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PORK QUALITY AND GENETIC ASSOCIATION STUDY OF PORCINE LEPTIN AND CATHEPSIN F GENE POLYMORPHISMS

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Abstract. This study aims to assess the quality of raw pork intended for further processing, and also to analyse associations of genotypes, determined by single-nucleotide polymorphisms (SNPs) in leptin (*LEP* g.2845 A > T) and cathepsin (*CTSF* g.22 C ≤ G) genes, with meat and fat quality traits. Meat and fat products as raw materials for further processing, produced from pigs of French origin bred on the pig farm "Artsyz Meat Company Ltd" located in Artsyz district of Odesa region, are considered as the object of this study. The total number of pigs used to perform DNA analysis is 350 heads. DNA tests and physicochemical analyses of meat and fat products were conducted at the research laboratory of the Institute of Pig Breeding and Agricultural Production of the National Academy of Agrarian Sciences of Ukraine in Poltava. Genomic DNA was extracted from pig bristle using the Chelex 100 ion exchange resin. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used for genotyping. Fattening pigs were assessed for leptin (*LEP* g.2845) and cathepsin F (*CTSF* g.22) genes. Upon the young stock's reaching the target live weight of 100 kg, 60 pigs in each group genotyped for the target genes were slaughtered using conventional methods. The results of genetic tests and association studies performed have shown that leptin and cathepsin F gene polymorphisms produce an effect on particular pork and backfat quality attributes. Statistically significant associations of the genetic marker *LEP* SNP g.2845 A > T with the investigated quality attributes of meat and fat products, in particular water-holding capacity, meat tenderness, intramuscular fat content, backfat moisture content and melting point, have been detected. Meanwhile, the genetic marker *CTSF* SNP g.22 C ≤ G is found to be associated with such pork quality attributes as water-holding capacity, tenderness, weight loss during thermal processing (cooking loss), intramuscular fat content, calcium and phosphorus levels, and energy value.

Key words: pork, quality, meat and fat products, genetic markers, SNP, LEP g.2845, CTSF g.22

Introduction. Formulation of the problem

Meat is an essential protein food for humans. Versatility and uniqueness of porcine muscle tissue consist in its high energy value, balanced amino acid composition of proteins, and level of bioactive compounds. Altogether, it ensures the normal physiological state and nutrient absorption by the human body. Meat contains all the nutrients a human needs [1].

A relatively large number of candidate genes that are located at loci (called quantitative trait loci – QTL), which contribute to a greater or lesser extent to reproductive, fattening, slaughter and carcass traits in pigs and even quality attributes of raw pork intended for further processing, have been identified so far. At the same time, among these candidates, not so many genes and respective DNA markers are known to be effectively used in practice by a modern animal breeder with respect to their informativeness and strength of association with traits of interest in swine selection [2].

Over the past 10-15 years, marker-assisted selection (MAS), amid adequate nutrition and proper housing system, has been increasingly used as the basis for improving pig populations of different breed-of-origin via modern selective breeding. Adopting the afore-mentioned approach to selective breeding entails

rather reliable genotyping of species at specific loci, which are associated with economically relevant traits in pigs, and further incorporation of the obtained molecular and genetic data to correctly select individuals for their subsequent breeding, aimed at choosing parents to produce offspring of the desired type or even products of the desired quality [3-6].

Candidate genes *ACLY*, *ADIPOQ*, *ELOVL6*, *LEP* and *ME1* were identified through real-time or quantitative PCR (qPCR) as the most expressed genetic factors that affect meat composition and quality [7].

Analysis of recent research and publications

A morphological study of carcasses of young pigs of different origin proved that implementation of up-to-date crossbreeding schemes with the use of terminal sire meat-type breeds would result in improved carcass dressing percentage and optimised lean-to-fat ratio. As evident from the physical and chemical analyses of meat produced from pigs of different breed-of-origin, all investigated parameters were within the respective physiological limits. No significant difference was detected for the most parameters, though they tended to exhibit some peculiarities associated with the impact of genotype on the manifestation of one or another physical or chemical characteristic. The use of Pietrain breed as a terminal sire line resulted in decreased intramuscular fat content, and hence the energy value of pork, with the backfat having the highest melting point, which indicated its extended shelf life, though slightly impaired cooking properties as compared to similar products from the offspring of Large White and Landrace parents. With respect to its pH level and water-holding capacity, meat from Pietrain-sired progeny was quite similar to PSE (pale, soft, exudative) meat, being slightly less tender and paler in colour and exhibiting greater weight loss during thermal processing (cooking loss). Also, a comprehensive sensory evaluation of boiled pork and pork broth obtained from a group of Pietrain-sired young stock got the lowest score, which was consistent with most of the physical and chemical properties of meat from pigs of that genotype; it was concluded that it was 75% purebred Landrace which should be favoured as a terminal sire line in crossbreeding programmes in order to obtain pork and bacon of improved quality in intensive commercial swine production systems. It was recommended to preliminary combine Pietrain and Duroc lines to produce terminal sires (½ (Pietrain + Duroc)), which would be further mated with two-breed-cross dams (½ (Large White + Landrace)) [2]. It was also suggested that it would be quite feasible to produce pork of the desired quality by technological and cooking criteria, provided that MAS approach was adopted, and optimal nutrition, housing and handling were ensured for animals during their rearing, fattening, transport and slaughter.

