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COMPARATIVE PROTEOMIC STUDY OF PIG MUSCLE PROTEINS DURING GROWTH AND DEVELOPMENT OF AN ANIMAL

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Abstract

The production of high-quality pork is closely related to the growth and development of muscle tissue. The present article provides a comparative proteomic research of l. dorsi, b. femoris, m. brachiocephalicus during the pigs' growth and development (at age of 60 days and 180 days). This work was supported by data of electrophoretic methods: one-dimensional electrophoresis according to Laemmli with densitometric assessment in the ImageJ software and two-dimensional electrophoresis according to O'Farrell method with its further processing on the software ImageMaster. The mass spectrometric identification was conducted with the help of the high-performance liquid chromatography (HPLC) system connected to a mass spectrometer; further the data were interpreted by search algorithm Andromeda. When comparing frequency diagrams of one-dimensional electrophoregrams of all three muscle tissues of weaned pigs, the greatest difference was observed for the muscle sample l. dorsi. Comparison of diagrams of muscle tissue samples taken for mature pigs showed a great similarity of all three studied muscles samples. Within the framework of the research, the Fold indicator was calculated. The exceeding its value by more than 2 units is generally considered to be a statistically significant difference. When analyzing two-dimensional electrophoretograms of weaned pigs' muscles, 18 protein fractions were revealed with Fold > 2. When examining the muscle tissue of mature pigs, 15 of those proteins were found; the differences were mostly detected in the minor protein fractions. The mass spectrometric analysis of the cut bands with well-pronounced differences from the one-dimensional electrophoretogram revealed 214 proteins involved to a greater extent in cellular and metabolic processes, physical activity and localization. Growth and development protein — semaphorin-6B (96.78 kDa) — was revealed in muscle tissue of l. dorsi, a. Also in l. dorsi and b. femoris the growth and development proteins were found: cadherin-13 (78.23 kDa), cadherin-7 (87.01 kDa), the F-actin-cap protein beta subunit (30.66 kDa), and two uncharacterized proteins at 65.60 kDa and 63.88 kDa.

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Introduction

The way of application of existing and new technologies for forecasting of some definite characteristics in many food products, including meat, is now of a great interest [1.2]. Proteomic methods enable successful forecasting the quality of meat, and make it possible to improve stability of food parameters and increase the nutrition value of branded products [3]. For instance, the tenderness of pork is known to be difficult to forecast, probably because it is influenced by many factors, like pH [4.5], lipids [6], content of collagen [7] and muscle proteins degradation [8.9]. The researchers also found differences in the expression of metabolic and stress response proteins in tough and tender beef [10] and pork [11]. The studies run by various scientists demonstrate quite significant relationship between muscle metabolism and the quality of meat [12]. At the same time, differences in the proteomic profile of muscle tissues correlate with differences in meat quality [13]. Carlson et al. [14] have documented that even if pH, color and lipid content are the same, the tenderness of meat can vary quite significantly. These differences are associated with postmortem degradation of troponin-T, desmin, filamin and titin. Thus, there is a suggestion that the tenderness variation and the observed postmortem proteolysis in pork of the similar pH, lipid and color is explained by differences in the proteomic profile of sarcoplasm [15]. This research examines the proteome of pig muscles during pig's growth and development in order to determine the differences in protein composition to find additional potential biomarkers of meat quality.

Recently it has become popular to use biomarkers-based food tests to verify the authenticity of food and ensure its quality. The metabolic parameters of meat products were checked by several analytical platforms, mainly electrophoretic methods, chromatography-mass spectrometry (HPLC–MS and GC–MS) [16] and isotope ratio mass spectrometry (IRMS) [17], Fourier transformation infrared spectroscopy (FT-IR) [18] and nuclear magnetic resonance (NMR) [19]. In this article the proteome of pig muscles in the process of growth and development was studied with

the help of electrophoretic methods (one- and two-dimensional gel electrophoresis) in combination with bioinformatic processing and mass spectrometric identification.

Objects and methods

The samples of muscle tissue: *l. dorsi, b. femoris, m. brachiocephalicus* of pigs (aged 60 days and 180 days) of the Vietnamese pot-bellied breed were used as an object of research.

