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A COMPARATIVE ANALYSIS OF THE DIAGNOSTIC METHODS FOR STRESS SENSITIVITY IN CONNECTION WITH THE FRESH MEAT QUALITY*

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SUMMARY

The aim of this study was the comparative analysis of selected methods of identification HAL^n gene which were used for diagnosis of stress susceptibility and pork meat quality. The object of the investigations were: halothane test, haplotyping method and PCR/RFLP test. It has been confirmed low efficiency of halothane test in the diagnosis of stress sensitivity as well as small usefulness of this test for the results of diagnosis of pork meat quality. The comparative analysis of investigated methods for meat quality has shown better precision of PCR/RFLP test then haplotyping method. The HAL genotype within tested material identified by haplotyping method was as follows: 30 NN (28,6%); 43 Nn (40,9%); 32 nn (30,5%) while by PCR/RFLP analysis 25 NN (23,8%); 51 Nn (48,6%); 29 nn (27,6%). The genotype of 28 (26.7%) pigs was corrected by PCR/RFLP test. The PSE meat, was identified in 71,9% of animals of nn genotype defined by haplotyping method, whereas within group of the same HAL genotype but stated by PCR/RFLP 79,3% of pigs showed PSE meat post mortem.

INTRODUCTION

At the present time it is well known that the HAL^n gene is closely connected with the existing of the PSE meat after the slaughter of hogs (SELLIER 1987). Estimated incorrection of halothane test is caused by incomplete penetration of the gene [from 50% to 95% in the dependence from the breed or line (WEBB 1981)], lack of the possibility for the recognize of the resistance on the stress of homozygous and heterozygous as well as not sufficient results of the selection work (WEBB et al. 1987). Above mentioned reasons have created necessity of utilization in the breeding practice new and additional testes. Haplotyping method has been widely used which was developed by GAHNE and JUNEJA (1985) by using genetic test based on the investigation of polymorphism at least 3 among 5 locies (S, GPI, H, A1BG, PGD) linkage with locus HAL^n. The association between HAL genotype determined by haplotyping method and meat quality has been shown by many laboratories (SELLIER, 1987).

The ryanodine receptor of the sarcoplasmic reticulum calcium channel gene has been postulated to be the candidate for predisposition

* The research was supported in part by Committee of Scientific Investigations research grants: 5 5314 92 02 (PCR/RFLP test) and 5 0520 91 01 (HAL-GPI-AlBG-PGD haplotyping and meat quality evaluation).

to porcine malignant hyperthermie. The genotyping test developed by FUJI et al. (1991) involved an amplification of RYR 1 gene fragment comprising 1843 nucleotide followed by HgiAI restriction endonuclease digestion. Electroforetic separation of digested DNA fragments allowed HAL genotype identification (PCR/RFLP test). The effect of RYR1 gene on the quality of meat is at the present time the aim of several investigations (POMMIER and HOUDE 1993, HILBERT et al. 1994, KURYŁ et al. 1994a, RUSSO et al. 1994).

The aim of this work is the comparison analysis of halothane test, haplotyping method and PCR/RFLP test used for the identification of HAL^n gene in the field of the effectiveness of pig sensitivity on the stress in the connection with the quality of fresh meat.

MATERIAL AND METHODS

The investigations covered 105 German Landrace pigs from one herd. The HAL genotype was identified by HAL-GPI-A1BG-PGD haplotyping method (according to GAHNE and JUNEJA 1985) and by PCR/RFLP test (according to FUJI et al. 1991). The pigs were killed by the live weight about 100kg. Meat quality was evaluated on the basis of value of parameters: pH_1 and R_1 measured in M. longissimus dorsi at 45 minutes post mortem. PSE meat was classified on the basis of pH_1 and R_1 (HONIKEL and FISHER 1977 in modification of KOĆWIN-PODSIADŁA and CHMURA-JANOWIAK (1988).

