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## MARKER-ASSISTED SELECTION FOR GENOTYPING HERDS OF HYBRID GILTS BASED ON POPULATION-GENETIC VARIABILITY

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*In the study, the allelic effect of single nucleotide polymorphisms of SNPs was determined to estimate fattening productivity in a herd of hybrid gilts (n=101) (Large White × Landrace) × Maxgro. The studied herd of hybrid gilts was obtained as a result of direct (Large White × Landrace) and reciprocal crossing (Landrace × Large White) with boars of the Maxgro terminal line. We conducted a population analysis of polymorphisms by melanocortin genes 4 MC4R (c.1426 A>G), cathepsin D CTSD (g.70 G>A), and ryanodine receptor 1 RYR1 (g.1843 C>T) using software GenA1EX6. To genotyping a herd of hybrid gilts, 2 experimental groups were selected: control (uncastrated) in quantity (n=56) and experimental (immunologically castrated) (n=45) grown in conditions of LLC SPE «Globinsky Pig Farm». Laboratory tests are carried out in the laboratory of genetics in the Institute of Pig Breeding and Agro-Industrial Production of the NAAS of Ukraine. Due to the fact that information about polymorphism on the above SNPs in the population uncastrated and immunologically castrated herd of hybrid pigs which are breed in Ukraine is absent, this requires breeding work involving marker-associated selection on the inside of the breed base, to find out which alleles will be determined as desirable in the marker selection of hybrid pigs. Marker breeding is an effective tool in improving fattening qualities for the pig industry - modern commercial lines of pigs. Therefore, we decided to carry out the typing of an experimental herd of hybrid gilts. The results of genotyping will provide useful information for the selection of the commodity population of hybrid pigs for fixing the desired useful signs of the genotype. Polymorphism of the SNPs studied MC4R (c.1426 A>G), CTSD (g.70 G>A), and RYR1 (g.1843 C>T) is determined by the method of PCR-RFLP analysis. Thus, a breed character was established distribution of frequencies of encounter ability of alleles of investigated SNPs.*

**Keywords:** hybrid gilts, (Large White × Landrace) × Maxgro, native, immunologically castrated, SNP, MC4R (c.1426 A>G), RYR1 (g.1843 C>T), CTSD (g.70 G>A), PCR-RFLP analysis, ADG, AGE100.



Public appearance in banning surgical castration of pigs rises over animal welfare fears. Therefore, it is relevant to study possible methods for preventing the smell of boar in the meat and fat of pigs. A sustainable alternative as opposed to surgical and immunological castration of pigs there is selection at the genetic level in the pleiotropic period. Since the smell of boar is associated with reproductive hormones, it is important to take into account the possible negative lateral effects: change in behavior, and delayed puberty. Scientists (A Van den Broeke, M Aluwé, et al. 2015) it is reported that the marker of the receptor melanocortin-4 (MC4R) can be used to reduce the smell of boar in pig fat [8]. The concentration of three components is investigated (A Van den Broeke, M Aluwé, et al. 2015) causing the smell of boar: androstenone (0.044); indole (0.006), and scatole (0.049) were significantly higher in fat pigs with genotype  $MC4R^{AA}$  compared to  $MC4R^{GG}$  [9]. MC4R gene polymorphism can be used as a marker without a negative impact reproductive characteristics of pigs. Genetic selection in preventing the production of a specific smell of boar requires a management strategy to reduce the perverse effects in the herd of hybrid pigs. Cathepsin *D* gene localized at the p-telomere end of SSC2. The CTSD gene covers about 9 thousand base pairs and has 9 exons and 9 introns - *GT/AG* [10,5]. CTSD gene encodes lysosomal-aspartyl protease. Transcription of the gene with sites that serve as a start site for estrogen-regulating transcript [1]. Pigs with AG genotype had lower average daily growth and more days in reaching a live weight of 100 kg different from pigs with monomorphic genotype. Pigs with the AA genotype had higher levels of meat qualities and lower spitz thickness compared to pigs with the GG genotype [5].