One-dimensional gel electrophoresis (1-DE)

One-dimensional gel electrophoresis was conducted in 12.5% polyacrylamide gel in the presence of sodium dodecyl sulfate (SDS-PAGE) in an electrophoresis chamber (Helicon, USA) at a constant current amperage and voltage of 55V and 130V for 2 hours. As standard solution, a marker was used. The marker consisted of standard preparations with a molecular weight of 250, 150, 100, 70, 50, 40, 30, 20 kDa (Thermo, USA).

Two-dimensional gel electrophoresis (2-DE)

The samples were studied by two-dimensional electrophoresis [20]. At the first stage, isoelectric focusing (IEF) was run at 3,650 V/h in tubular gels of 2.4 mm × 160 mm. 0.01 M orthophosphoric acid and 0.02 M sodium hydroxide were used as anodic and cathodic buffers, respectively. After IEF, the gels were incubated for 10 minutes in 2.5 ml of equilibration buffer solution I (6 M urea, 20% glycerol, 2% SDS, and 1% DTT in 50 mM Tris-HCl buffer, pH 8.8), then in equilibration buffer II (6 M urea, 20% glycerol, 2% SDS, and 4% iodoacetamide in 375 mM Tris-HCl buffer, pH 8.8) [21].

Next, electrophoresis was run using a buffer solution, containing 25 mM Tris-HCl, 192 mM glycine, and 0.1% SDS; for this, the equilibrated gels were transferred into a 12.5% polyacrylamide gel (170 mm×180 mm×1.5 mm). The process was conducted at a current of 30 mA per gel, until the front of the dye reached the edge of the gel.

Visualization of protein fractions and image analysis

Protein stains were visualized by their staining with Coomassie brilliant blue dye G-250 (PanReac, Spain). When determining the density of stains (ImageJ, USA) [22, 23], at least 3 one-dimensional electrophoretograms of equal application were used.

The computerized densitometry of 2D-electrophoregrams was conducted in a wet condition. Their digital images were obtained via a Bio-5000 plus scanner (Serva, Germany). The scanned images were analyzed using *ImageMaster ™ 2D Platinum* software powered by Melanie 8.0 (GE Healthcare and Genebio, Switzerland).

Statistical analysis

Experimental data were analyzed using Student's t-criteria and one-way analysis of variance (between gels of various samples) using *ImageMaster ™ 2D Platinum* software based on Melanie 8.0 (GE Healthcare and Genebio, Switzerland). If P value is less than 0.05 (P <0,05), it is considered as a sign of significant difference. As part of the work, protein stains were compared by their volume, and the Fold index was calculated. If Fold index value is exceeded by more than 2 units, it is generally considered to be a statistically significant difference. All results are presented as mean ± standard deviation, obtained from at least three independent experiments.

Mass spectrometric analysis

The analysis was conducted using the equipment of the Human Proteome Core Facility: the peptides obtained by trypsinolysis were analyzed using an Ultimate 3000 RSLCnano HPLC system (Thermo Scientific, USA) connected to a Q-exactive HFX mass spectrometer (Thermo Scientific, USA)) in positive ionization mode, using an NESI source (Thermo Scientific, USA). Proteins were revealed, using the software *MaxQuant v.1.6.3.4*, search algorithm *Andromeda* using the *Uniprot* database with restrictions relevant to the species of the organism.

Results and discussion

Three different muscles of *l. dorsi, b. femoris* and *m. brachiocephalicus* of weaned pigs were studied by electrophoretic methods in order to reveal significant differences in composition of proteins. At the initial stage one-dimensional electrophoretograms of muscle tissue samples were obtained. Those samples were subsequently analyzed in the *ImageJ* software. During data processing of one-dimensional electrophoregrams of *l. dorsi* samples (Figure 1) a wide range of compounds was revealed of various molecular weights ranging from 13 kDa and more. The major peaks were found on the diagrams corresponding to high molecular weight fractions of 250 kDa and more, 150 kDa, 110–115 kDa, as well as smaller proteins: 93–98 kDa, 78–83 kDa, 62–66 kDa, 41–46 kDa, 40 kDa, 37–38 kDa, 34–35 kDa, 30 kDa, 24 kDa, 17 kDa. The distinct peaks of lower height should also be noted: 95–97 kDa, 70–72 kDa, a number of peaks, incl. minor peaks within the range of 50–62 kDa, 36 kDa, 33–34 kDa, 28–29 kDa, 23 kDa, 20 kDa and 14 kDa. The high sensitivity of the software used during densitometric analysis also made it possible to detect the smallest peaks corresponding to the bands 135–137 kDa, 103–106 kDa, 51–52 kDa, 39 kDa, three peaks within the range 31–33 kDa, 22–23 kDa, 21 kDa, as well as several peaks of 13–15 kDa. The above-listed compounds were generally typical for both mature animals and for young animals with some exceptions. Thus, the mentioned smallest peaks of 103–106 kDa and 51–52 kDa were observed in the longest muscle of only young individuals, while peaks at 44 and 80 kDa were found in tissues of mature animals only. The samples also differed in strength of some individual bands. A high degree of protein expression, which manifested itself in increase in the peaks height, was peculiar for the samples taken from

