

## RELATIONSHIP BETWEEN CARCASS AND MEAT QUALITY TRAITS AND POLYMORPHISM OF *CLPS* AND *RYRI* GENES IN ZŁOTNICKA SPOTTED PIGS

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### Abstract

The aim of the study was to characterize the polymorphism of *CLPS* and *RYRI* genes in Złotnicka Spotted breed pigs and to analyse the relation between the *CLPS* and *RYRI* genotypes and carcass and meat quality traits. The study was carried out on 32 fatteners which were slaughtered at approximately 100 to 110 kg body weight. Genomic DNA was extracted using standard molecular biology methods. Polymorphism in the *colipase* gene (*CLPS*) was identified with *DdeI* restriction enzyme as described by Baskin and Pomp (1998) and *RYRI* gene with *HinPI* according to Fujii et al. (1991). In the studied pig population the allele frequencies of *CLPS* gene were 0.28 for *A* and 0.72 for *B* and those of *RYRI* gene 0.75 for *N* and 0.25 for *n*. Three genotypes at locus *CLPS/DdeI* and two genotypes at locus *RYRI/HinPI* were found. Genotype *nn/HinPI* was not found. Meat quality traits were determined in the *longissimus lumborum* muscle. Such traits as the meat pH, colour, drip loss and intramuscular fat content were analyzed. None significant differences in carcass and meat quality traits with respect to *CLPS* or *RYRI* pig genotype effects were found. Interactions between *CLPS* and *RYRI* genes were also statistically not significant for all performed values.

**Key Words:** *CLPS* and *RYRI* genes, carcass, pork quality

The techniques of molecular biology and molecular genetics are rapidly developed. These methods have opened possibility to detected *quantity trait loci* - QTL (quantitative genes) which control important, production traits. Swine chromosome 7 contains the *Major Histocompatibility Complex* (MHC) also called *Swine Leukocyte Antigens* (SLA). A major QTL for fatness, backfat thickness and growth has been identifying around SLA complex, in a region situated between microsatellites *Sw1354* and *S0102* (Genêt et al., 2001). In this region has been found by Baskin and Pomp (1998) *colipase* gene (*CLPS*). This gene is a possible candidate gene affecting fat deposition.

The relationship between an increased carcass lean meat content and pork quality deterioration has been explained mainly by the presence of the recessive form of the *RYRI* gene. Recessive homozygote (*nn*) are marked with higher carcass leanness, which at the same time is of a worse quality.

The aim of the study was to determine the relationships between the *CLPS* and *RYRI* genotypes and quality of carcass and meat values in Złotnicka Spotted pigs.

### Material and methods

The experiment was carried out on 32 purebred Złotnicka Spotted pigs (gilts to barrows 1:1). Maintenance and feeding were similar for all animals and in accordance with obligatory standards. Fatteners were slaughtered at about 100 to 110 kg live body weight according to the meat industry standards.

From all animals blood samples were drawn into test tubes containing K<sub>3</sub>EDTA which then were kept at -25°C until required. Genomic DNA was isolated using standard molecular biology methods. PCR-RFLP polymorphism of *CLPS/DdeI* gene was determined according to Baskin and Pomp (1998). *RYRI/HinPI* genotypes were identified with the method of Fujii et al. (1991).

After 24 hours cooling the right carcass-sides were dissected and evaluated using the simplified methods applied to a Pig Testing Stations (Różycki, 1996). Value of pH<sub>1</sub> was measured using the R. Matthaüs pH-meter, and ultimate pH<sub>u</sub> was determined in an aqueous suspension of muscle tissue. Meat colour first was measured on minced meat samples on Spekol 11 with a reflectance attachment and using regression equations (Różycka et al., 1968) to estimate brightness and again using a Minolta CR 310 colorimeter to obtain L, a\* and b\* values (Itten, 1997). Drip loss was estimated for approximately 150g slice of meat (Honikel, 1987). Basic chemical composition (intramuscular fat content) was assayed according to AOAC (1990). Earlier mentioned measurements in samples of *longissimus lumborum* (LL). Statistical evaluation and estimation of significant differences (Duncan's test) were carried out using the computer programme STATISTICA 5.5 PL, 2000.

