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LIPID COMPOSITION OF MUSCLE AND FAT TISSUES OF DUROC PIGS (*Sus scrofa domesticus* Erxleben, 1777) — FEATURES AND CORRELATIONS

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Abstract

A key direction in animal genetics and animal breeding is currently the study of metabolic characteristics of animals in combination with their genotyping, which leads to the development of the new specific markers for prediction the individual phenotypic characteristics of animals based on correlation studies. This would further make possible to create new methods for animal phenotyping using lipid analysis techniques to assess the complex effect of environmental and genetic factors (D.P. Lo Fiego et al., 2002; D.P. Lo Fiego et al., 2005; R. Rossi et al., 2002). Data obtained as a result of the analysis of the genetic features of the animal and its metabolic characteristics make it possible to create predictive models for accurate phenotyping of animals and their offspring. In this work we have for the first time performed the comparative non-targeted mass-spectrometry study of the lipid composition of muscle and adipose tissue in Russian Duroc pigs using positive ion registration mode. The aim of the work was to carry out lipidomic analysis of the pig muscle and adipose tissue as an input for predictive models for animal phenotyping. The study was carried out on the samples of adipose and muscle tissue collected post-mortem from 150-180 day-old Duroc boars (n = 9). Samples were taken from three regions of the longest back muscle, three regions of the biceps femoris, and two regions of subcutaneous dorsal fat (72 samples in total). Analysis of the lipid compounds was performed by liquid chromatography coupled with high-precision time-of-flight mass spectrometry, preceded by the methyl tert-butyl ether and methanol extraction of lipids. Type of ionization used was electrospray. A total of 844 mass spectrometry peaks satisfied the quality criteria and were used for the statistical analysis. Peaks were annotated using the LIPID MAPS database search (http://www.lipidmaps.org), with an accuracy of 10 ppm. Statistical analysis shows significant differences in the Pearson correlation for adipose and muscular tissue samples compared or the same tissue samples compared. Correlation coefficients between lipid patterns of adipose and muscular tissue samples are lower (from 0.48 to 0.86, r = 0.69 on average with 95 % confidence interval from 0.61 to 0.79). Correlation coefficients between lipid patterns in two samples of muscle or adipose tissue are higher (from 0.73 to 0.99, r = 0.93 on average with 95 % confidence interval from 0.86 to 0.97). Unpaired t-test shows differences at p-value < 0.01 Data clustering confirms the difference between muscle samples and subcutaneous fat samples. The main classes of lipids detected in the samples were triglycerides (TAG), diglycerides (DAG), phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylserines (PS), phosphatidic acids (PA), phosphatidylinositols (PI), and lysophosphatidylcholines (LPC). We have found that adipose tissue samples are enriched in triacylglycerols, while muscle tissue samples are enriched in phospholipids. To summarize, we have identified the main lipid types present in different regions of muscle and adipose tissue of pigs, and revealed the similarities and differences in the lipid composition between the two analyzed tissue types, as well as between two different types of

muscles (biceps femoris and longisimus dorsi), and also between muscle and fat tissues. Considering the results obtained in this work we may conclude that liquid chromatography coupled with high-precision time-of-flight mass spectrometry efficiently produces accurate and reproducible lipidomes data. These data may be used in animal breeding, in the search for new genetic markers associated with economically important traits and in breeding programs to evaluate the traits determined by lipid composition.

Keywords: lipidome, animal phenotyping, *Sus scrofa domesticus*, pigs, Duroc boars, high-performance liquid chromatography, mass spectrometry, muscle tissue, adipose tissue

The development of molecular-genetic methods opens up new possibilities for accelerating animal breeding. Since the 2000s, genomic selection has been replacing traditional selection on the basis of BLUP-AM (Best Linear Unbiased Prediction — Animal Model) [1]. The introduction of a genomic assessment system in dairy cattle breeding increased the accuracy of predicting the breeding value of young animals by 15-25 % [2]. In modern animal breeding, the technologies of genotyping of tens and hundreds of thousands of single nucleotide polymorphisms (SNPs) are developed and applied; however, the question of their relationship with certain phenotypic traits often remains open [3]. The synthesis of the most important compounds in the body of an animal directly depends on the presence of mutations in the genes of the corresponding enzymes. In turn, enzyme activity may be associated with certain SNPs and manifest itself in the relative amount of metabolites of a certain type. Often, the function of a defective enzyme is performed by another related enzyme based on the compensating biochemical reactions. A study on the diversity and representation of metabolites in biological samples will help to clarify the issue of the participation of certain SNPs in regulating the activity of biochemical processes in an organism [4], and in the future, to identify associations between SNPs and certain economically valuable traits. This is the reason for the importance of modern high-performance technologies of metabolome research for the development of genomic breeding methods in animal farming.