adult animals. This is evidenced and confirmed by increase in saturation of the molecular weight fractions of 250 kDa, 60 kDa, 39–40 kDa, 36 kDa, 33–34 kDa, 30 kDa, 28–29 kDa, 20 kDa, 17 kDa, 15 and 14 kDa (Figure 1, No. 2). The decrease in the bands expression was not peculiar for the process of animal's growth, and was expressed as decrease in the height of a single peak of 78–83 kDa. This fact can be explained by the absence of the need for expression of proteins included in the above-specified fraction, which leads to a decrease in proteins concentration in tissues.

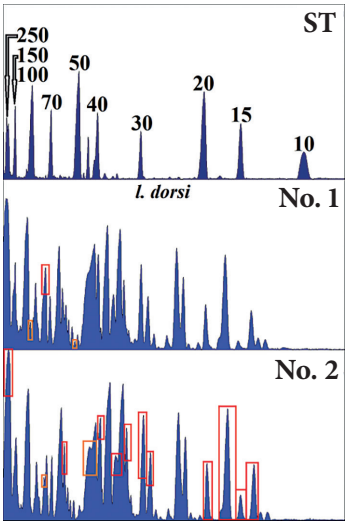

Figure 1. Diagrams of protein fractions frequency in the samples of *l. dorsi*.

Legend: ST — standard of molecular weights, expressed in kDa; No. 1 — sample of *l. dorsi* taken from young pigs; No. 2 — sample of *l. dorsi* taken from adult pigs. Red frames indicate the increase in the corresponding peaks height; changes in the fractional composition are shown in orange

Basing on the data obtained from densitometric analysis of the diagrams, that represent the muscle densities of *brachiocephalicus* (Figure 2), a large difference in the proteomic profile and intensity of individual fractions manifestation was revealed. Both diagrams were characterized by the peaks of 250 kDa and more, 145 kDa, 105 kDa, a series of minor peaks of 85–97 kDa, a peak with an average height of 73–78 kDa, minor bands of 69 kDa and 66–67 kDa, a series of peaks within the ranges 53–63 kDa and 48–52 kDa, major fractions 40–45 kDa, 39 kDa and 36 kDa, as well as the spectrum of lower peaks, incl. the smallest, within the range less than 35 kDa down to the last manifested peak that appeared within 11–12 kDa. Comparative analysis of the diagrams of the protein fractions saturation in the samples showed the proteomic profile peculiar for the muscle proteins of young animals with a molecular weight of less than 31 kDa, namely 16–17 kDa, 18–19 kDa, 20–21 kDa, 22 kDa, 25 kDa and 31 kDa. (Figure 2, # 1, marked in orange). In a sample taken from mature pigs (Figure 2, No. 2), there was a general trend to an increase in intensity of manifestation of most of minor and major peaks. High-molecular-weight proteins remained, in general, identical in both cases; and a striking difference was noted medium- and low-molecular-weight bands in a greater degree.

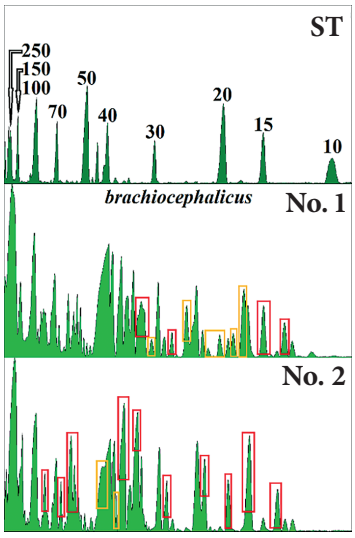

Figure 2. Diagrams of protein fractions frequency in the samples of *brachiocephalicus*.

