 0.01$). While, in a study conducted by Pieszka et al. (2006) did not shown any statistical differences in relation to wateriness values with regard to the RYR1 gene effect.

The most significant traits influencing the taste of meat is it's the intramuscular fat content. As suggested Bejerholm and Barton-Gade (1986), the optimal intramuscular fat content level is considered to oscillate around 2-3%. While, the decrease in intramuscular fat content below 1% can be considered as unacceptable (Schwörer et al., 2000). The mean of the IMF content of longissimus dorsi below 2% are characterized by the Polish Landrace, the Polish Large White and the Pietrain pigs raised in Poland (Tyra and Żak, 2010). In the experiment presented herein, the IMF content was on very low level and was not varied in all subgroups. The previous studies (Jankowiak et al., 2010a) on the Polish Large White and crossbreds with the Polish Landrace pigs confirm the influence of the CPLS genotypes on the intramuscular fat content.

Conclusion

On the basis of the present study conducted on three-breed crossbred pigs, it may be stated that no significant associations were identified between the polymorphism of the CLPS and the RYR1 genes and the meat quality traits of pigs. These studies have confirmed the effect of the major gene (RYR1) on the meat quality of pork (pH_1, pH_u, WHC, drip loss and IMF content).

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