The RYR1 gene (receptor ryanodine 1) of pigs is the main gene responsible for the quality of meat. But its effect on fat content and the place of fat deposition are less well known. The mutation (PSS) in this gene is responsible for a sharp deterioration in the quality of meat. Mutation (c.1843T) impairs fat distribution between dorsal and abdominal fat which have a different value in the processing of meat [6]. PSS - is inherited as an autosomal recessive dysfunction that has been clearly demonstrated and associated with a single point mutation (c.1843T) (Arg615Cys) in the  $Ca^{2+}$  release channel at the sarcoplasmic reticulum RYR1, where the susceptible mutant allele is denominated  $RYR1^{TT}$  [2,7]. Scientists (Fujii et al. 1991, Bates et al. 2012) it has been investigated that the recessive version of the (c.1843T) is responsible for the tendency to stress and the occurrence of PSE meat (pale, soft, exudative). Compared to pigs that are homozygous by the genotype  $RYR1^{CC}$  allele  $RYR1^T$  is associated with the deposition of fat in the lower abdomen. Heterozygous pigs  $RYR1^{CT}$  tended to secrete adipose tissue in dorsal fat. However, the effect on the distribution of fat does not depend on the overall fatness of the pig [4,6]. The result of the study (MacLennan et al. 1990) on variants of RYR1 pigs helped explain the molecular background of a malignant human tumor - hyperthermia. The fatness of pigs is considered as a model phenotype for the study of complex human obesity similarity in body size, physiology and diet between humans and pigs (Lunney 2007, Switonski et al. 2010). The benefits of this species as a model in studies on obesity-related diseases, including diabetes type 2, it was justified (Kogelmann et al. 2013). (Neeland et al. 2012) it has been shown that a person has an excessive accumulation of abdominal fat is the best marker of risk of prediabetes and type 2 diabetes mellitus than general obesity. Therefore, the analysis of the effects of genotypes  $RYR1$  (c.1843) on weight and deposition of visceral and subcutaneous fat in modern commercial pig lines is still an urgent issue. This is closely related to the well-being of pigs and people consuming pork products which is the result of a mutant allele (c.1843T). Therefore, the search and identification of DNA markers associated with



performance indicators in the studied groups of hybrid pigs are of particular relevance due to the intensification of pork production technologies.

**The aim of our work is to study** the polymorphism of melanocortin genes 4 *MC4R* (c.1426 A>G), cathepsin D *CTSD* (g.70 G>A), and ryanodine receptor 1 *RYR1* (g.1843 C>T) in the herd of hybrid pigs (Large White × Landrace) × Maxgro based on indicators of population-genetic variability.

**Materials and methods of research.** Gene polymorphism *MC4R* (c.1426 A>G), *CTSD* (g.70 G>A), and *RYR1* (g.1843 C>T) studied in the stage of hybrid pigs (Large White × Landrace) × Maxgro: uncastrated (n=56), immunologically castrated (n=45). As a genetic material, bristles (hair follicles) were used from the ears of the pigs studied. DNA release from bristles was carried out using Chelex-100 ion exchange resin according to the method (Korinnyi S. M., Pochernyaev K. F., Balatsky V. M., 2005) [3]. The obtained samples were subjected to PCR amplification using primers (Table 1).

Table 1

**Structure of genetic marker *MC4R* (c.1426 A>G), *CTSD* (g.70 G>A) and *RYR1* (g.1843 C>T) for PCR amplification**

Target genes	Primer structure (5'→3')	Amplification program	Endonuclease	Size (pb)	GenBank accession
<i>MC4R</i> (c.1426 A>G)	298R-5'-TACCCTGACCATCTTGATTG 298F-5'-ATAGCAACAGATGATCTCTTTG	94°C–3min.;31cycle: 94°C-25min.;64°C-26sec.;72°C–40sec., 72°C–2min.	<i>Taq I</i>	220, 150, 70	NC_010443 .5/397359
<i>CTSD</i> (g.70 G>A)	F: GCTGTGCACCCTAGGA ACC R: TCGTCAGGTCCAGGAC AAAC	94°C–3min.;31cycle: 94°C–30sec.;58°C–26sec.;72°C–40sec., 72°C–2min.	<i>Msc I</i>	184, 117, 67	NC_010444 .4/494568
<i>RYR1</i> (g .1843 C>T)	F:5/GTGCTGGATGTCC TGTGTTCCCT-3 / R:5/CTGGTGACATAGT TGATGAGGTTTG-3 /	94°C–3min.;31cycle: 94°C25sec.;68,5°C–26sec.;72°C–40sec., 72°C–2min.	<i>Hha I</i>	134, 84, 50	NM_001001 534.1/3967 18