In pigs, the metabolites that are involved in the formation of the main physicochemical and organoleptic properties of muscle and adipose tissue, which form the indicators of the quality of meat raw materials, are of the greatest interest [5]. The quality and nutritional value of meat depend on its chemical composition influenced by both genetic and paratypical factors. The nutritional value of pork is determined by the fatty acid composition, in particular, the quantitative ratio of free and bound long-chain fatty acids in muscle and adipose tissue.

The composition of intramuscular fat is influenced by several main factors: genetic characteristics, sex, the ratio of body weight and age of an animal, and the diet composition [6]. In pigs, the lipid composition of the tissues, reflecting the phenotypic characteristics of a specimen, directly depends on the method of animal feeding and the individual characteristics of the digestion of the fodder [7]. The composition of the diet also has a great influence [8]. For example, an increased content of polyunsaturated fatty acids in the fodder may increase their content in muscle tissue [9]. It is known that linoleic and linolenic acids coming with the fodder are metabolized in the liver with the formation of polyunsaturated fatty acids that are sensitive to the oxidative process. These changes in the fatty acid composition of intramuscular fat, due to the composition of the diet, can increase the sensitivity of meat to the effects of oxidizing agents [9]. The lipid composition of muscle and adipose tissue, which determines the qualitative characteristics of meat, can be considered as a unique individual characteristic of an animal, reflecting not only its genetic predisposition to assimilate and synthesize lipids but also phenotypical signs [10, 11].

The pool of lipids (lipidome) presented in the muscle and adipose tissue of pigs, in addition to the known fatty acids, includes cholesterol esters, triacyl-,

diacyl- and monoacylglycerols, free cholesterol and its intermediates, as well as various classes of phospholipids, including phosphatidylethanolamines, etc. [12]. It should be noted that the effect of the characteristics of absorbing fatty acids on the composition of muscle and fatty lipidomes is most reflected in the phospholipid fraction, as well as in the length and degree of saturation of fatty acid chains in triacylglycerides [12-14].

To assess the phenotypic lipid and other metabolic characteristics in mammals, the methods of nuclear magnetic resonance spectroscopy and gas or liquid chromatography combined with mass spectrometry detection are most often used [3, 15-18]. Attention is paid to mass spectrometry as the main method of lipid detection, and in particular liquid chromatography coupled with mass spectrometry which makes it possible to simultaneously identify up to several tens of thousands of compounds in a single biological sample [19].

The current trends in selection research in modern pig breeding are assessing the metabolic characteristics of animals in combination with whole-genome genotyping, detecting specific markers to predict an individual phenotype based on the identified correlations, and developing the methods of phenotyping using lipid analysis technologies to assess the complex influence of factors causing phenotypic features of an animal [7, 20, 21]. A quantitative analysis of lipids in the muscle and adipose tissue of pigs in combination with genotyping can be the basis for determining the genes associated with the lipid composition of tissues, which will optimize selection (in particular, for meat quality traits).

In this work, we for the first time applied liquid chromatography with mass-spectrometric detection in the registration mode of positive ions for a detailed study of the lipid profiles of muscle and adipose tissue (molecular phenotyping) in Duroc pigs of domestic reproduction. The similarities and differences in the composition and content of lipids between the studied tissue types were determined; in particular, it was shown that the samples of muscle tissue are enriched in phospholipids, whereas an increased content of triacylglycerols is typical of adipose tissue.

The goal of the study is the lipid analysis of muscle and adipose tissue of pigs using ultra-high performance liquid chromatography—high-precision time-of-flight mass spectrometry (UPLC-MS) to further create predictive models of animal phenotyping.

