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ASSOCIATION BETWEEN LEPTIN RECEPTOR GENE POLYMORPHISM AND QUALITY OF BOTH MEAT AND BACK FAT IN LARGE WHITE PIGS OF UKRAINIAN BREEDING

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Leptin receptor is one of the components of the system of regulating energy homeostasis of the organism. Leptin receptor gene (*LEPR*) polymorphism is associated with pig carcass index of the content of intramuscular fat in its valuable parts, which is particularly important when assessing the quality of their carcasses for processing. Intramuscular fat is associated with meat flavor characteristics and partly determines its tenderness, juiciness, and other parameters. **Aim.** To analyze *LEPR* gene (SNP NM001024587.1, p. 1987 C > T) polymorphism in populations of various pig breeds and to establish its relationship with the quality of both meat and fat of pigs of Large White breed of Ukrainian breeding. **Methods**. Genetic-population analysis of nine pig breeds, associative analysis on the search connection of *LEPR* gene polymorphism with quality of both meat and fat of pigs of Large White breed of Ukrainian breeding. *LEPR* locus genotyping was performed by High Resolution Melting (HRM). **Results.** All the studied breeds are characterized by polymorphism of the leptin receptor gene (SNP NM001024587.1, p. 1987 C > T), significant breed specificity in the distribution of frequencies of alleles was established. Statistically confirmed effect ($p < 0.05$) of genotypes *LEPR* on the content of intramuscular fat, total dry matter and moisture in the meat, as well as the moisture content in the back fat of pigs of Ukrainian Large White breed was revealed. Higher content of intramuscular fat was found in the animals with genotype TT, while a smaller amount of intramuscular fat and more moisture in fat was revealed in heterozygotes. **Conclusions**. Genetic marker *LEPR* SNP NM001024587.1, p. 1987 C > T can be used in the marker-assisted selection to predict and improve the performance quality of the meat of pigs of Large White breed of the Ukrainian breeding. These results suggest that porcine leptin receptor gene controls the quality of fat complex – inside muscles and in the dorsal part of the carcass.

Keywords: pigs, SNP, DNA-typing, *LEPR*, meat quality, the quality of the fat.

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INTRODUCTION

The transition to intensive pig breeding presupposes the use of animals, remarkable for high growth energy and increased carcass meat yield. However, the selection, aimed at creating such genotypes, is accompanied with a considerable aggravation of meat quality – the reduction in active acidity (pH), moisture-retaining capacity, meat tenderness, content of intramuscular fat and other biochemical, organoleptic and sanitary and hygienic indices. Along with the study of the impact of such traditional factors as the content of feeds, the technology of keeping, transporting and organizing slaughter of animals on the meat quality, in recent years greater attention is paid to the genetic constituent, in particular, the search for and use of gene markers of meat quality with the purpose of solving this problem.

A number of gene loci, associated with the indices of pig meat quality, have been established. In particular, these include the leptin receptor gene (*LEPR*). A leptin receptor belongs to the family of cytokine receptors, class I, and is one of components of the system, regulating the energetic homeostasis of the organism. It modulates the effects of leptin, adipocyte-derivative protein hormone, which participates in the control over the intensity of food consumption, regulation of the bodyweight of animals and fat accumulation. In pigs the gene *LEPR* is localized in chromosome 6 and has 20 exons. At least 26 single nucleotide polymorphisms (SNP), localized in introns, exons and areas, adjacent to the genes, were found in its structure [1−7].

For some *LEPR* gene SNPs of pigs allele frequencies and polymorphism levels were established. Their association with such performance traits as fat thickness, growth intensity and morphometric quality of the carcass was estimated [8, 9]. Especially noteworthy is the fact that *LEPR* polymorphism is associated with the index of intramuscular fat content, on which taste characteristics of meat, in particular, its tenderness, juiciness, and other parameters, depend greatly [10].

There have been no studies on polymorphism and association of gene *LEPR* with the performance parameters and meat quality in pigs, conducted on domestic and foreign breeds of pigs, bred in Ukraine. There is no information on the association of *LEPR* polymorphism with quality indices of pig fat. At the same time, genetic markers, developed on the basis of polymorphism of gene *LEPR*, may be included into the selection programs, designed for the purpose of obtaining commercial herd with high levels of meat and fat quality, corresponding to the consumers' requirements. Taking into consideration the fact that Large White is the breed, most frequently used in selection programs and different cross-bred combinations, it seems relevant to establish associations between *LEPR* polymorphism and the indices of meat and fat quality for this pig breed in particular. A necessary prerequisite of such an associative study should be a sufficient level of *LEPR* polymorphism by the analyzed SNP.