Associations of polymorphisms in the cholecystokinin A receptor (*CCKAR*), melanocortin 4 receptor (*MC4R*), leptin (*LEP*) and porcine cathepsin (*CTS*) genes with feed efficiency and growth rates are known in research practice. The *MC4R* AA genotype is strongly associated with daily feed intake (DFI) at $p<0.01$. Polymorphisms in porcine cathepsin D (*CTSD*) and cathepsin Z (*CTSZ*) genes are reliably associated with feed efficiency, gain to feed ratio (G/F) and residual feed intake (RFI) at $p<0.05$. Low feed conversion ratio (FCR) is related to the *CTSZ* and *CTSD* GG genotype [8].

The porcine cathepsin F gene (*CTSF*) is mapped to chromosome 2(SSC2)p14-p17; it comprises 12 exons and 11 introns. Through expression the gene is converted into a protein containing 474 amino acid residues. Due to physiological function of this protein and localisation of the gene within the QTL region of the pig genome, the *CTSF* gene is responsible for meat quality and fat deposition traits. It has also been found out that the cathepsin F gene polymorphism plays a major role in determining traits of economic importance in swine, such as average daily gains (ADG; in g), carcass lean percentage (%) and backfat thickness (in mm) [1].

In particular, in the known research by V. Russo [3], a significant association of the *CTSF* SNP g.22 G > C with ADG and backfat thickness in Italian Large White pigs was shown. The mentioned *CTSF* polymorphism involved a G to C single nucleotide substitution (in rs1113132904) [1] that resulted in replacement of glutamic acid with aspartic acid in the cathepsin F enzyme polypeptide chain. Pigs of the cathepsin F g.22 CC genotype exhibited higher growth rates during ontogenesis and noticeably lower carcass fat percentage [3].

A genetic analysis of a population of Large White pigs of Ukrainian selection using genetic markers *LEP* SNP g.3469 T > C, *LEP* SNP g.2845 A > T and *CTSF* SNP g.22 C > G was carried out. The measured informativeness of *LEP* SNP g.2845 A > T and *CTSF* SNP g.22 C > G, or more exactly the calculated Polymorphism Information Content (PIC) value, was optimal for association studies (PIC = 0.311 and 0.373, respectively). Meat samples were classified by their quality as PSE, normal and DFD (dark, firm, dry) meat. Most of the samples exhibited moderate (n = 22) or mild (n = 59) signs of the PSE defect. Chuprov's coefficients of contingency between genotypes calculated for the studied SNPs and meat quality classes showed that genotypes for *LEP* SNP g.2845 A > T and *CTSF* SNP g.22 C > G were moderately correlated with meat quality levels (K = 0.26 and 0.24, respectively). The Analysis of Variance (ANOVA) tests of the composite meat quality index revealed differences between homozygous and heterozygous genotypes for *CTSF* SNP g.22 C > G. As reported, the composite quality index for the pork sampled from the *CTSF* SNP g.22 C > G (g.22 GC) heterozygous pigs

was 4.6, which was higher than that for the samples from the g.22 GG and g.22 CC homozygous pigs (4.2, $p \leq 0.05$ and 3.9, $p \leq 0.01$, respectively) [4].

Another study was conducted in a sub-population of Ukrainian Large White pigs with the aim of analysing associations of the *CTSF* SNP g.22 G > C with performance traits, in particular the age when a pig reaches target live weight of 100 kg, backfat thickness at the P1 position near the $6^{th}/7^{th}$ thoracic vertebrae and at the 10^{th} rib, rump fat thickness and average daily gain. The statistical association analysis showed that the *CTSF* SNP g.22 G > C genetic marker tended to be associated with the age of a pig's reaching the target live weight of 100 kg ($p = 0.07$) [5].

Leptin (*LEP*) and cathepsin F (*CTSF*) genes have been considered as potential candidate genes for marker-assisted selection due to their direct contributing to fat deposition and meat quality traits in swine. A genetic association analysis of four SNPs – namely, *LEP* g.3469 SNP T > C, *LEP* SNP g.2845 A > T, *LEP* SNP g.3996 T > C and *CTSF* SNP g.22 C ≤ G – was performed in a population of Ukrainian Large White pigs. In the pig group under investigation, three of four analysed genetic variations in *LEP* and *CTSF* genes were proved to be polymorphic, whereas *LEP* g.3996 T > C was not found to be polymorphic as the T allele was the only observed one. Associations of SNPs with pork and backfat quality attributes were investigated for 16 parameters in total. It was reported that *LEP* SNP g.3469 T > C affected the protein content, moisture loss during thermal processing (cooking loss) and water content of backfat; *LEP* SNP g.2845 A > T was associated with water-holding capacity, intramuscular fat and water content of backfat; *CTSF* SNP g.22 C ≤ G was associated with the fat percentage and calcium level in meat. The following trends in SNP effects were observed: *LEP* SNP g.3469 T > C tended to be associated with meat tenderness ($p = 0.06$) and fat percentage ($p = 0.07$); *CTSF* SNP g.22 C ≤ G – with the total moisture ($p = 0.07$) and protein content of meat ($p = 0.07$); *LEP* SNP g.2845 A > T – with the energy value ($p = 0.08$) and protein in meat ($p = 0.08$) [6].