Techniques. In Duroc boars of domestic reproduction grown on automated feeding stations GENSTAR (Cooperl, France) (CGTs OOO, Verkhnaya Khava, Voronezh Region) and aged 150-180 days (n = 9, carcass weight 89.4±5.67 kg), tissue samples were taken post mortem from the longest back muscle at three points (the 5th-6th rib, LM1; 8th-9th rib, LM2; belt, LM3), biceps femoris at three points (upper part, BF1; middle part, BF2; lower part, BF3), as well as subcutaneous dorsal fat at two points (the 5th-6th ribs, inner and outer layers, respectively scat1 and scat2). A total of 72 samples were analyzed. The tissues were dissected no later than 10 min after the slaughter of an animal. To reduce the activity of enzymes, the samples were taken at a temperature not higher than 4 °C and immediately frozen in liquid nitrogen vapor (–196 °C). The biomaterial was stored and transported at a temperature not higher than –80 °C. For the analysis, 130-145 mg of muscle tissue and 50-65 mg of adipose tissue from each point were used.

The solutions used during extraction were preliminary cooled to 0 °C. The extraction method is based on the use of mixtures of methyl tert-butyl ether (MTBE) with methanol (MeOH) (3:1, v/v) and methanol with water (1:3, v/v) (Scharlau, Spain). Oleic acid $^{13}C_{18}$, palmitic acid $^{13}C_{16}$, stearic acid $^{13}C_{18}$ (Sigma-Aldrich, Germany); 15:0-18:1-d7-diacylglycerol, cholesterol (D7) (Avanti

Polar Lipids, Inc., USA) were isotope-labeled internal standards added at a concentration of 3 μg/ml to the MTBE:MeOH solution. MTBE:MeOH extraction mixture (1000 μl) was added to the sample aliquots, and the tubes were placed in a Precellys® Evolution homogenizer cooled to 7 °C (Bertin Technologies, France). Three cycles of 30 s at 10000 rpm with a 10 s break were used to homogenize muscle tissues. The samples were vortexed for 5 s, transferred to an ultrasonic bath filled with ice (Sonorex Super RK 103 H, BANDELIN electronic GmbH & Co. KG, Germany) and kept for 30 min at 3000 rpm and 4 °C; then the ultrasound treatment was repeated. From each tube (Precellys, Bertin Technologies, France), the liquid phase was carefully recovered and transferred to new tubes. The MeOH:H_2O extraction mixture (700 μl) was added to each tube and vortexed for 5 s. The samples were centrifuged for 10 min at 15000 rpm and 4 °C. After the centrifugation, 200 μl were recovered from the upper phase into the Safe-lock tubes (Eppendorf AG, Germany). The samples were dried with an open lid for 1.5 h in a vacuum centrifuge concentrator (Concentrator plus, Eppendorf AG, Germany) at 30 °C and 1400 rpm (vacuum 20 hPa, V-HV mode). The dry extracts were stored at a temperature not higher than –80 °C.

To prepare the extracts for mass spectrometry analysis, 200 μl of acetonitrile:isopropanol mixture (70:30, v/v) (LC-MS purity) were added to each sample and vortexed for 5 s. The samples were placed in an ultrasonic bath for 10 min. Then they were shaken at 4 °C and 3000 rpm for 10 min. The solutions were centrifuged for 10 min at 15000 rpm and 4 °C. The obtained lipid extracts were diluted by adding acetonitrile:isopropanol mixture (70:30, v/v) (1:50 for muscle tissue and 1:100 for adipose tissue). Prior to the analysis, the 100 μl aliquots of the resultant solutions were transferred to mass spectrometry vials and loaded into the autosampler of a chromatograph.

The lipid extracts were analyzed using UHPLC/Q-TOF-MS, an ultra-high performance liquid chromatography (UPLC) (Acquity I-class, Waters, USA) coupled to a high-resolution time-of-flight mass spectrometry Q-TOF (Maxis Impact II, Bruker Daltonik GmbH, Germany). To separate the extracts, the reversed-phase chromatography was used (an analytical column Acquity UPLC BEH C8 2.1×100 mm, the size of particles 1.7 μm, Waters, USA; a guard column Acquity UPLC BEH C8 VanGuard 2.1×5 mm, 1.7 μm, Waters, USA). The eluent A was 0.1 % formic acid, 10 mM ammonium acetate and 100 % water (LC-MS grade), the eluent B was 0.1 % formic acid, 10 mM ammonium acetate in the acetonitrile:isopropanol (70:30, v/v) (LC-MS grade). The eluent gradient was as follows: 0 min — 45 % A, 1st min — 45 % A, 4th min — 20 % A, 12th min — 15 % A, 15th min — 0 % A, 19.50 min — 0 % A, 19.51 min — 45 % A, 24th min — 45 % A (A + B = 100 %), at a flow rate of 0.4 ml/min. The analytical column was thermostated (60 °C), the autosampler temperature was 4 °C; the volume of injected samples was 3 μl.