The aim of the study was to analyze gene *LEPR* polymorphism (c. 1987 C > T) in the populations of pigs of different breeds and to establish its association with the quality of meat and fat of Ukrainian Large White pigs.

MATERIALS AND METHODS

The DNA samples of pigs of nine breeds from the DNA bank of the Genetics laboratory of the Institute of Pig-Breeding and Agro-Industrial Production of NAAS: Ukrainian Large White (ULW, $n = 93$), Ukrainian White Steppe (UWS, $n = 9$), Ukrainian Spotted Steppe (USS, $n = 18$), Myrgorod (Myr, $n = 36$), Poltava Meat (PM, $n = 28$), Landrace (L, $n = 29$), Pietrain (P, $n = 8$), Meishan (M, $n = 5$) and Large Black (LB, $n = 48$), were used in genetic-population analysis.

The associative studies were conducted on 60 animals of ULW breed (the ratio of barrows and gilts is approximately 1:1), which were fed till the living weight of 109 kg at the experiment farm "Stepnoe" of the Institute of Pig-Breeding and Agro-Industrial Production. The type of feeding was without restrictions. During the growth phase of animals from 40 to 60 kg of live weight the diet, calculated on dry basis, contained 12.9 MJ/kg energy, 19.1 % crude protein and 1.1.4 % lysine; from 60 to 90 kg of live weight − 12.8 MJ/kg energy, 18.0 % crude protein and 1.0 % lysine; from 90 to 109 kg − 12.6 MJ/kg energy, 17.1 % crude protein and 0.8 % lysine. The permit for the use of animals was approved by the Scientific Council of the Institute of Pig-Breeding and Agro-Industrial Production in accordance with the European Convention of Protection of Vertebrates, used for Experimental and Other Scientific Purposes (Strasbourg, 18.03.1986).

The blood samples (1 ml) were obtained in morning hours prior to feeding, when the animals gained 109 kg. The blood was mixed with 0.05 M of EDTA solution and kept at 4 °C till it was used for DNA isolation (not longer than 7 days).

Genomic DNA was isolated from meat samples using the sorbent method with the set "Diatom^{TM} DNA Prep 100" ("Isogen", RF).

LEPR SNP NM001024587.1, c. 1987 C > T polymorphisms were determined by the high resolution melting method (HRM) [5] using the reaction mixture Luminaris Color HRM qPCR ("LifeTechnologies", USA) on the thermocycler BioRad CFX96 using F: AGAGGACCTGAATTTGGAG and CATAAAAATCAGAAATACCTTCCAG primers.

To define the indices of meat quality the meat samples were taken from the longest dorsal muscle (*M. longisimus dorsi*) of the right semi-carcass between thoracic vertebra 9−12 after the maturation for 48 h in the regime of gradual cooling down to the temperature of +2…−4 °C. Fat samples were also selected above the longest muscle.

The active acidity pH was registered at the portable digital LF-Meter "LF-Star CPU-Pistole" (Ing.-Büro & Klassifizierungsservice Rudolf Matthäus, Klausa, Germany). The total moisture in the meat was defined while drying it at 105 °C till the establishment of permanent weight. The protein content was analyzed by Kjeldahl's method, that of intramuscular fat –

by Soxhlet's method (Official Methods of Analysis (AOAC, 1990) [11].

The moisture-retaining capacity of meat was found by the pressing method [12].

The moisture content in fat was determined by the method of drying 0.5 g of weighed portion in glass weighting bottles for 2.5 h till the permanent mass at 105 °C, the temperature of melting fat – in straight capillary, open on both sides, with the 1.5 mm diameter using the digital thermometer AMA-digit ad 14th (Germany) according to the methodological recommendations [13].

The frequencies of alleles and genotypes, the levels of heterozygosity (both observed and expected one) were estimated using GenAlEx 6.0 software [14]. The experimental data to determine the association of genotypes and the indices of meat quality were processed by the method of single-factor dispersion analysis using the computational medium of MS Office Excel 2007 Spreadsheet.