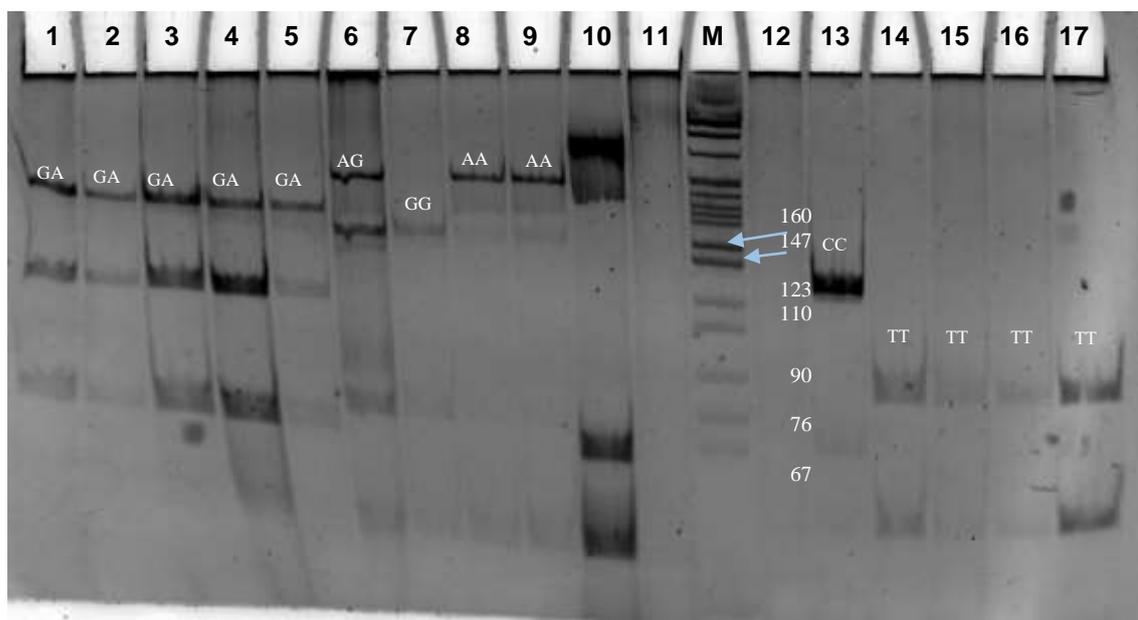
The complete set of samples underwent PCR amplification. The reaction was set to a final volume of 20µL, 1.0 µL MgCl<sub>2</sub>, 0.25 µL of each primer, 1.25 µL of dNTP, 1X reaction buffer NH<sub>2</sub>SO<sub>4</sub>, 0.5 µL of Taq DNA polymerase, ultrapure sterile water – 5.0 µL and the final volume of the template DNA 10.5 µL. Thermocycling conditions were installed according to (Table 1.). All amplifications were performed in conjunction with negative control (distilled water). DNA fragment amplification was confirmed by electrophoresis on 2% m/v agarose gel, stained with ethidium bromide (10 mg/ml), and visualized under UV light.

Enzymatic digestion was performed in a final volume of 15 µL, including 5 µL (~ 0.1–0.5 µg of DNA) of the PCR product, 0.2 µL of Taq I, Msc I, Hha I endonuclease (Thermo Fisher Scientific™) and 2.8 µL Buffer 10X, together with nuclease-free water to reach final volume 7.0 µL. Samples were incubated at 65 °C, time - 1 hour 50 minutes for *MC4R* (*TaqI*), and at 37 °C, time – 3 hours 10 minutes for *CTSD* (*MscI*) and *RYR1* (*HhaI*). Electrophoretic separation of DNA fragments was carried out in 8% pol-



yacrylamide gel in 1xTBE buffer, at current strength (5V/cm) gel length. Visualization of restriction products was carried out by dyeing bromide ethidium and viewing on the transilluminator in UV light.