Therefore, at the current stage of scientific research evolution, the role and potential of genomics for strengthening control of meat quality attributes is increasing. It promotes improvement of key performance traits in swine, further increase in meat productivity of commercial pigs and hence enhancement to raw material source for production of high-quality meat products [9].

Genetic structures of populations of numerous swine breeds reared in Ukraine have been determined by cathepsin genes *CTSS*, *CTSL*, *CTSB* and *CTSK*. The informativeness and feasibility of usage of the respective genetic markers in MAS have been assessed and their association links with particular pig performance traits have been identified [11,12].

Recently, the market for commercial pig products has been increasingly supplied at the expense of foreign selected lines of swine breeds. Pietrain breed has gained considerable popularity, and today it is extensively used in crossbreeding and hybridisation schemes in commercial swine industry in Ukraine. Yet, there is no data on the cathepsin F gene association with meat quality traits in Pietrain pigs of French origin.

The aim of the study was to assess the quality of raw pork intended for further processing produced from Pietrain pigs of French origin, and also to analyse associations of genotypes, determined by single-nucleotide polymorphisms in leptin (*LEP* g.2845 *A* > *T*) and cathepsin (*CTSF* g.22 *C* ≤ *G*) genes, with meat and fat quality traits.

In order to achieve the afore-stated aim, the following **objective** was set: to study the effect of polymorphisms *LEP SNP* g.2845 A > T and *CTSF* SNP g.22 C ≤ G on physical and chemical quality attributes of pork and backfat produced from Pietrain pigs.

Research materials and methods

Meat and fat products as raw materials for further processing, produced from Pietrain pigs of French origin bred on the pig farm "Artsyz Meat Company Ltd" located in Artsyz district of Odesa region, are considered as the object of this study. The total number of pigs used to perform DNA analysis was 350 heads. DNA tests and physicochemical analyses of meat and fat products were conducted at the research laboratory of the Institute of Pig Breeding and Agricultural Production of the National Academy of Agrarian Sciences of Ukraine in Poltava.

Genomic DNA was extracted from pig bristle using the Chelex 100 ion exchange resin [11]. A PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method was used for genotyping.

The following primers were used in PCR of the *CTSF* gene: AGGGAGGGCTGGAGACGGAGTA as a forward primer and TCATTCTGGCTCAGCTCCAC as a reverse primer [12]. Restriction digestion of the PCR product DNA was accomplished by incubation of endonuclease Rsa I following the manufacturer's instructions (Thermo Fisher Scientific Baltics, Lithuania).

The following primers were used in PCR of the *LEP 2845* gene: TTGGCGAGCCTGGAGCAGT as a forward primer and GCAGCCTCCATCCCTAAGTGGG as a reverse one [13]. Restriction digestion of the PCR product DNA was accomplished by incubation of endonuclease Xba following the manufacturer's instructions (Thermo Fisher Scientific Baltics, Lithuania).

The restriction fragments were separated using 3% agarose gel electrophoresis. Visualisation of DNA fragments stained with ethidium bromide was performed using UV transilluminator.

Genotyping for *LEP* SNP g.2845 *A* > *T* and *CTSF* SNP g.22 *C* ≤ *G* was performed using a PCR-RFLP technique, taking into consideration protocols described in research papers [12-14]. The FastPCR software was employed to design primers for the PCR amplification of the target SNPs [16].

The PCR amplification was conducted via the following thermal cycling steps: an initial step at 94°C for 2 minutes, then three 40 rounds of a three-step temperature cycle – at 94°C for 30 sec, at 63°C for 30 sec and at 72°C for 40 sec. The restriction digestion of amplicons was performed following the manufacturer's protocol (Thermo Fisher Scientific Baltics, Lithuania) for each of the endonucleases used. The restriction fragments were separated using 2% agarose gel electrophoresis run at 120 V.

The allele and genotype frequencies, as well as the levels of *Ho* (observed) and *He* (expected) heterozygocity, were computed using the GenALEX 6.0 software tools and techniques described in [13] with the PIC value calculated as in [17]. The deviation of the actual distribution of genotype frequencies from Hardy-Weinberg Equilibrium was statistically evaluated using a $\chi 2$ test.

Upon the young pig stock's reaching the target live weight of 100 kg, 60 pigs in each group genotyped for the target genes were slaughtered using conventional methods [18,19]. Sampling for physicochemical tests was carried out after 24-hour ageing of half-carcasses chilled conventionally at +2–4 °C in the slaughterhouse of "Artsyz Meat Company Ltd". The samples were collected from the longissimus muscle and subcutaneous fat at the $9^{th}/11^{th}$ thoracic vertebrae location.