RESULTS AND DISCUSSION

LEPR gene single nucleotide polymorphism NM001024587.1 SNP c. 1987 C > T (defined in some works as c. 2002 C > T), p. Leu663Phe, was selected for genotyping, since its significant associations with performance traits and meat quality of pigs of some crossbred lines were determined [5, 15]. The results of our genetic population analysis regarding this gene for nine breeds of pigs of Ukrainian and foreign selection are presented in Table 1.

Both alleles of the gene were found in all the breeds. At the same time, if allele C was predominant in breeds ULW, PM, L and UWS, allele T prevailed in the USS breed. As for the rest of the breeds the frequencies of alleles were found to be almost equal. The highest frequency of allele C was found in the population of Landrace breed (0.880) and the least – in Meishan breed (0.400). There is a statistically reliable difference in the frequencies of alleles between the breeds which testifies to certain genetic differentiation of breeds by this locus.

Noteworthy are statistically reliable deviations in actual distribution of genotypes from the equilibrium one in the breeds of Poltava Meat, Ukrainian Spotted Steppe and Landrace – the shortage of heterozygotes and the excess of both homozygous genotypes was found. Obviously, in the investigated populations there is a certain selection pressure on the locus, related to the selection of animals by the performance traits, the control over which is executed with the involvement of *LEPR*. Such selection pressure gives an advantage to homozygous genotypes.

Table 1. The frequencies of alleles and genotypes of *LEPR* gene (c. 1987 C > T) in the breeds of pigs of different trends of performance and origin

Breed	*n*	Genotype frequency			Frequency of allele		χ^2	PIC
		CC	CT	TT	C	T		
Ukrainian Large White	93	0.46 (0.44)	0.41 (0.44)	0.13 (0.12)	0.67	0.33	0.097	0.344
Poltava Meat	28	0.61 (0.51)	0.21 (0.41)	0.18 (0.08)	0.71	0.29	6.318	0.327
Landrace	29	0.86 (0.77)	0.03 (0.21)	0.11 (0.02)	0.88	0.12	20.342	0.189
Ukrainian Spotted Steppe	18	0.28 (0.15)	0.22 (0.48)	0.50 (0.37)	0.39	0.61	5.103	0.363
Ukrainian White Steppe	9	0.44 (0.44)	0.44 (0.44)	0.12 (0.12)	0.67	0.33	0.000	0.344
Myrgorod	36	0.36 (0.31)	0.39 (0.49)	0.25 (0.20)	0.56	0.44	1.626	0.371
Large Black	48	0.40 (0.35)	0.40 (0.48)	0.20 (0.17)	0.59	0.41	1.546	0.367
Pietrain	8	0.25 (0.25)	0.50 (0.50)	0.25 (0.25)	0.50	0.50	0.000	0.375
Meishan	5	0.00 (0.16)	0.80 (0.48)	0.20 (0.36)	0.40	0.60	2.222	0.365

Note. The expected frequencies of genotypes, estimated by Hardy-Weinberg's formula, are presented in brackets. The frequencies of genotype CT correspond to the heterozygosity levels: observed (Ho) – without brackets, expected (He) – in brackets. The values χ^2 were determined for the estimation of the fidelity of deviation of the observed distribution of genotypes from the expected one.

At the same time the breeds vary in the level of locus variability. For example, the lowest level of actual heterozygosity (one of the indices of genetic variability) was noted for Landrace breed (0.03) and the highest level – for Meishan breed (0.80).

The level of locus polymorphism by any genetic marker, necessary for associative studies, may be estimated using the index of polymorphism information content (PIC). The average values of locus PIC (from 0.25 to 0.75) are optimal for associative studies and ensure required variety of genotypes for the determination of their relation to the performance traits [16]. It should be noted that PIC does not exceed 0.375 for diallel systems. The level of PIC fluctuates from 0.327 to 0.375 for the majority of analyzed breeds (Table 1). PIC was found to be low (0.189) only in Landrace, and thus the level of locus polymorphism by this genetic marker was insufficient for associative studies. Conducting such studies on Landrace requires the use of a considerably higher number of animals, compared to the studies of other breeds, which is required for the representation of all the genotypes in the experimental group.