**Results of the study and their discussion.** DNA typing of the studied groups was carried out on the uncastrated ( $n=56$ ) and immunological castrated hybrid pigs ( $n=46$ ) for SNP *MC4R* ( $c.1426 A>G$ ), *RYR 1* ( $g.1843 C>T$ ) and *CTSD* ( $g.70 G>A$ ). DNA typing involves identifying allele gene variants alleles of which are characterized by restriction fragments the size of base pairs (bp). Fragments of the resulting electrophoregram (Fig. 1.): Track 1-5 with genotype  $g.70^{GA}$  (184, 117, 67 bp.). Track 6 with genotype  $c.1426^{AG}$  (70 bp.), 7 with genotype  $c.1426^{GG}$  (150 bp.), 8,9 with genotype  $c.1426^{AA}$  (220 bp.). Track 13 with genotype CC (134 bp.), 14-17 with genotype TT (84, 53 bp.).



**Fig.1. Electrophoregram of restriction products Taq I locus DNA *MC4R* ( $c.1426 A>G$ ); *MscI* locus DNA *CTSD* ( $g. 70 G>A$ ); *HhaI* - *RYR1* ( $g. 1843 C>T$ ) y 8% PAAG. Molecular mass marker pBR322/*MspI*.**

The analysis of fattening productivity was carried out according to the results of control cultivation up to 100 kg according to the following indicators: age of reaching live weight 100 kg (AGE100); fattening duration, days; average daily growth, g (ADG) (Table 2).

Analyzing the results of fattening indicators, it can be argued that uncastrated ( $n=5$ ) (ADG=0.928g/144day) and immunologically castrated gilts ( $n=5$ ) (ADG=0.966g/144day) with  $MC4R^{AA}$  genotype are characterized by a uniform growth rate (AGE100) – 144 days. Immunologically castrated gilts ( $n=2$ ) with  $MC4R^{GG}$  genotype (ADG=0.735g/167day) have a difference in indicator (AGE100) – 8 days, compared to uncastrated gilts ( $n=5$ ) (ADG=0.778g/159day). Immunologically castrated gilts ( $n=12$ ) with  $MC4R^{AG}$  genotype (ADG=0.924g/145day) predominate uncastrated ( $n=8$ ) by age reaching a live weight of 100 kg in 4 days and average daily growth of 0.72 g - (ADG=0.852g/149day). The results prove that hybrid gilts 1 and 2 groups with the genotypes  $MC4R^{AA}$  and  $MC4R^{AG}$  have a high rate (ADG) unlike gilts 1 and 2 groups with  $MC4R^{GG}$  genotypes with a total difference (ADG=0.162g/17days).



Table 2

**Results of fattening indicators of herds of hybrid pigs (Large White × Landrace) × Maxgro progenotyped by locus MC4R (c.1426 A>G), CTSD (g. 70 G>A) and RYR1 (g. 1843 C>T)**

Locus genotypes	Uncas-trated pigs	ADG, g	AGE 100kg/day	Immuno-castrated pigs	ADG, g	AGE 100kg/day
<i>MC4R<sup>AA</sup></i>	5	0.928	144	5	0.966	144
<i>MC4R<sup>GG</sup></i>	5	0.778	152	2	0.735	167
<i>MC4R<sup>AG</sup></i>	8	0.852	149	12	0.924	145
<i>CTSD<sup>GG</sup></i>	6	0.770	159	1	0.808	151
<i>CTSD<sup>GA</sup></i>	25	0.858	147	15	0.879	147
<i>RYR1<sup>TT</sup></i>	6	0.811	157	10	0.875	150
<i>RYR1<sup>CC</sup></i>	1	0.677	163	-	-	-

Progenotyped by a DNA marker *CTSD<sup>GG</sup>* immunologically castrated pigs (n=1) predominate uncastrated (n=6) in terms of average daily growth of 0.808g.per 38g. and the age of reaching a live weight of 100 kg. 151 days 8 days earlier than uncastrated (ADG=0.770g/159day). With the *CTSD<sup>GA</sup>* (n=15) genotype, the research team prevails only by an average daily increase of 0.879 g. for 21 g. with the same indicator of reaching the age of live weight of 100 kg (ADG=0.858g/147day).