Electrospray was used to ionize the substance and deliver it to the mass spectrometer. The drying and spraying gas was N_2, supplied by the generator (flow rates of 2 and 6 l/min, respectively); the voltage of the ionizing spray was 4000 V, with nitrogen was used as the collision gas in the collision cell at a pressure of 0.04 bar. The desolvation line temperature was 180 °C, the radio-frequency of the ion focusing was 300 Vpp in the focusing funnels and 1000 Vpp in the collision cell, the ion accumulation time was 10 ms, and the ion transmission time was 45 ms. The ion spectra were recorded in positive and negative full-scan mode. Mass spectrometry data was processed using the functions from the specialized software packages IPO [22], XCMS [23] and Camera in the R programming language (http://www.r-project.org).

To analyze the mass spectrometry data, the Pearson correlation, Stu-

dent's *t*-test (identifying differences for two samples of the same size with the assumption of equal variances), and the Benjamini-Hochberg method [24] were used to reduce the number of false-positive results for multiple testing. According to the Benjamini-Hochberg method, for p_1, p_2, ..., p_M with the number of conducted tests M, without loss of generality, we can take $p_1 \le p_2 \le ... \le p_M$. Taking a chosen significance level Q, the critical value $c_i = (iQ/M) \cdot N$ is calculated for each p_i and the largest p-value p_k ($p_k < c_k$) is found. Then all p_i for $i \le k$ are considered statistically significant even at $p_i > Q$.

Using the principal component analysis, the clusters of similar samples were identified. The principal component analysis [25] allows constructing a linear transformation that transmits the data into the space with a smaller dimension, where the new coordinate axes are called the main components. The first main component was chosen so that the data dispersion was maximum along it, and the second one was orthogonal to the first one with the maximum possible dispersion along the axis. The subsequent components are chosen in such a way as to be orthogonal to all previous components with maximum data dispersion along them. To calculate the main components, the "scikit-learn" package was used of the version 0.19.1 for the Python 3.5 programming language.

Results. A schematic representation of the sampling points and the results of the studies of their lipid composition are presented in Figure 1, A. A total of at least 10000 chromatographic and mass-spectrometric peaks were detected, each of which can correspond to specific lipid compound.

The peaks were annotated with an accuracy of 10 ppm using the LIPID MAPS database (http://www.lipidmaps.org) [26]. For this, the M+H, $M+NH_4$, M+Na adducts were chosen. A total of 1397 peaks were annotated by at least one lipid, many peaks received multiple annotation, the maximum number of lipids assigned to one peak was 134. In the subsequent work, we used only annotated peaks. After the annotation and filtration of peaks by retention time in the chromatographic column (from 0.6 to 19 min), as well as the exclusion of probable contaminants, 844 peaks remained.

The comparison of lipid profiles showed significant differences between the adipose and muscle tissue samples, whereas the differences between the two different muscle or adipose tissue samples were not significant. The values of the Pearson correlation coefficients for lipid composition for all pairs of samples are presented in Figure 1, B. The dark left upper square and lower right squares show that muscle tissue was characterized by a strong correlation in the pairs of the samples. The same is true for adipose tissue. The correlations found when comparing different types of tissues are significantly weaker. Thus, the correlation coefficients between the pairs of samples from the same tissue (muscle tissue samples were combined into one group) were, on average, 0.93 (the range is 0.73-0.99; 95 % confidence interval is from 0.86 to 0.97). When comparing the pairs of samples of different tissues, the correlation coefficients on average amounted to 0.69 (the range is 0.48-0.86; 95 % confidence interval is from 0.61 to 0.79). The confidence intervals do not overlap; therefore, the correlations between the samples of the same tissue along the lipid profiles are significantly stronger than between the samples of different tissues. The unpaired Student's test also showed that the correlations of lipid composition in the same and in different tissues differ at $p < 0.01$.