Legend: ST — standard of molecular weights, expressed in kDa; No. 1 — a sample of *brachiocephalicus* taken from young pigs; No. 2 — a sample of *brachiocephalicus* taken from adult pigs. Red frames indicate the increase in the corresponding peaks height; changes in the fractional composition are shown in orange

Comparison of results of software processing of *b. femoris* electrophoregrams (Figure 3) showed almost identical qualitative protein composition of muscle samples taken from pigs at the age of 60 and 180 days, as well as a wide profile of compounds. The major peaks of 250 kDa and more, 150 kDa, 98 kDa, as well as 39–43 kDa, 37–38 kDa, 36 kDa and 33–34 kDa were detected. In addition, there were lower peaks, including minor peaks in the entire presented range of molecular weights. It is necessary to note these peaks at 130–140 kDa, 76–85 kDa, 68–69 kDa, 65–66 kDa, as well as compounds within the range of 51–58 kDa and 11–49 kDa. The fractional composition of the considered samples differed by the smallest peak of 61–62 kDa in young animals;

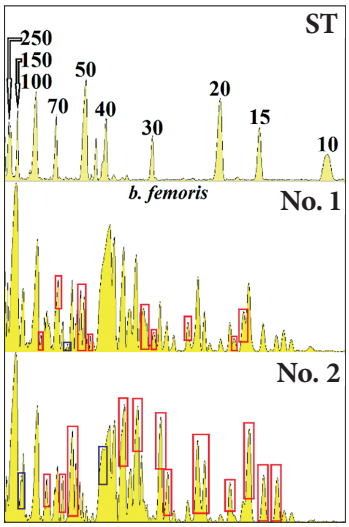

Figure 3. Diagrams of frequency of protein fractions of samples *b. femoris*.

Legend: ST — standard of molecular weights, expressed in kDa; No. 1 — sample of *b. femoris* of young pigs; No. 2 — sample of *b. femoris* of adult pigs. Red frames indicate the increase in the corresponding peaks height; changes in fractional composition are shown in blue

and more distinct bands of 143–145 kDa and 41–43 kDa in mature animals respectively. A distinctive factor when comparing the diagrams was, as in the previously considered cases, precisely the change in the height of the peaks, or the saturation of the corresponding protein fractions. So, on the diagram of the protein fractions density in reference to the young animals (Figure 3, No. 1), the peaks 84–90 kDa, 68–69 kDa, 53–54 kDa, 51 kDa, 32 kDa, 17 kDa, and also some others to a lesser extent (47–48 kDa, 31–32 kDa, 25 kDa and 18 kDa). In the case of mature pigs, an increase in the number of peaks was observed (58 kDa, 57 kDa, 36 kDa, 33–34 kDa, 29 kDa, 28 kDa, 24 kDa, 22–23 kDa, 18–19 kDa, 16 kDa, 14–15 kDa and 13 kDa, etc.), which witnesses a greater degree of accumulation of the relevant proteins in tissues of *b. femoris*.

When comparing the diagrams of the densities of all three samples (Figure 4), obviously the greatest difference was observed for muscle *l. dorsi* taken from other weaned piglets. This fact was confirmed by the clear peak observed at 93–98 kDa compared to a number of peaks within this range in the samples of *brachiocephalicus* and *b. femoris*; a higher peak 62–66 kDa and subsequent peaks within the range 58–61 kDa, as well as bands 34–35 kDa, 37–38 kDa, 29 kDa and 24 kDa; a distinctive 33 kDa peak, corresponding to less pronounced 32–33 kDa bands in the other two muscles. At the same time, a clear difference was determined in the low molecular weight proteins fraction in the sample of 11–20 kDa (Figure 4, *l. dorsi* No. 1). *Brachiocephalicus* and *b. femoris* tissue samples, according to the corresponding diagrams, were the most similar among