The study group (immunological castrated) with the genotype *RYR1<sup>TT</sup>* (ADG=0.875g/150day) on 7 days earlier reached a live weight of 100 kg from the control group (uncastrated) (ADG=0.811g/157day), with an advantage of 64g of average daily growth. With the genotype *RYR1<sup>CC</sup>*, there were only uncastrated pigs (1 head) with an average daily increase of 677g at the age of 163 days. No *RYR1<sup>CC</sup>* genotype was found among immunologically castrated pigs. This genotype in the study population among the uncastrated pigs - 7 heads and immunologically castrated - 10 heads is an isolated case. However, animals with the polymorphic genotype *RYR1<sup>TC</sup>* have also not been detected.

We counted the connection of genotypes by loci CTSD, MC4R and RYR1 with an (ADG) during the fattening period of hybrid gilts (Large White × Landrace) × Maxgro (Table 3.).

Intra-breed average (X) for hybrid gilts with genotype c.1426<sup>GG</sup> 0.776 g with arithmetic mean deviation ±Sx 0.024198. For animals with genotype c.1426<sup>AG</sup> 0.897 g with arithmetic mean deviation ±Sx0.020992. The average (ADG) by intra-breed type is 0.947 g with an arithmetic mean deviation of ±Sx0.015786 for animals with genotype c.1426<sup>AA</sup>. For hybrid pigs with genotypes c.1426<sup>GG</sup>, c.1426<sup>AG</sup>, c.1426<sup>AA</sup>, Fisher's reliability criterion is 0.00017\*\*\* (\*\*\*) - p≤0.01), what the indicator of the force of influence indicates D2 – 66.61. Hybrid pigs with genotype g.70<sup>GG</sup> indicator (ADG) is 0.776 g with ±Sx 0.024198. For animals with the genotype g.70<sup>GA</sup>, the indicator (ADG) is 0.866 g of the ± Sx 0.013478. For hybrid pigs with g.70<sup>GA</sup> genotypes, g.70<sup>GG</sup> Fisher's reliability criterion is 0.010\*\*\* (\*\*\*) - p≤0,01), what the indicator of the force of influence indicates D2 – 15.83. The intra-breed average (X) for hybrid gilts with genotype g.1843<sup>TT</sup> (ADG) is 0.851 ± ±Sx 0.02088. For animals with genotype g.1843<sup>CC</sup> (ADG) is 0.677. Fisher's credibility criterion for hybrid gilts with genotypes g.1843<sup>CC</sup>, g.1843<sup>TT</sup> is 0.0613, what the indicator of the force of influence indicates D2 – 27.27.



Table 3

**Connection of genotypes by loci MC4R, CTSD and RYR1 with an average daily increase during the fattening period of hybrid gilts (Large White × Landrace) × Maxgro (n=101)**

Locus	$X \pm Sx$	$X \pm Sx$	$X \pm Sx$	D <sup>2</sup> , %	p
	GG/CC	GA	AA/TT		
CTSD 47 goals	0.776±0.024198	0.866±0.013478	-	15.83	0.010 ***
MC4R 37 goals	0.766±0.022172	0.897±0.020992	0.947±0.015786	66.61	0.00017 ***
RYR1 17 goals	0.677	-	0.851±0.02088	27.27	0.0613

Note: X - average for intra-breed type;

±Sx - arithmetic mean error;

p - probability level;

\*\*\* -  $p \leq 0,01$ , \*\* -  $p \leq 0,01$ , \* -  $p \leq 0,05$  - by the criterion of Fisher's reliability;

D<sup>2</sup> – indicator of the force of influence.