Figure 2, A shows the first two main components. It is seen that the samples are grouped in two clusters, i.e. LM + BF and scat1 + scat2, which are linearly separable. This confirms the differences in muscle and adipose tissue samples in the lipid composition.

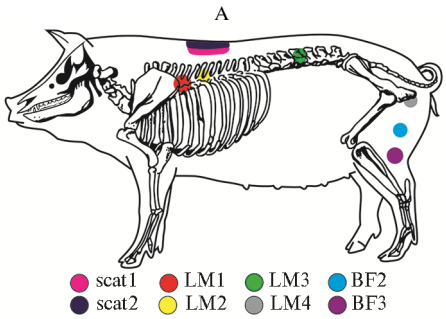
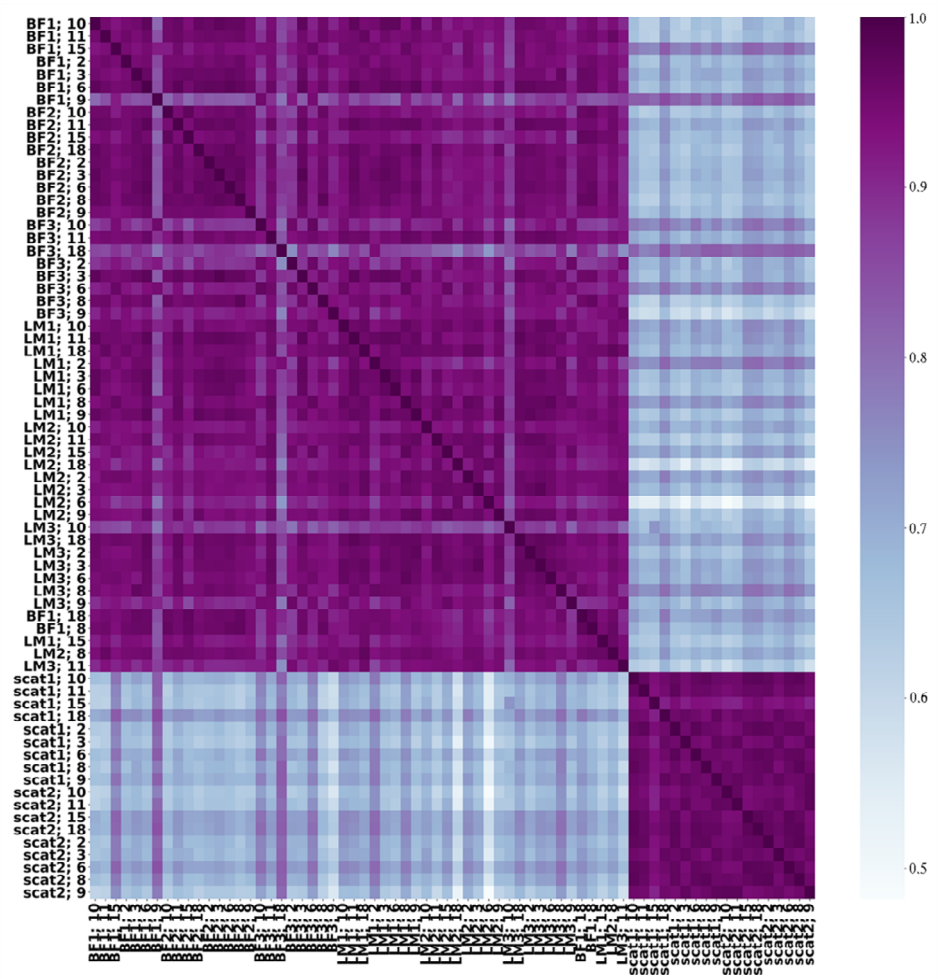


Fig. 1. Lipid composition of tissues in Duroc boars (n = 9, weight 89.4±5.67 kg, age 150-180 days). A: The pattern of sampling for analysis (scat1 and scat2 — subcutaneous dorsal fat, the upper and lower layer, respectively; BF1, BF2 and BF3 — biceps femoris above the back, in the middle part and by a knee; LM1, LM2 and LM3 — the longest muscle between the 5th-6th, 8th-9th vertebrae and at the base of the tale). B: Calculation of the Pearson correlations (r) for the pairs of samples of adipose and muscle tissue (the intensity of violet color reflects the strength of the positive correlation, blue is a weak correlation, white is zero). The r values corresponding to the scale of the intensity of color are indicated in the right. The lipid composition was analyzed by liquid chromatography—time-of-flight mass spectrometry (Acquity I-class, Waters, USA, Maxis Impact II, Bruker Daltonik GmbH, Germany).