The assessment of physical and chemical characteristics of muscle tissue was performed in accordance with regulatory documents (ISO 2917:1999, IDT), such as the National Standard of Ukraine (NSU) ISO 2917:2001.

The following analyses of the samples were performed 48 hours after slaughter: the pH level (NSU ISO 2917:2001) was measured using a portable pH-meter pH-150M (Belarus); water-holding capacity was determined by the Grau & Hamm filter paper press method; pork tenderness was evaluated through the method of D. L. Levantin using the Warner-Bratzler shear machine [18]; colour intensity was measured with photocolorimetric technique using a KFK-3 photocolorimeter (Russian Federation) [18]; moisture loss during thermal processing (cooking loss) was calculated as a difference in the sample weight before and after heating in water bath for 50 minutes. The following parameters were measured in freshly rendered lard from subcutaneous fat layers: moisture content was determined via drying at 105^0C; the melting point was measured using a straight capillary tube of 1.5 mm diameter open at both ends with an Amarell Digital Thermometer ama-digit ad 14^{th} (Germany) [18].

The chemical composition of pork was determined following standard procedures [18] and regulations as per the National Standard (GOST) 23042-86 and 9793-74. To this end, a number of parameters were measured in pork samples as follows: the total moisture content was determined by drying at 100–105^0C; intramuscular fat was extracted by the Soxhlet method with petroleum ether as a solvent; the mineral ash content was measured after the samples had been subjected to a temperature of 450^0C in a muffle furnace; crude protein was determined using the Kjeldahl method. The energy value of pork (longissimus muscle) was derived from the chemical analyses results; it equalled 4.1 kcal per gram of protein and 9.5 kcal per gram of fat [18].

In another study, the determination of optimal values of key pork quality parameters up to standards reported in [20,21] yielded the following results: water-holding capacity 53–65%; tenderness by Warner-Bratzler shear force (WBSF) 23.55–83.49 N/cm^2; intramuscular fat content 1.2–3.3% and the melting point of backfat 29.7–42.0°C. Optimal pH levels calculated as per [21] were pH_{24} 5.6-6.2 and pH_{48} 5.2–5.8 [22].

The present study provides information about the quality of meat and backfat products as raw materials for further processing, produced exclusively from young Pietrain pigs, in the context of their distribution by SNPs in *LEP* g.2845 and *CTSF* g.22 genes.

Statistical analysis of the results was conducted with GenAlex cross-platform packages [17] run within latest versions of Microsoft Excel 2010 application using conventional methods [23].

Results of the research and their discussion

The results of the genetic structure analysis of a population of Pietrain pigs of French origin using single nucleotide polymorphisms *LEP* SNP g.2845 A > T and *CTSF* SNP g.22 C ≤ G as genetic markers have proved that, among all investigated genetic markers, it is *LEP* SNP g.2845 *A* > *T* (PIC ≥ 0.25) and *CTSF* SNP g.22 *C* ≤ *G* (PIC ≥ 0.25) that yield the informativeness PIC values which can be reckoned as optimal for association studies. Given a uniform distribution of genotypes by genetic markers of optimal informativeness in the studied pig population, all genotyped variants can be represented to the number of species sufficient for association analyses.

The PIC values optimal for association studies, which ensure a variety of genotypes to the extent required for identifying their associations with pig performance traits, range from 0.25 to 0.75. As follows from our studies performed using *LEP* SNP g.2845 and *CTSF* SNP g.22 as genetic markers, it is PIC values of Pietrain pigs that fall within the afore-specified optimal range.

Table 1 presents the results of our investigation of associations of SNPs in *LEP* g.2845 with particular parameters of carcass morphology, as well as meat and backfat quality, in Pietrain pigs of French origin.

Table 1. – The effects of the polymorphism *LEP* SNP g. 2845 A > T on physical and chemical quality attributes of pork and backfat, $\overline{X} \pm S_{\overline{x}}$