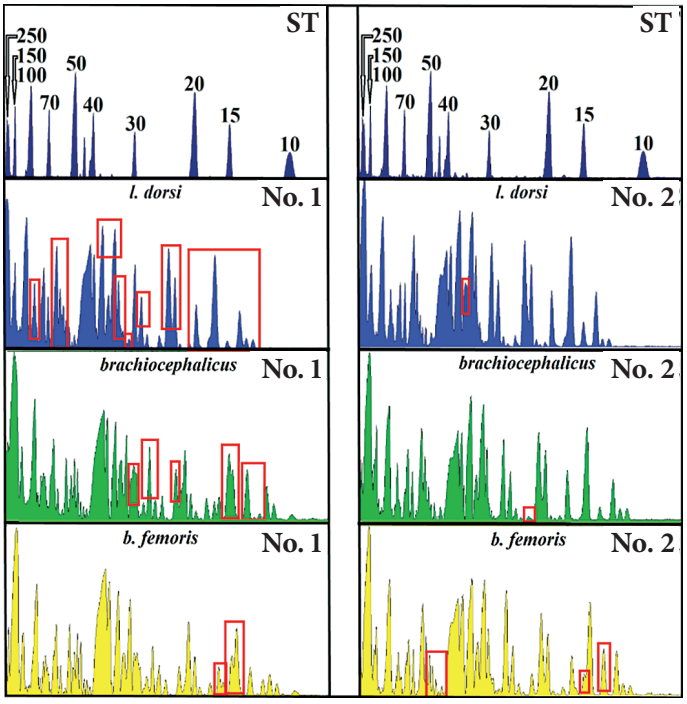

Figure 4. Frequency diagrams of protein fractions of samples l. dorsi, brachiocephalicus, b. femoris.

Legend: ST — standard of molecular weights, expressed in kDa; No. 1 — tissue sample taken from young pigs; No. 2 — tissue sample taken from adult pigs. Red frames show peculiar differences on the diagram of the corresponding sample

the young piglets. The difference was found only in higher peaks of 32–34 kDa, 29 kDa, 25 kDa, and 15 kDa in the case of *brachiocephalicus* muscle, as well as in the fractional composition of the low-molecular proteins fraction in both samples. Thus, the diagram for *brachiocephalicus* showed better visible bands of 20 kDa, 18 kDa, 17–18 kDa, 15 kDa, and 13 kDa, while in tissue of *b. femoris* the bands were more distinct at18–19 kDa, 16–17 kDa and 14 kDa. Comparison of diagrams of muscle tissue samples taken from mature pigs (Figure 4, No. 2) showed a great similarity of all three muscles under consideration. The only exceptions were some separate peaks within the range 53–57 kDa, 17 kDa, and 14–15 kDa in case of *b. femoris*, 35 kDa — in case of *l. dorsi*, and the absence of an expressed peak of 25 kDa in the diagram for *brachiocephalicus*.

For a more detailed analysis of muscle tissues proteome, a study was conducted by the method of two-dimensional electrophoresis of three different muscles: *l. dorsi, b. femoris* and *m. brachiocephalicus*. Fragments of two-dimensional electrophoretograms of weaned pigs muscles, which display 18 fractions with Fold > 2, are presented below in the Figure 5, and their integrated optical densities are shown in the Table 1.

Biological information analysis of the revealed proteins stains suggests that the fractions numbered 1 and 2 in the Figure 5 correspond to myosin light chains of fast (MLC1f) and slow (MLC1s / v) skeletal muscle. These fractions were quite distinct in *l. dorsi*. It is interesting to note that in samples of *b. femoris* tissues, only a slow chain was detected, while in *m. brachiocephalicus* a vaguely expressed fraction of MLC1s was found. The authors V. Montowska and Pospiech [24] used MLC in their studies as a marker to authenticate the meat products made from pork and other kinds of meat.

An interesting distribution of integral optical density was observed in a group of proteins within the range of molecular weights from 50 to 60 kDa, marked with numbers 3–7 in the Figure 5 below. Fractions No. 4 and No. 7 were more pronounced in muscles *b. femoris*, were less pronounced in *m. brachiocephalicus* and muscles of *l. dorsi*. Protein compounds No. 3 and No. 6 were 4 times more pronounced in the muscles *l. dorsi* in comparison with and *m. brachiocephalicus*.