We annotated the main groups of lipids: tri- and diacylglycerols (TAG and DAG), phosphatidylcholines (PC, including LPC lysophosphatidylcholines), phosphatidylethanolamines (PE), phosphatidylserines (PS), phosphatidic acids (PA), and phosphatidylinositols (PA) and phosphatidylinositols (PI) for all samples of adipose and muscle tissue (see Fig. 2, B). As it turned out, the adipose tissue samples are enriched in triacylglycerols, and muscle tissue in phospholipids.

The graph of m/z vs. the exit time (Fig. 3, A) shows the differences in the intensity of the peaks of different lipids. The size of the dots on the graph corre-

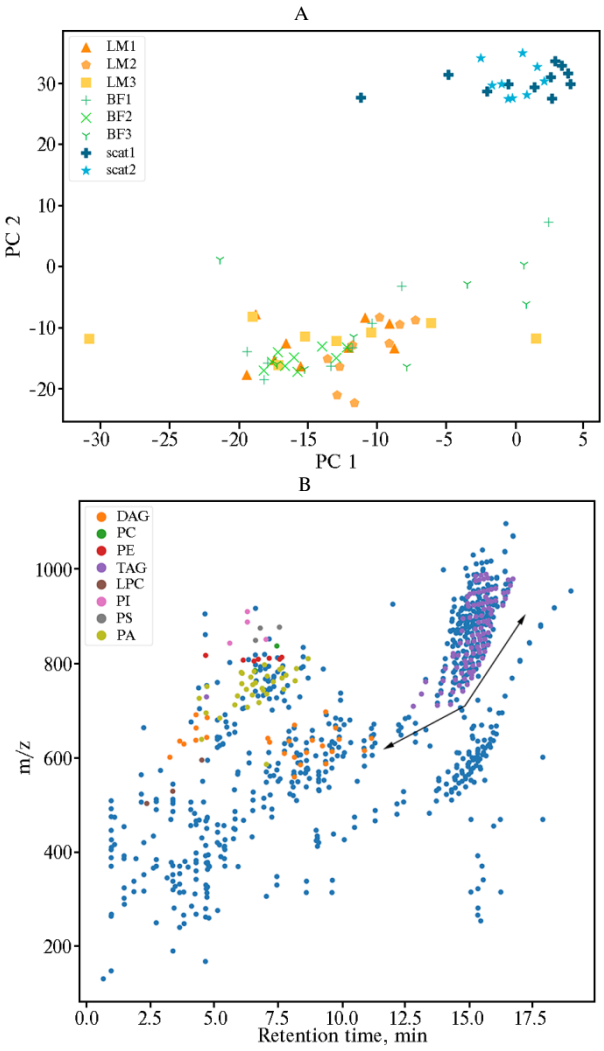


Fig. 2. Clusterization of adipose and muscle tissues sampled from Duroc boars (n = 9, weight 89.4±5.67 kg, age 150-180 days) **according to the lipid composition:** A — principal component analysis (the first two components are shown, see the *Techniques* section), B — distribution of m/z over the time of exit for the main groups of lipids; scat1 and scat2 — subcutaneous dorsal fat, the upper and lower layer, respectively, BF1, BF2 and BF3 — biceps femoris above the back, in the middle part and by a knee, LM1, LM2 and LM3 — the longest muscle between the 5th-6th, 8th-9th vertebrae and at the base of the tail; TAG — triacylglycerols, DAG — diacylglycerols, PC — phosphatidylcholines, PE — phosphatidylethanolamines, PS — phosphatidylserines, PA — phosphatidic acids, PI — phosphatidylinositols, LPC — lysophosphatidylcholines. The arrows indicate the direction of the lengthening of the chain (right, up) and increasing the number of double bonds (left, down). The point corresponds to one sample (B). The measurements were performed by liquid chromatography—time-of-flight mass spectrometry (Acquity I-class, Waters, USA, and Maxis Impact II, Bruker Daltonik GmbH, Germany).

sponds to the average peak intensity for all samples. To find the differences between the tissues, a multiple t-test was used. For each pair of tissues and for each peak, the p-value for the hypothesis of equal averages was calculated. The p-values were adjusted for multiple testing in accordance with Benjamini-Hochberg, the peaks with corrected p-values less than 0.05 were considered statistically significant (see Fig. 3, B). It can be seen that the samples of adipose tissue did not differ significantly from each other, but were significantly different from the samples of muscle tissue. Although in general the differences between muscle and adipose tissue prevailed, some lipids were also statistically significantly different in the two muscles, BF2 and LM2.