Parameters (attributes)	Genotype *LEP* SNP g.2845			P-value		
	AA	AT	TT	AA/AT	AT/TT	AA/TT
Meat quality traits:						
Water-holding capacity, %	55.8 ± 0.69	52.6 ± 0.64	51.0 ± 0.96	***	–	***
Tenderness (WBSF) N/cm^2	76.70 ± 0.31	72.56 ± 0.29	85.68 ± 0.48	–	***	*
pH_{48}	5.4 ± 0.02	5.4 ± 0.03	5.3 ± 0.02	–	**	***
Weight loss during thermal processing (cooking loss), %	17.6 ± 0.19	16.4 ± 0.31	14.8 ± 0.46	***	**	***
Total moisture, %	74.5 ± 0.21	74.6 ± 0.19	74.0 ± 0.28	–	–	–
Dry matter, %	25.5 ± 0.21	25.4 ± 0.19	26.0 ± 0.28	–	–	–
Ash, %	1.18 ± 0.016	1.20 ± 0.024	1.15 ± 0.009	–	–	–
Protein, %	22.9 ± 0.36	22.4 ± 0.29	22.8 ± 0.19	–	–	–
Intramuscular fat, %	1.4± 0.19	1.8± 0.18	2.1± 0.16	–	–	**
Calcium, %	0.19 ± 0.003	0.19 ± 0.002	0.18 ± 0.002	–	***	**
Phosphorus, %	0.38 ± 0.004	0.39 ± 0.003	0.39 ± 0.001	*	–	*
Energy value, kcal	107.2 ± 0.97	108.9 ± 1.37	111.2 ± 0.85	–	–	*
Backfat quality traits:						
Moisture content, %	7.0 ± 0.11	7.42 ± 0.16	8.51 ± 0.37	*	**	***
Melting point, ^oC	34.4 ± 0.47	35.0 ±0.51	32.9 ±0.59	–	*	**

Note: the p-value hereinafter determines the level of statistical significant difference between the genotypes from a Student's t-test: * The 1st level of significance ($p \leq 0.05$); ** The 2nd level of significance ($p \leq 0.01$); *** The 3rd level of significance ($p \leq 0.001$).

Capacity to hold water in sufficient amounts is an essential quality attribute and cooking characteristic of pork. Water-holding capacity is directly related to the presence of free water and bound water held by chemical bonds to meat proteins. It is known that sufficient content of bound water in pork contributes to its enhanced juiciness, tenderness and flavour. In this experimental study, the water-holding capacity of the pork sampled from pigs of the homozygous AA genotype for *LEP* g. 2845 was 3.2% higher ($p \leq 0.001$) than that of the samples from the heterozygous AT genotype carriers and 4.8% higher than for the pork sampled from pigs of the homozygous genotype TT.

Meat tenderness is also a meaningful quality attribute influenced by water-holding capacity, extent of autolytic processes (ageing) and the percentage of fat and connective tissues. In particular, pork with an increased percentage of connective tissue cannot be distinguished by proper tenderness and hence it requires longer time for ageing. Generally, tenderness scores of pork from Pietrain pigs are worse than those of pork from the most common swine breeds, such as Large White, Landrace, Duroc, etc., which are extensively used in the known classical schemes of genotype combinations when producing final commercial hybrids. Our investigations have not detected any consistent pattern of the association of tenderness of pork from Pietrain pigs of a given genotype for *LEP* g.2845. However, it is worth noting that, as in our previous studies, pork produced from Pietrain pigs is in general firmer as compared to similar products from other swine breeds [2]. Actual scores of tenderness of pork from Pietrain pigs are close (in genotypes AA and AT) or somewhat higher (in the genotype TT) than upper limits of the quality scoring scale [21]. As can be inferred from our research findings, more tender pork is an attribute of the heterozygous AT genotype pigs, which tend to outperform carriers of the homozygous genotype AA by 5.4% and have a significant advantage of 15.3% ($p \leq 0.001$) over carriers of the TT genotype. The difference between tenderness scores of homozygous genotypes AA and TT is 10.5% at $p \leq 0.05$.

Active acidity (pH) of meat intended for further processing is one of the key criteria for raw meat quality evaluation; it determines the rates of biochemical processes in carcass or muscle tissue and directly contributes to eating quality and technological properties of pork. The 48-hours post-slaughter pH of the pork sampled from Pietrain pigs is within the current technological standard (pH = 5.2-5.8). However, the pork from carriers of the homozygous TT genotype tends to be 0.1 units or 2.0% more acidic as compared to that of the samples from carriers of the heterozygous AT ($p \leq 0.01$) and homozygous AA ($p \leq 0.001$) genotypes.

As regards moisture loss during thermal processing (cooking loss), it has been observed that this parameter is lower for the pork samples from pigs of the homozygous genotype TT, which outperformed carriers of the heterozygous genotype AT by 1.6% ($p \leq 0.01$) and those of the homozygous genotype AA by 2.8% ($p \leq 0.001$). Moisture loss during thermal processing (cooking loss) of the pork sampled from

Pietrain pigs of the heterozygous AT genotype is 1.2% lower ($p \leq 0.001$) than that of the samples from the homozygous genotype AA carriers.

Let us discuss the chemical composition of muscle tissue, total moisture and dry matter content of pork from Pietrain pigs differentially genotyped for the gene *LEP* g.2845. There is only one trend pattern that can be traced (with a statistically significant difference between the genotypes): increased moisture amid lower dry matter content of the pork from pigs of the homozygous genotype AA. On the contrary, the dry matter content of the pork samples from pigs of the homozygous TT genotype is slightly increased, while the observed values in carriers of the heterozygous genotype AT fall in between those of the other two genotypes.

Any consistent pattern or statistically significant difference between the studied Pietrain pigs of different genotypes for the *LEP* g.2845 gene with regard to the ash content of pork has not been detected either.