To understand the distribution of allele frequencies in the population of hybrid gilts (Large White × Landrace) × Maxgro, we counted the frequency distribution of genotypes by DNA markers MC4R (c.1426 A>G), RYR1 (g.1843 C>T) and CTSD (g.70 G>A) (Table 4.).

Table 4

**Distribution of frequencies of alleles and genotypes of DNA marker MC4R (c.1426 A>G), RYR1 (g.1843 C>T) and CTSD (g.70 G>A) in gilts (Large White × Landrace) × Maxgro**

Locus	SNP	Frequencies of alleles	Frequencies of genotypes			$\chi^2$	F
			GG/CC	AG/GA	AA/TT		
MC4R	c.1426 A>G	A=0,554 G=0,446	0,19 (0,31)	0,51 (0,49)	0,30 (0,19)	0.057ns	-0.039
CTSD	g.70 G>A	G=0.595 A=0.405	0.19 (0.33)	0.85 (0.49)	- (0.18)	25.789** *	-0.682
RYR1	g.1843 C>T	C=0.059 T=0.941	0.06 (0.003)	- (0.11)	0.94 (0.89)	17.000** *	1.000

In the SNP MC4R (c.1426 A>G) allele frequency A 0.554 is higher than the frequency of the allele G 0.446. At the same time, the frequency of the  $MC4R^{AG}$  genotype 0.51 over the genotype  $MC4R^{AA}$  0.30 and  $MC4R^{GG}$  0.19 of the gene prevailed. For SNP behind locus RYR1 (g. 1843 C>T) found both homozygous alleles. Allele T (0.941) higher in frequency allele C (0.059). At the same time, the frequency of the  $RYR1^{TT}$  genotype 0.94 over the genotype  $RYR1^{CC}$  0.06. For SNP behind locus CTSD (g.70 G>A) in the studied breed found both heterozygous alleles. Allele frequency G (0.595) in SNP CTSD was higher than allele A frequency (0.405). According to the SNP, there was no reliable deviation of frequencies with DNA-typed Genotypes under the Hardy-Weinberg Equilibrium Law. At the same time, the frequency of the  $CTSD^{GA}$  genotype 0.85 over the genotype  $CTSD^{GG}$  0.19 of the gene prevailed. Reliable deviations of the observed dispersion of genotypes from the expected ones were recorded in the SNP in frequency at  $\chi^2 = 25.789^{***}$  and  $17.000^{***}$ . Wright Fixation



Index also showed an excess of heterozygous genotypes at the level of  $F=-0.682$  in the investigated micro population. A positive value of the fixed index  $F=1.000$  shows the overwhelming number of homozygous genotypes.

We have calculated the indicator of the information content of loci (PIC) (*Polymorphism Information Content*) by identifying the levels of locus polymorphism required for associative research by any genetic marker (Table 5).

Table 5

**Actual and theoretically expected heterozygousness and indicator PIC of gene MC4R, CTSD and RYR1 in gilts (Large White × Landrace) × Maxgro**

Locus	Homozygous	Heterozygous	PIC
<i>MC4R</i>	0.514	0.494	0.37
<i>CTSD</i>	0.811	0.482	0.37
<i>RYR1</i>	0.000	0.111	0.10

Optimal indicators for associative research which provide the necessary variety of genotypes to establish their relationship with performance indicators are from 0.25 to 0.75. Thus, the *SNP MC4R* and *CTSD* gene has an optimal value of 0.37 of the corresponding (PIC) level for associated studies. For the *RYR1* locus alone, the (PIC) level is low at 0.10, which limits its use for associated studies.

Of the sample of 17 samples studied, only one of the gilts with the genotype *RYR1<sup>CC</sup>*. 16 pigs were found to have the genotype *RYR1<sup>TT</sup>* - this indicates a tendency to stress, accompanied by aggressive behavior in the herd, and resistance to diseases.

As a result of genotyping by DNA markers of productivity polymorphic genotypes found among herds of uncastrated gilts *MC4R<sup>AG</sup>* ( $n=8$ ) and *CTSD<sup>GA</sup>* ( $n=25$ ) and immunologically castrated gilts polymorphic genotypes *MC4R<sup>AG</sup>* ( $n=12$ ), *CTSD<sup>GA</sup>* ( $n=15$ ) this result shows that the studied groups effectively absorb feed and are characterized by precocity and high daily growth.