Table 2. The impact of the genotypes of *LEPR* (c. 1987 C > T) on the investigated indices of meat and fat quality of Ukrainian Large White

Index	Genotype			η^2, %	p CC/CT CC/CT CT/TT
	g CC Average (s. d.; *n*)	g CT Average (s. d.; *n*)	g TT Average (s. d.; *n*)		
pH_{48}	5.45 (0.162; 27)	5.49 (0.218; 27)	5.53 (0.103; 6)	2.215	0.237 0.432 0.648
Moisture-retaining capacity, %	56.60 (2.665; 17)	58.77 (2.903; 6)	56.23 (1.403; 4)	4.428	0.790 0.364 0.593
Total moisture, %	75.05 (1.807; 27)	76.37 (2.144; 27)	75.43 (3.893; 6)	7.896	0.715 0.012 0.411
Air dry substance, %	26.41 (1.803; 27)	25.12 (2.187; 27)	26.098 (3.841; 6)	7.577	0.758 0.021 0.399
Protein, %	21.84 (1.400; 27)	21.13 (1.794; 27)	20.97 (3.229; 6)	4.297	0.297 0.107 0.871
Intramuscular fat, %	1.99 (1.113; 27)	1.38 (0.837; 27)	2.61 (1.179; 6)	14.212	0.229 0.029 0.005
Content of moisture in fat, %	6.26 (2.140; 24)	8.08 (2.549; 21)	6.19 (0.980; 6)	14.619	0.960 0.013 0.091
Temperature of fat melting, ºC	28.09 (1.610; 24)	28.66 (3.096; 21)	29.00 (2.495; 6)	2.005	0.806 0.488 0.278

Note. s. d. – standard deviation; *n* – number of animals; η^2 – force of genotype impact on the index; p – level of significance of differences between genotypes CC/TT, CC/CT, CT/TT.

In our work the associative studies were performed using Ukrainian Large White pigs, for which PIC by the genetic marker of leptin receptor gene NM001024587.1 SNP c. 1987 C > T has the optimal value of 0.344.

The results of the study of the impact of genotypes of leptin receptor gene on the analyzed indices of meat and basting fat of Ukrainian Large White pigs are presented in Table 2.

The genotypes of *LEPR* have considerable impact on the content of intramuscular fat in the meat, $\eta^2 = 14.212$ %, ($p < 0.05$), total moisture, $\eta^2 = 7.896$ %, ($p < 0.05$) and dry substance, $\eta^2 = 7.577$ %, ($p < 0.05$). It should be noted that the content of fat in the meat of pigs with heterozygous genotype was considerably smaller compared to the animals with homozygous genotypes, and the latter did not differ by this index. This fact allows assuming the additive interaction of alleles of this locus. Our results are in agreement with the data, obtained in the works [4, 15] on the cross-bred Duroc x Iberian breed pigs and in the publication [5] – using the experimental group of Iberian x Landrace pigs, testifying to the fact that allele c. 1987 T is associated with higher content of fat in meat compared to allele c. 1987 C.

The impact of *LEPR* genotypes (c. 1987 C > T) on the indices of carcass fat quality in pigs was studied. Their considerable association with the melting temperature was not revealed, but the impact on the index of moisture content was determined – $\eta^2 = 14.619$ % ($p < 0.05$). The moisture content in pigs with the heterozygous genotype CT was higher than in pigs with genotypes CC and TT. In general the results obtained testify to the fact that the leptin receptor gene in pigs controls the quality of fat depositions in a complex – inside muscles and on the back. The heterozygotes are remarkable for a smaller amount of intramuscular fat and a larger amount of moisture in fat.

CONCLUSIONS

All the investigated breeds of pigs are characterized by polymorphism by the leptin receptor gene (SNP NM001024587.1, c. 1987 C> T). There is evident breed-wise specificity in the distribution of frequencies of its alleles. There is a determined statistically relevant impact ($p < 0.05$) of genotypes of *LEPR* gene on the content of intramuscular fat, total moisture and dry substance in meat as well as on the moisture content in the blasting fat of Ukrainian Large White pigs. The genetic marker *LEPR* SNP NM001024587.1, c. 1987 C > T may be used in marker selection for the purpose of forecasting and improving the indices of meat quality of Ukrainian Large White pigs.