The only trend observed with respect to the protein content of pork from Pietrain pigs is that carriers of the homozygous genotype AA tend to outperform pigs of the heterozygous AT genotype by 0.4% and those of the homozygous genotype TT by 0.1%.

At the same time, another trend pattern can be traced with respect to intramuscular fat content: Pietrain pigs of the homozygous TT genotype can be distinguished by somewhat increased fat content of pork, which is 0.7% ($p \leq 0.01$) and 0.3% higher than that in carriers of the homozygous AA and heterozygous AT genotypes, respectively.

The results obtained in this study can be used when performing selection of Pietrain pigs of French origin for increased intramuscular fat content. Choosing such a target trait for selective breeding will enable enhancing flavour and eating quality of commercially produced raw pork for further processing. It is especially important for pork produced from Pietrain pigs as according to sensory taste-test scores it tends to lag far behind similar products from other swine breeds.

Some statistically significant differences in calcium and phosphorus levels in pork from Pietrain pigs of one or another genotype have been established; however, no consistent pattern of associations or correlations with a particular genotype for *LEP* g.2845 have been detected.

Pork is one of the highest in calories when compared to traditional meats from livestock bred in Ukraine. An interesting fact has been revealed in this study, which is increased energy value of pork from Pietrain pigs genotyped for the gene *LEP* g.2845. The energy value of the pork sampled from carriers of the homozygous TT genotype is 4.0 kcal or 3.7% higher ($p \leq 0.05$) than that of the samples from pigs of the homozygous AA genotype and 2.3 kcal or 2.1% higher than the energy value of the pork from the heterozygous AT genotype pigs. Therefore, as observed, *LEP* SNP g.2845 A > T tends to affect the energy value of pork, which is mainly due to the differences in intramuscular fat content.

Quality attributes of backfat are quite important for domestic customers. Besides, the backfat quality attributes that we have taken into consideration in this study – namely, moisture content and melting point – play an essential role in terms of shelf life and technological properties of products. The investigation of backfat quality attributes has shown that the lowest backfat moisture content is observed in carriers of the homozygous genotype AA; this parameter is 1.51% ($p \leq 0.001$) and 1.09% lower ($p \leq 0.01$) lower than that of the backfat from the homozygous TT and heterozygous AT genotype pigs. At the same time, the higher-moisture backfat from carriers of the homozygous TT genotype has the lowest melting point, which tends to be 2.1 ^{o}C or 6.0% ($p \leq 0.01$) and 1.5 ^{o}C or 4.0% ($p \leq 0,05$) lower than that of the backfat from pigs of the heterozygous AT and homozygous AA genotypes, respectively.

Therefore, we have detected several statistically significant associations of the genetic marker *LEP* SNP g.2845 A > T with the target quality traits of meat and fat products, in particular intramuscular fat content, water-holding capacity, meat tenderness, backfat moisture content and melting point (Table 1). In general, the results obtained in this study are consistent with findings reported by other Ukrainian [4] and foreign authors [12]; however, the mentioned studies, in particular those conducted by Ukrainian researchers in Poltava region, focused mainly on Large White pigs and their reaching the target live weight of 120 kg at slaughter.

Table 2 presents the results of the study of associations of SNPs in the cathepsin F gene (*CTSF* SNP g.22 C ≤ G) with particular parameters of carcass morphology, as well as meat and fat quality, in Pietrain pigs of French origin. The lowest water-holding capacity of 51.2% is observed in pigs of the GG genotype. This parameter is 0.7% and 2.4% higher ($p \leq 0.01$) in carriers of the homozygous genotype CC and heterozygous genotype GC, respectively. In carriers of the heterozygous genotype GC, it is 1.7% higher than in pigs of the homozygous CC genotype ($p \leq 0.05$).

No difference has been detected in meat tenderness between the heterozygous GC genotype carriers and pigs of the GG genotype. Pigs of the GG and GC genotypes have slightly better meat tenderness as compared to carriers of the homozygous CC genotype, for which this parameter is 4.38% lower ($p \leq 0.01$) than in the other two genotypes.

No difference has been found in the acidity (pH) of pork between the studied genotypes.

Table 2. – The effects of the polymorphism *CTSF* SNP g.22 *C* ≤ *G* on physical and chemical quality attributes of pork and backfat, $\overline{X} \pm S_{\overline{x}}$