This work was done with the support of the National Academy of Agrarian Sciences of Ukraine 31.01.00.07.F. «To investigate the pleiotropic effect of genes which SNP is used in marker-associated pig breeding». DR No. 0121U109838. Conclusion. Typing by selected genes provided useful information for the selection of hybrid pigs according to the desired fattening characteristics. It is worth noting that in Ukraine, the study of the distribution of frequencies and associations of DNA markers of the performance of these genotypes among hybrid gilts (Large White × Landrace) × Maxgro. We see the prospect of continuing research in the direction of a comprehensive analysis of the impact of the above genes on fattening qualities in the stages of hybrid pigs for the use of maxgro terminal line boars.

**Conclusions:**

1. Assessed DNA marker melanocortin genes 4 c.1426<sup>AG</sup> and cathepsin *D* g.70<sup>GA</sup> demonstrated the presence of polymorphism. It is promising is to conduct associative studies on DNA markers of productivity in the herd of hybrid gilts (Large White × Landrace) × Maxgro of LLC SPE "Globinsky Pig Farm".

2. Uncastrated ( $n=5$ ) (ADG=0.928g/144day) and immunologically castrated gilts ( $n=5$ ) (ADG=0.966g/144day) with c.1426<sup>AA</sup> genotype are characterized by a uniform growth rate (AGE100) – 144 days.



3. It is established that pigs of groups 1 and 2 with the genotype MC4R<sup>AA</sup> and MC4R<sup>AG</sup> have an ADG +0.162g indicator higher, other than pigs with MC4R<sup>GG</sup> genotype. and prevail in growth rate (AGE100) for 17 days.

4. It was clarified that immunologically castrated gilts with genotype CTSD<sup>GG</sup> were characterized by a higher indicator of (ADG=0.808g/151day) and the average growth rate other than uncastrated pigs (ADG=0.770g/159day). Pigs of control and research groups with genotype CTSD<sup>GA</sup> differ only in indicator (ADG) - (ADG=0.858g; 0.879/147day).

5. Gilts with genotype RYR1<sup>TT</sup> are stress-sensitive. Indicator (ADG) for immunologically castrated gilts is (ADG=0.875g/150day) with a significant difference with uncastrated pigs in indicator of – average growth rate 156days. The genotype RYR1<sup>CC</sup> was found in only one native pig with (ADG=0.677g/163day).

6. Hybrid gilts with genotype CTSD<sup>GA</sup>=0.85 genotypes prevail CTSD<sup>GG</sup> with a frequency of 0.19. Using the cathepsin D gene in the MAS with other DNA markers of fattening performance is possible. In hybrid gilts, the frequency of the MC4R<sup>AG</sup> genotype 0.51 over the genotype MC4R<sup>AA</sup> 0.30 and MC4R<sup>GG</sup> 0.19 of the gene prevailed. The genotype RYR1<sup>TT</sup> with a frequency of 0.94 genotypes prevails RYR1<sup>CC</sup> =0.06.

7. Average actual heterozygousness in the studied population as the main indicator of genetic variability detected an excess of heterozygous genotypes at (F=-0.741) for the CTSD locus, In the case of SNP RYR1, homozygous genotypes of RYR1<sup>TT</sup> with a frequency of 0.94 prevailed. The data indicate a high level of genetic consolidation of the studied herd of hybrid gilts.

**Prospects for Further Research.** We see the prospect of continuing research in the direction of a comprehensive analysis of the impact of the above genes on fattening qualities in the stages of hybrid pigs for the use of Maxgro terminal line boars.