### Results and discussion

Table 1 shows the frequency of particular genotypes and alleles of the *CLPS* and *RYRI* genes. Within the analysed population of the Złotnicka Spotted breed of pigs, the existence of two alleles of the *colipase* gene (*A* and *B*) and

three of its genotypes (*AA*, *AB* and *BB*), as well as two alleles of the *RYRI* (*N* and *n*) major gene and two of its genotypes *NN* and *Nn*, has been confirmed. The frequency of appearance of specific *CLPS* genotypes turned out to be considerably diversified: 0.09 (*AA*), 0.38 (*AB*) and 0.53 (*BB*). In the course of the studies by Kurył et al. (2001), carried out on a numerous population of pigs covering the Złotnicka Spotted breed and the researches performed by Jankowiak (2005) among three-breed [*P* x (*PLW* x *PL*)] crossbreds, the *AB/CLPS* individuals were the most numerous; and the experiment conducted by Blicharski et al. (2004), on Polish Large White gilts, proved the most numerous presence of homozygous *AA* animals. The *RYRI* genotype frequency was on the other hand identical (0.50 *NN* and 0.50 *Nn*). There were no instances of animals with a recessive *nn* genotype within the whole analysed population. The results obtained confirm the outcome of previous studies which revealed, with regard to the population of the Złotnicka Spotted pigs, inconsiderable proportion of *nn/RYRI* animals or their lack of the genotype conditioning pigs' susceptibility to stress (Bogucka and Kapelański, 2005; Buczyński et al., 2006).

The animal fattening was terminated with a body weight from 100 to 110 kg. The pig slaughtering weight in *CLPS* genotype groups amounted to: 105.00 kg (*AA*) – 109.58 kg (*AB*) (Table 2). The animals carrying the *Nn* stress susceptibility gene were heavier at slaughter than the group of the *NN* resistant animals (respectively: 108.38 kg and 105.81 kg), however, the differences were not confirmed as statistically significant.

The *colipase* gene is viewed as one of the many candidate genes which may influence on quantitative traits and in particular those of the traits relating directly to the carcass fatness (Demeure et al. 2003). In the experiment presented herein, linear measurements of the backfat thickness were taken in 5 sections: above the shoulder, behind the last rib and at the height of the I, II and III sacral vertebra (Table 2). The average backfat thickness was calculated from the 5 measurements and was similar in all of the analysed genotype groups. No influence of the analysed polymorphism of *CLPS* and *RYRI* genes on the fatness of examined pigs has been therefore confirmed. In the course of the studies carried out by Blicharski et al. (2004) there was also no indication of any influence of *colipase* polymorphism on the backfat thickness. Dissimilar results were however obtained from an experiment carried out by Kurył et al. (2001), where the tendencies of larger fatness of carcasses of the animals with *BB/CLPS* genotype have been observed, as compared with the *AA/CLPS* group. The compared backfat thickness taken from the 5 measurements in *RYRI* genotype groups was also levelled (2.77 cm *NN* and 2.72 cm *Nn*). The Złotnicka Spotted pigs revealed larger fatness in the study by Kapelański et al. (2002).

In the cross section of the *longissimus dorsi* muscle, the cross sectional area of the loin was also established (Table 2). There has been no influence of the *colipase* gene

polymorphism identified in this scope and the values obtained were equal in all three genotype groups (from 31.43 cm<sup>2</sup> for *AA* to 32.83 cm<sup>2</sup> for *AB*). The previous own studies did not confirm the analysed correlations either, with regard to the loin cross section area (Jankowiak, 2007). The pig stress susceptibility gene did not differed the value of this particular feature (31.21 cm<sup>2</sup> for *NN* and 33.49 cm<sup>2</sup> for *Nn*). The obtained values of the cross sectional area of the loin of the Złotnicka Spotted breed are identical to the data presented by Kapelański et al. (2006) and slightly lower than the results provided by Rak et al. (1999).

The results of the features affecting meat quality of the analysed fatteners are presented in Table 3. The level of meat acidification in the first hour after slaughter was considerably differed in case of both *CLPS* and *RYRI* genotype groups; however, the differences revealed were not confirmed as statistically significant. Meat of the *AB/CLPS* heterozygous individuals was of a lower pH<sub>1</sub> value (6.14), compared with the remaining two groups (6.25 *BB* and 6.47 *AA*). In case of polymorphism of *RYRI* gene, the heterozygous animals (*Nn* 6.06 compared with 6.39 in *NN*) also revealed lower pH<sub>1</sub> value measured 45 minutes after slaughter.

Meat brightness measurement was carried out with the aid of the Spekol 11 spectrophotometer. The values obtained within this area of research were similar in all respective genotype *CLPS* (from 19.03% for *AA* and *AB* to 19.61% for *BB*) and *RYRI* (19.25 for *Nn* and 19.43 for *NN*) groups. Meat of the Złotnicka Spotted pigs had higher brightness in the studies carried out by Kapelański et al. (2002 and 2006). The results collected in the experiment presented herein and in this particular scope, describe the meat obtained as a very good quality and the values obtained via measurement of brightness with the aid of Minolta CR 310 fotocolorimeter (approximately 48% L\*) may serve as a verification of the above statement.