The differences between muscle and adipose tissue for all filtered peaks were estimated by the natural logarithm of the ratio between the peak intensity for the averaged muscle and the averaged adipose samples (Fig. 4, A). The differences between the biceps tissues (BF) and the longest muscle (LM) were assessed in a similar way (see Fig. 4, B).

The results we obtained show the effectiveness of liquid chromatography combined with high-resolution time-of-flight mass spectrometry in molecular phenotyping of farm animals, in particular, pigs.

In general, the fatty acid composition only indirectly reflects the fat composition of meat and is insufficient to describe its lipid components [6, 27]. Mass spectrometric analysis in the mode of positive ions gives a more detailed

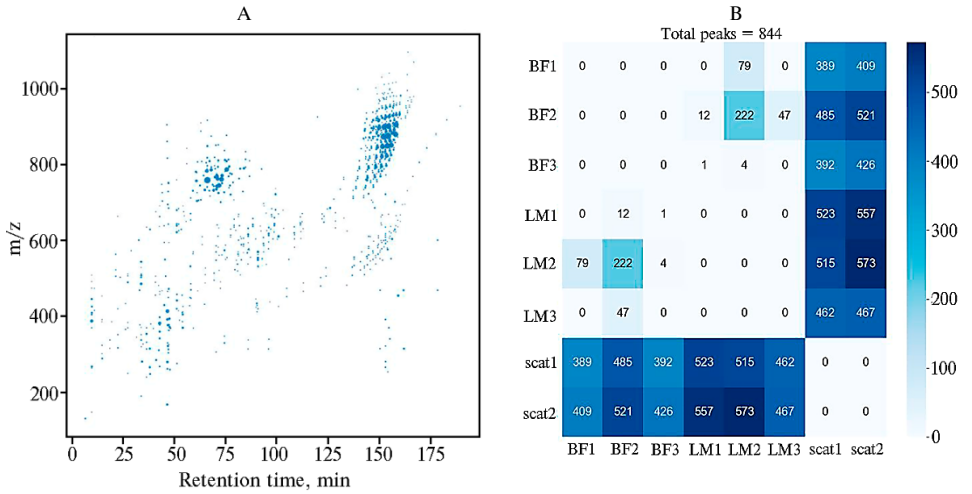


Fig. 3. The diversity of the lipid composition of muscle and adipose tissues in Duroc boars (n = 9, weight 89.4±5.67 kg, age 150-180 days): A — the distribution of m/z vs. the exit time (size of dots corresponds to the average peak intensity for all samples); B — the table reflecting the number of statistically significantly different peaks for the pairs of tissues (at p = 0.05), estimated by Student's *t*-criterion with the Benjamini-Hochberg amendment. The total number of estimated peaks is 844. The intensity of the blue color corresponds to the number of differing peaks, white is the absence or the minimum number of such peaks; scat1 and scat2 — subcutaneous dorsal fat, the upper and lower layer, respectively, BF1, BF2 and BF3 — biceps femoris above the back, in the middle part and by a knee, LM1, LM2 and LM3 — the longest muscle between the 5th-6th, 8th-9th vertebrae and at the base of the tail. The measurements were performed by liquid chromatography—time-of-flight mass spectrometry (Acquity I-class, Waters, USA, Maxis Impact II, Bruker Daltonik GmbH, Germany).