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**МАРКЕР АСОЦІЙОВАНА СЕЛЕКЦІЯ ДЛЯ ГЕНОТИПУВАННЯ СТАДА ГІБРИДНИХ СВИНОК НА ОСНОВІ ПОКАЗНИКІВ ПОПУЛЯЦІЙНО-ГЕНЕТИЧНОЇ МІНЛИВОСТІ**

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У проведеному дослідженні було визначено алельний ефект однонуклеотидних поліморфізмів SNPs для оцінки відгодівельної продуктивності в стаді гібридних свинок ( $n=101$ ) (велика біла  $\times$  ландрас)  $\times$  Махгро. Досліджуване стадо гібридних свинок отримано в результаті прямого (велика біла  $\times$  ландрас) та реципрокного схрещування (ландрас  $\times$  велика біла) з кнурами термінальної лінії Махгро. Нами був проведений популяційний аналіз поліморфізму генів меланокортину 4 MC4R (с.1426 A>G), катепсину D CTSD (г.70 G>A) і ріанодинового рецептора 1 RYR1 (г.1843 C>T) за допомогою програмного забезпечення GenAlEX6. Для генотипування стада гібридних свинок були відібрані 2 експериментальні групи: контрольна (некастровані) ( $n=56$ ) та дослідна (імунологічно кастровані) ( $n=45$ ), вирощених в умовах ТОВ НВП «Глобинський свинокомплекс». Лабораторні дослідження проводяться в лабораторії генетики Інституту свинарства і Аграрно-мислового виробництва НААН України. У зв'язку з тим, що інформації про поліморфізм за вищевказаними SNPs в популяції некастрованих та імунологічно кастрованого стада гібридних свиней, які розводяться в Україні, відсутні, це вимагає проведення селекційної роботи із залученням маркер асоційованої селекції на



внутріпородній основі, для з'ясування того, які саме алелі будуть визначатися, як бажані при маркерній селекції гібридних свиней. Маркерна селекція є ефективним інструментом в оцінці відгодівельних якостей для галузі свинарства – сучасних комерційних ліній свиней. Тому нами було прийнято рішення провести типування експериментального стада гібридних свинок. Поліморфізм досліджуваних генів *MC4R* (с.1426 A>G), *CTSD* (г.70 G>A) та *RYR1* (г.1843 C>T), визначали методом ПЛР-ПДРФ аналізу. Таким чином, встановлено породний характер розподілу частот зустрічальності алелей досліджуваних SNPs.

Ключові слова: гібридні свинки, (велика біла × ландрас) × Махgro, нативний, імунологічно кастровані, SNP, *MC4R* (с.1426 A>G), *RYR1* (г.1843 C>T), *CTSD* (г.70 G>A), ПЛР-ПДРФ аналіз, ADG, AGE100

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## GENETIC ANALYSIS OF LOCAL UKRAINIAN HORSE BREEDS BY POLYMORPHISMS IN LY49B, EDNRB AND CSN3 GENES

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*Marker-Assisted Selection is getting increasing attention in animal breeding as an effective tool for choosing animals with desirable traits. Identification of molecular markers which are related to candidate genes is a promising approach for improving economic traits and has to be evaluated for further gene-trait associations. Single nucleotide polymorphisms are genetic markers that can be associated with production traits. SNP genotyping has to be done additionally for each breed to see if they are polymorphic and have significant associations with certain traits. Among the candidate genes that influence the expression of productive traits, special attention is drawn to LY49B, EDNRB and CSN3 genes. Gene EDNRB is associated with lethal white foal syndrome, LY49B is responsible for induction of immune response and CSN3 gene is responsible for reproduction traits in horses.*

*SNPs LY49B c. 1763 C>T, EDNRB g.118 TC/AG and CSN3 g. 66 A>G have been receiving increasing attention as potential markers which are responsible for developing important selection traits in horses. The study was conducted on Ukrainian Riding Horse, Russian Trotter and Orlov Trotter horse breeds (50 animals for each breed). Genotyping was performed using PCR-RFLP method. EDNRB polymorphism g. 118 TC/AG was polymorphic only in the Ukrainian Riding horse breed. This indicates carriers of the lethal mutation of the White Foal Syndrome (LWFS) only in the mentioned breed. SNP CSN3 g. 66 A>G turned out to be polymorphic (with the predominance of A g. 66 allele) and low-informative (i.e., PIC=0,090-0,122) in Ukrainian Rid-*