One of the most significant traits influencing the meat's taste is its intramuscular fat content (IMF). The value between 2.0% and 3.0% remains to be seen as the optimal level (Bejerholm and Barton-Gade, 1986), and decrease in intramuscular fat content below 1% is considered unacceptable (Schwörer et al. 1999). In the course of studies presented herein animals with a *CLPS/BB* genotype stood out as those with a higher and more advantageous intramuscular fat content in meat (2.42%), compared with *CLPS/AA* (2.06%) and *CLPS/AB* (2.06%) individuals, which is in accordance with the previous own researches (Jankowiak, 2006). In the experiment by Blicharski et al. (2004), carried out on Polish Large White gilts, an overall lower intramuscular content has been observed, with its level oscillating from 1.07% for the *BB* group to 1.18% for the *AA* group. A similar low IMF level in case of the Złotnicka Spotted pigs was reported by Rak et al. (1999). In case of the pig stress susceptibility gene, there has been no significant effect confirmed with regard to the level of this component (2.28% *Nn* and 2.23% *NN*).

**Table 1. Frequency of genotypes and alleles of the CLPS and RYR1 genes**

Genotype <i>CLPS</i>			Alleles		Genotype <i>RYR1</i>			Alleles		
Number	<i>AA</i>	<i>AB</i>	<i>BB</i>	<i>A</i>	<i>B</i>	Number	<i>NN</i>	<i>Nn</i>	<i>N</i>	<i>n</i>
	3	12	17	0.28	0.72		16	16	0.75	0.25
Frequency	0.09	0.38	0.53			Frequency	0.50	0.50		

**Table 2. Means and their standard deviations for carcass traits as related to genotype at locus *CLPS* and *RYR1***

Trait		Genotype <i>CLPS</i>			Genotype <i>RYR1</i>	
		<i>AA</i>	<i>AB</i>	<i>BB</i>	<i>NN</i>	<i>Nn</i>
Body weight at slaughter, kg	mean	105.00	109.58	105.71	105.81	108.38
	SD	6.56	8.90	6.10	6.10	8.41
Cold carcass weight, kg	mean	74.87	79.35	76.25	75.84	78.73
	SD	7.20	8.96	6.86	7.09	8.19
Backfat thickness, cm over shoulder	mean	3.57	4.01	3.71	3.91	3.70
	SD	0.64	0.84	0.98	0.79	0.99
over last rib	mean	2.50	2.47	2.24	2.31	2.38
	SD	1.28	0.56	0.74	0.66	0.78
over sacrum I	mean	2.80	2.79	2.61	2.66	2.73
	SD	1.01	0.64	0.97	0.97	0.72
over sacrum II	mean	2.27	2.28	2.02	2.15	2.14
	SD	0.91	0.41	0.67	0.66	0.55
over sacrum III	mean	2.97	2.89	2.58	2.81	2.65
	SD	0.96	0.63	0.72	0.84	0.57
Average of 5 measurements, cm	mean	2.82	2.89	2.63	2.77	2.72
	SD	0.95	0.55	0.75	0.73	0.66
Loin cross section, cm <sup>2</sup>	mean	31.43	32.83	32.17	31.21	33.49
	SD	2.06	4.33	5.02	4.59	4.22

**Table 3. Means and their standard deviations for meat quality traits as related to genotype at locus *CLPS* and *RYR1***

Trait		Genotype <i>CLPS</i>			Genotype <i>RYR1</i>	
		<i>AA</i>	<i>AB</i>	<i>BB</i>	<i>NN</i>	<i>Nn</i>
pH <sub>1</sub>	mean	6.47	6.14	6.25	6.39	6.06
	SD	0.45	0.42	0.48	0.40	0.45
pH <sub>u</sub>	mean	5.49	5.53	5.48	5.51	5.49
	SD	0.03	0.09	0.09	0.08	0.10
Drip loss, %	mean	2.27	2.31	2.73	2.35	2.70
	SD	1.26	1.50	1.28	1.32	1.38
Brightness, %	mean	19.03	19.03	19.61	19.43	19.25
	SD	4.11	4.04	3.33	3.39	3.83
Minolta L*	mean	47.99	48.01	48.65	48.62	48.08
	SD	1.61	2.51	3.00	2.58	2.85
a*	mean	17.10	16.88	17.01	17.10	16.84
	SD	0.31	0.56	0.59	0.57	0.52
b*	mean	2.05	2.36	2.76	2.50	2.58
	SD	0.64	1.04	1.33	1.03	1.35
Intramuscular fat, %	mean	2.06	2.06	2.42	2.23	2.28
	SD	0.83	0.45	0.60	0.65	0.51

## Conclusion

To sum up, it has to be stated that the analysed polymorphism of the *CLPS* candidate gene as well as of the *RYR1* major gene did not influence in any significant way the quality of carcass as well as meat quality of Złotnicka Spotted pigs. Furthermore, there has been no significant interaction between the analysed genes identified. Meat obtained was of usually good quality and the attention should be paid on the optimal intramuscular fat content and the desirable, dark meat colour.

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