Table 1
The comparative analysis of diagnostic methods for stress sensitivity in connection with the PSE meat

Methods	Genetical groups	The frequency of occurence animals		The frequency of animals with PSE meat	
		n	%	n	%
Halothane test	H^-	74	70.47	19	25.67
	$H^{+/-}$	11	10.48	5	45.45
	H^+	20	19.00	11	55.00
	Total	105	100.00	35	33.30
Haplotyping method HAL-GPI-AlBG-PGD	NN	30	28.57	2	6.67
	Nn	43	40.95	10	23.25
	nn	32	30.48	23	71.87
	Total	105	100.00	35	33.30
PCR/RFLP analysis	NN	25	23.81	1	4.00
	Nn	51	48.57	11	21.57
	nn	29	27.62	23	79.31
	Total	105	100.00	35	33.30

RESULTS AND DISCUSSION

The effectiveness of the pigs sensitivity diagnosis on the stress by using halothan test based on the genetic methods in the connection with the frequency of the PSE meat is shown in table 1. It has been estimated that disproportion between the results of halothane test on the ground of haplotyping method and DNA test are effected by uncompleted penetration of gene (tab. 2). So low noted efficiency of tested method is caused by lack of its ideally. To the parameters which determinate the possibility of the positive reaction on the halothan according to WEBB (1981) and MARBRY et al. (1981) influence among others age, mass of body and energetic reserve at the moment of testing of sensitive animals. The next element is the estimated positive reaction on the halothan of some animals which are carriers of gene and even of some cases of genetic resistant animals (tab. 2). The sensitivity on the halothan observed for heterozygous animals which were identified by haplotyping

method and PCR/RFLP test was described in literature by GROBET et al. (1992), HOUDE et al. (1993), SOUTHWOOD et al. (1988) and KURYŁ et al. (1994b).

The HAL genotypes within tested material identified by HAL-GPI-A1BG-PGD haplotyping were as follows: 30 NN (28,6%), 43 Nn (40,9%), 32 nn (30,5%) while by PCR/RFLP test - 25 NN (23,8%); 51 Nn (48,6%); 29 nn (27,6%). The genotype of 28 pigs was corrected by PCR/RFLP test (tab. 3). The PSE meat (qualified on the basis of pH_1 and R_1) was identified in 71,9% of animals of nn genotype defined by haplotyping whereas within by PCR/RFLP test - 79,3% of pigs - showed PSE meat post mortem (tab. 1). About 20% of Nn animals developes PSE meat post mortem (tab. 1).

Noted incorrectness in diagnosis of animals genotype is reflected in the frequency of occurrence of PSE meat for analogical genetical group which were identified by investigated methods (tab. 1, 2 and 3). Early made comparative anaysis of haplotyping method with PCR/RFLP test on the base of the pig meat quality has shown better precision of test which is based on the analysis of gene RYR1 mutation (KURYŁ et al. 1994a).

In the group of stress resistant animals (NN) and heterozygous (Nn), which were identified by test DNA has been pointed out lower about 2% quantity of PSE meat but in the group of animals which were stress sensitive (nn) nearly 8% more than in analogous groups identified by haplotyping method (tab. 1). The linkage disequilibrium coefficient values shown by this same authors confirmed the association between nn genotype and values of pH_1 as well as PSE meat occurence. However, the D_S value determined for HAL^nHAL^n - pH_1 pair (0,92) suggested more significant association between pH_1 and HAL genotype defined by PCR/RFLP test (KURYŁ et al. 1994a).

Table 2
The results of halothane test on the basis of genetical methods used for the diagnosis for stress sensitivity

Methods	Genotype	Number of animals	Halothane test		
			+	+/-	-
Haplotyping method HAL-GPI-AlBG-PGD	NN	30	2	2	26
	Nn	43	1	3	39
	nn	32	17	6	9
	Total	105	20	11	74
PCR/RFLP analysis	NN	25	2	2	21
	Nn	51	4	3	44
	nn	29	14	6	9
	Total	105	20	11	74

Table 3
The efficiency of identification of animals genotype HAL by haplotyping method and PCR/RFLP test

PCR/RFLP test	Haplotyping method HAL-PHI-AlBG-PGD			Total (pieces)
	NN	Nn	nn	
NN	**20**	10	-	30
Nn	5	33	5	43
nn	-	8	24	32
Total (pieces)	25	51	29	105

animals identified the same by both methods: 20(NN)+33(Nn)+24(nn)=77
28 animals, which genotypes changed by PCR/RFLP test 26.7%

CONCLUSIONS

1. The low efficiency of halothan test for diagnosis the stress sensitivity and the PSE syndrome has been confirmed.
2. The haplotyping method based on the four linkage loci (HAL-GPI-AlBG-PGD) applied for family material does 73.3% corrected defined genotypes with comparison to PCR/RFLP test.
3. The comparative analysis of genetic methods for the need of meat quality diagnostic showed the best precision of PCR/RFLP test.

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