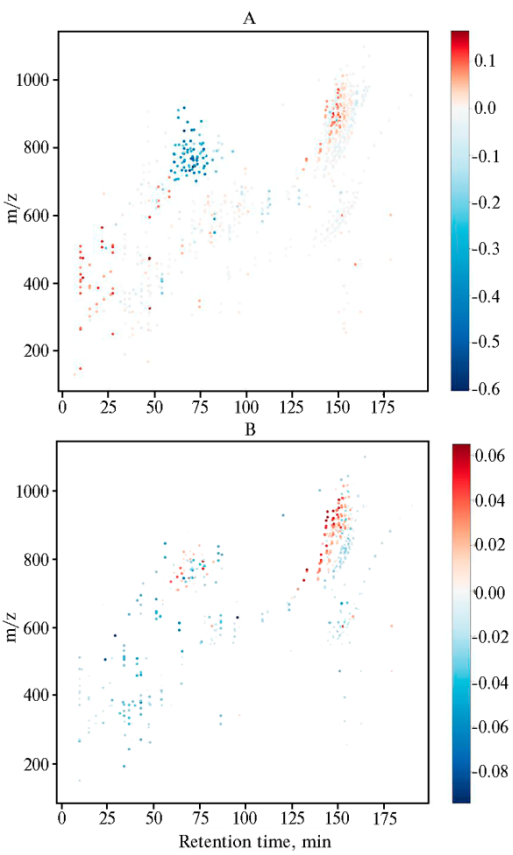


Fig. 4. The graphs of m/z vs. the exit time of lipid peaks for muscle and adipose tissues (A) **and for two fragments of muscle tissue, the averaged sample of biceps tissue BF and averaged sample of the longest muscle LM** (B). The graphs are based on the natural logarithms (ln) of the peak intensity relationships. The color indicates the differences in peak intensity: red — the peak intensity is higher in the fat sample (A) and in the biceps (B), blue — in the muscle sample (A) and the longest muscle (B); white color means that the peak intensities in the compared samples are equal. The measurements were performed by liquid chromatography—time-of-flight mass spectrometry (Acquity I-class, Waters, USA, Maxis Impact II, Bruker Daltonik GmbH, Germany).

characteristic of fat composition of tissues and covers most classes of lipids, i.e. TAG, DAG, PC, PE, PS, PA, PI, LPC, etc. [28]. The results of the analysis show the absence of significant differences in the total fat composition between the muscle areas, but manifest the expected significant differences in the lipid profiles of muscle and adipose tissues. Based on this analysis, it is possible to optimize assessing fat composition of the muscle tissue of animals.

It should be noted, that in pigs, the lipid analysis of tissue samples by UHPLC/Q-TOF-MS was never performed in the mode of positive ion registration. We have found the difference between the correlation coefficients of the lipid composition for the samples from the same tissue ($r = 0.93$) and from different tissues ($r = 0.69$). The reliability of these differences was confirmed by non-overlapping confidence intervals at a significance level of $p < 0.05$ and an unpaired Student's test with a p-value < 0.01. The high correlation of the lipid profiles between the samples from one organ or from two different muscles indicates a good reproducibility of the method. The results show that the muscle tissue samples are enriched in phospholipids, and adipose tissue in triacylglycerols. Triacylglycerols differ in the total length of the chains of their fatty acid residues and in the total number of double bonds. An increase in the total length leads to an increase in the retention time in the chromatographic column, while the addition of a double bond, on the contrary, shortens it. Due to this, chromatographic and mass-spectrometric peaks related to triacylglycerols form the patterns similar to a network on the m/z vs. retention time graph [29]. Unsaturated triacylglycerols are grouped into a single cluster of peaks in the upper left part of the cloud of all peaks belonging to triacylglycerols (see Fig. 2, B). It is interesting that the differences between muscle tissue and adipose tissue, as well as the differences between the two muscles, mainly affect polyunsaturated triacylglycerols, while 0-2-unsaturated fats do not actually change. The use of automatic annotation of chromatographic and mass-spectrometric peaks makes it possible to substantially purify the data from contaminants. The annotation can be further improved by using internal standards or by taking into account the net-like patterns described above. These experiments are beyond the scope of this paper, but we plan to continue the studies in the future.

Thus, the results show that in pigs, muscle tissue is enriched in phospholipids, and adipose tissue in triacylglycerols. In general, the lipid profiles of different tissues differ, while in the same tissue they show similarity. The samples taken from different points of the same muscle do not differ significantly from each other, whereas the difference for different muscles is statistically significant at $p < 0.05$ (in unpaired Student's test, p-value < 0.01). UHPLC/Q-TOF-MS makes it possible to quickly, reliably, efficiently and objectively determine the molecular phenotypes of farm animals, in particular, their lipid profiles, to qualitative assess individuals during selection.

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