Parameters (attributes)	Genotype *CTSF* SNP g.22			P-value		
	GC	CC	GG	GC/CC	CC/GG	GC/GG
Meat quality trait:						
Water-holding capacity, %	53.6 ± 0.59	51.9 ± 0.48	51.2 ± 0.64	*	–	**
Tenderness (WBSF) N/cm^2	78.77 ± 0.11	82.23 ± 0.12	78.77 ± 0.15	**	**	–
pH_{48}	5.4 ± 0.02	5.4 ± 0.03	5.4 ± 0.01	–	–	–
Weight loss during thermal processing, %	20.2 ± 0.42	18.1 ± 0.38	20.9 ± 0.72	***	***	–
Total moisture, %	74.6 ± 0.24	75.1 ± 0.29	75.0 ± 0.34	–	–	–
Dry matter, %	25.4 ± 0.24	24.9 ± 0.29	25.0 ± 0.34	–	–	–
Ash, %	1.18 ± 0.01	1.16 ± 0.02	1.17 ± 0.02	–	–	–
Protein, %	22.7 ± 0.10	22.3 ± 0.11	22.8 ± 0.18	**	*	–
Intramuscular fat, %	1.5 ± 0.11	1.4 ± 0.10	1.0 ± 0.09	–	**	***
Calcium, %	0.19 ± 0.002	0.19 ± 0.003	0.18 ± 0.001	–	**	***
Phosphorus, %	0.37 ± 0.004	0.38 ± 0.002	0.39 ± 0.003	*	**	***
Energy value, kcal	107.3 ± 0.59	104.7 ± 0.53	103.0 ± 0.45	**	**	***
Backfat quality trait:						
Moisture content, %	7.3 ± 0.24	7.3 ± 0.18	6.8 ± 0.21	–	–	–
Melting point, ^{o}C	34.6 ± 0.43	34.7 ±0.55	33.0 ±0.51	–	*	*

The minimum moisture loss during thermal processing (cooking loss), which is obviously the most desired value, has been detected in the pork sampled from pigs of the homozygous CC genotype; this parameter is 2.1% ($p \leq 0.001$) and 2.8% lower ($p \leq 0.001$) than in the pork samples from carriers of heterozygous GC and homozygous GG genotypes, respectively.

The conducted analyses of the total moisture, dry matter and ash content of pork have not detected any statistically reliable difference between Pietrain pigs of a given genotype for *CTSF* SNP g.22 C ≤ G.

The protein content of the pork sampled from carriers of the homozygous GG genotype tends to be higher than in other genotypes; in particular, it is 0.5% higher ($p \leq 0.05$) than in the samples from pigs of the homozygous CC genotype and 0.1% higher than in the heterozygous GC genotype pigs. The crude protein level of the pork sampled from carriers of the heterozygous GC genotype is 0.4% ($p \leq 0.01$) higher than in the samples from pigs of the homozygous CC genotype, which is to a large extent associated with the effect of the G allele on the protein content of pork from Pietrain pigs.

As mentioned above, intramuscular fat content is an essential contributor to flavour compounds, eating quality and increased energy value of pork. A consistent trend for increased fat content has been traced in carriers of the homozygous CC genotype, in which this parameter is 0.4% higher ($p \leq 0.01$) than in pigs of the homozygous genotype GG. At that, intramuscular fat content of the pork sampled from carriers of the heterozygous genotype GC is at the maximum level, which is 0.5% higher ($p \leq 0,001$) than that of the pork from the homozygous CC genotype carriers. In our opinion, it is the presence of the C allele that is associated with this attribute.

With respect of the calcium level in pork, a statistically reliable advantage of carriers of the homozygous CC genotype over pigs of the homozygous GG genotype can be traced, with the former outperforming the latter by 0.01% ($p \leq 0.01$). As regards the phosphorus level in pork, there is a statistically reliable advantage of 0.01% ($p \leq 0.01$) of carriers of the homozygous GG genotype over the homozygous CC genotype carriers.

In Pietrain pigs genotyped for *CTSF* SNP g.22 *C* ≤ *G*, the pork samples from carriers of the homozygous CC genotype have an increased energy value, which is 1.7 kcal or 1.7% higher than for the pork samples from pigs of the homozygous genotype GG. The energy value of the pork sampled from the heterozygous GC genotype pigs is 4.3 kcal or 4.2% higher.

The lowest backfat moisture content has been recorded in carriers of the homozygous GG genotype; this parameter tends to be 0.5% lower than in the homozygous CC genotype carriers and similar to that in carriers of the heterozygous GC genotype. The lowest-moisture backfat in carriers of the homozygous GG genotype has the lowest melting point, which tends to be 1.7^{o}C or 5.0% ($p \leq 0.05$) lower than in the homozygous CC genotype pigs and 1.6 ^{o}C or 4.9% ($p \leq 0.05$) lower than in the heterozygous GC genotype pigs.

Therefore, *CTSF* SNP g.22 C ≤ G appears to be quite promising as a genetic marker associated with the quality of pork from Pietrain pigs, which has been substantiated by the findings of this study. In particular, it has been proved that *CTSF* SNP g.22 C ≤ G is associated with such pork quality attributes as water-holding capacity, tenderness, moisture loss during thermal processing (cooking loss), intramuscular fat content, calcium and phosphorus levels, and energy value.

Summarising the above, each of the two investigated genetic markers, which are polymorphic

genetic variations in Pietrain pigs, are associated with particular quality attributes of meat and fat products of the studied sub-population of Pietrain pigs of French origin.

In general, modern swine breeds that have been genetically improved for enhanced growth rates and lean meat deposition differ from domestic swine breeds in the rates of fat deposition, fat quality, specific features of lipid metabolism and other characteristics [1]. It is especially pronounced in the Pietrain breed as it is considered to be an ultra-muscular breed.