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Асоціація поліморфізму гена рецептора лептину з якістю м'яса і сала у свиней великої білої породи української селекції

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Рецептор лептину є одним із компонентів системи регулювання енергетичного гомеостазу організму. Поліморфізм гена рецептора лептину (*LEPR*) асоційований з таким показником туші свині, як вміст внутрішньом'язового жиру в її цінних частинах, який є особливо важливим при оцінці туш для їхньої якісної переробки. Внутрішньом'язовий жир пов'язаний із смаковими характеристиками м'яса і частково визначає його ніжність, соковитість та інші параметри. **Мета.** Проаналізувати поліморфізм SNP NM001024587.1, с. 1987 С > Т гена *LEPR* у популяціях свиней різних порід та встановити його зв'язок з якістю м'яса і сала свиней великої білої породи української селекції. **Методи.** Генетико-популяційний аналіз дев'яти порід свиней, асоціативний аналіз щодо пошуку зв'язку поліморфізму гена *LEPR* з показниками якості м'яса і сала свиней великої білої породи української селекції. Генотипування за локусом *LEPR* здійснювали методом High Resolution Melting (HRM). **Результати.** Всі досліджувані породи характеризуються поліморфізмом за геном рецептора лептину (SNP NM001024587.1, с. 1987 С > Т), встановлено виражену породну специфіку у розподілі частот його алелів. Виявлено статистично підтверджений вплив ($p < 0,05$) генотипів *LEPR* на вміст внутрішньом'язового жиру, загальної вологи та сухої речовини у м'ясі, а також на вміст вологи в хребтовому салі свиней української великої білої породи. У тварин з генотипом ТТ виявлено вищий вміст внутрішньом'язового жиру, а в гетерозигот − меншу кількість внутрішньом'язового жиру і більшу кількість вологи в салі. **Висновки.** Генетичний маркер *LEPR* SNP NM001024587.1, c. 1987 C > T можна використовувати у маркерній селекції для прогнозування і поліпшення показників якості м'яса свиней великої білої породи української селекції. Отримані результати дозволяють припустити, що у свиней ген рецептора лептину контролює якість жирових відкладень комплексно − всередині м'язів і на спинній частині туші.

Ключові слова: свині, SNP, ДНК-типування, LEPR, якість м'яса, якість жиру.

Ассоциация полиморфизма гена рецептора лептина с качеством мяса и сала у свиней крупной белой породы украинской селекции

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Рецептор лептина является одним из компонентов системы регулирования энергетического гомеостаза организма. Полиморфизм гена рецептора лептина (*LEPR*) ассоциирован с таким показателем туши свинки, как содержание внутримышечного жира в ее ценных частях, который является особенно важным при оценке туш для их качественной переработки. Внутримышечный жир связан с вкусовыми характеристиками мяса и частично определяет его нежность, сочность и другие параметры. **Цель.** Проанализировать полиморфизм SNP NM001024587.1, c. 1987 C > T гена *LEPR* в популяциях свиней различных пород и установить его связь с качеством мяса и сала свиней крупной белой породы украинской селекции. **Методы.** Генетико-популяционный анализ девяти пород свиней, ассоциативный анализ по поиску связи полиморфизма гена *LEPR* с показателями качества мяса и сала свиней крупной белой породы украинской селекции. Генотипирование по локусу *LEPR* осуществляли методом High Resolution Melting (HRM). **Результаты.** Все исследуемые породы характеризуются полиморфизмом по гену рецептора лептина (SNP NM001024587.1, c. 1987 C > T), установлена выраженная породная специфичность в распределении частот его аллелей. Выявлено статистически подтвержденное влияние ($p < 0,05$) генотипов *LEPR* на содержание внутримышечного жира, общей влаги и сухого вещества в мясе, а также на содержание влаги в хребтовом сале свиней украинской крупной белой породы. У животных с генотипом ТТ определено более высокое содержание внутримышечного жира, а у гетерозигот − меньшее количество внутримышечного жира и большее количество влаги в сале. **Выводы.** Генетический маркер *LEPR* SNP NM001024587.1, c. 1987 C > T можно использовать в маркерной селекции для прогнозирования и улучшения показателей качества мяса свиней крупной белой породы украинской селекции. Полученные результаты позволяют предположить, что у свиней ген рецептора лептина контролирует качество жировых отложений комплексно − внутри мышц и на спинной части туши.

Ключевые слова: свиньи, SNP, ДНК-типирование, LEPR, качество мяса, качество жира.

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