Taking into account physiological importance of the leptin and cathepsin F gene functions in the process of muscle tissue formation, as well as in intramuscular and subcutaneous fat deposition, it is likely that these genes are associated with the quality of raw meat for further processing. However, it is pretty unlikely that each of the polymorphic markers located at a given gene should be associated with the traits controlled by this gene. If MAS is based on a causative polymorphism called the Quantitative Trait Nucleotide (QTN), then such a genetic marker is reckoned as optimal, because it directly contributes to the total variation in the target quantitative trait. Vice versa, other polymorphisms may be characterised by a specific physical linkage to QTNs and thus can be not associated with a given trait at all.

Scientific research in swine breeding has proved that genetic markers within leptin and cathepsin genes are associated with a number of quality attributes of meat and fat products. Yet, there has been no reliable answer to the questions regarding whether these markers can be classified as QTNs or whether they are physically linked to QTNs and hence can be only segregated together in the studied sub-populations. In either case, there is quite a prospect that these markers will be used in marker-assisted selection of swine in order to produce meat and fat products as raw materials of the desired quality.

The results obtained and discussed in this study are fully consistent with research findings reported by other authors [3,6,24-32].

Conclusion

1. The results of genetic association studies performed in a sub-population of Pietrain pigs of French origin suggest that polymorphisms in leptin and cathepsin F genes produce an effect on performance traits, in particular on specific pork and backfat quality attributes. The prospects of adopting SNPs in *LEP* g.2845 A > T and *CTSF* g.22 C > G as genetic markers in marker-assisted selection in swine breeding are promising. Genome-wide association studies in other swine breeds can yield new associations of these markers with carcass, meat and fat quality traits.
2. Several statistically significant associations of the genetic marker *LEP* SNP g.2845 A > T with the studied quality attributes of meat and fat products, in particular water-holding capacity, meat tenderness, intramuscular fat content, backfat moisture content and melting point, have been detected.
3. It has been established that the genetic marker *CTSF* SNP g.22 C ≤ G is associated with such pork quality attributes as water-holding capacity, tenderness, weight loss during thermal processing, intramuscular fat content, calcium and phosphorus levels, and energy value.
4. The results obtained in this study can be used when performing selection of Pietrain pigs for increased intramuscular fat content, thus improving flavour compounds and eating quality of pork from Pietrain pigs, which, according to sensory taste-test scores, tends to lag far behind similar products from a number of other swine breeds.

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ЯКІСТЬ СВИНИНИ ТА ГЕНЕТИЧНИЙ АНАЛІЗ АСОЦІАЦІЙ ПОЛІМОРФІЗМІВ В ГЕНАХ ЛЕПТИНУ І КАТЕПСИНУ F СВИНЕЙ

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Анотація. Мета роботи – дослідити якість свинини, як вихідної сировини для переробки. Провести аналіз асоціацій генотипів з показниками якості м'яса і сала з урахуванням генетичної належності тварин за однонуклеотидними поліморфізмами (SNP) генів лептину (*LEP* g.2845 A > T) і катепсину F (*CTSF* g.22 C ≤ G). Об'єктом дослідження була м'ясо-сальна сировина, що одержували від свиней французького походження, що належали ТОВ «Арцизька м'ясна компанія» Арцизького району Одеської області. Загальна численність поголів'я, яку було задіяно для ДНК аналізу, склала 350 голів. ДНК дослідження та фізико-хімічні дослідження м'ясо-сальної продукції виконані в умовах наукових лабораторій Інституту свинарства та АПВ НААН України (м. Полтава). ДНК виділяли із щетини з використанням іонообмінної смоли Chellex-100. Генотипування проводили методом ПЛР-ПДРФ (полімеразна ланцюгова реакція, поліморфізм довжин рестрикційних фрагментів). Відгодівельне поголів'я оцінювалося за генами лептину (*LEP* g.2845) та катепсину F (*CTSF* g.22). По досягненню молодняком свиней живої маси 100 кг проводили контрольні забої по 60 голів кожної генетичної групи за загальноприйнятими методиками. Проведені генетичні і асоціативні дослідження свідчать про вплив поліморфізмів генів лептину і катепсину *F* на окремі параметри якості їх м'яса та сала. Встановлено статистично підтверджені асоціації генетичного маркера *LEP* SNP g.2845 A > T з досліджуваними показниками якості м'ясо-сальної продукції, а саме, із вологоутримуючою здатністю, ніжністю м'яса, вмістом внутрішньом'язового жиру, вмістом вологи в салі та температурою його плавлення. Поряд з цим, генетичний маркер *CTSF* SNP g.22 C ≤ G був асоційований з такими показниками якості м'яса свиней, як вологоутримуюча здатність, ніжність, втрати вологи за термічної обробки, вмістом внутрішньом'язового жиру; вмістом кальцію, фосфору та енергетичною цінністю м'яса

Ключові слова: свинина, якість, м'ясо-сальна продукція, генетичні маркери, SNP, LEP g.2845, CTSF g.22