The intensity of staining of protein No. 8 (Figure 5) decreased evenly from *l. dorsi* to *m. brachiocephalicus*. The same trend was observed in fractions No. 13 — No. 17, among which troponin fractions are presumably present. The skeletal muscle protein troponin I has already been characterized as a potential thermostable and species-specific biomarker of mammalian muscle tissue in raw meat and meat products [25], which makes it promising for identification of muscles of various localization. Possibly a lesser staining in *m. brachiocephalicus* was related to the biological function of this muscle, since it is less active in comparison with to *l. dorsi* and *b. femoris*. An interesting distribution of protein stains was observed in the fractions

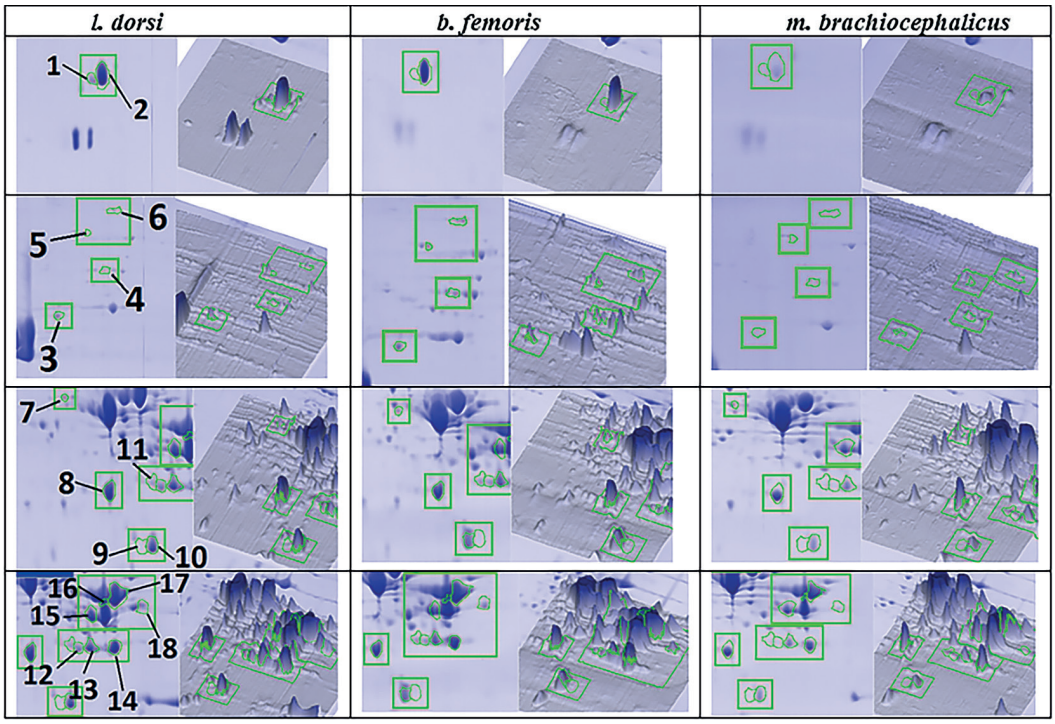

Figure 5. Fragments of 2-DE muscle tissue gels of weaned pigs

Table 1. Results of densitometric analysis of protein fractions of a piglet's muscle tissue (vol ± SD)

No. of a protein spot	l. dorsi	b. femoris	m. brachiocephalicus
1	$9.07 \pm 1.19 \times 10^6$	$2.60 \pm 1.63 \times 10^6$	$1.46 \pm 0.36 \times 10^6$
2	$0.11 \pm 1.35 \times 10^7$	$8.93 \pm 1.13 \times 10^7$	$2.78 \pm 1.77 \times 10^7$
3	$3.84 \pm 0.67 \times 10^6$	$2.74 \pm 1.15 \times 10^6$	$1.02 \pm 0.37 \times 10^6$
4	$1.15 \pm 0.34 \times 10^6$	$2.63 \pm 0.52 \times 10^6$	$1.18 \pm 0.57 \times 10^6$
5	$2.55 \pm 0.63 \times 10^5$	$1.18 \pm 0.29 \times 10^6$	$2.72 \pm 0.67 \times 10^5$
6	$6.88 \pm 3.57 \times 10^5$	$2.42 \pm 0.40 \times 10^6$	$1.42 \pm 0.47 \times 10^6$
7	$1.49 \pm 0.79 \times 10^6$	$5.96 \pm 1.67 \times 10^6$	$3.49 \pm 0.22 \times 10^6$
8	$6.21 \pm 0.29 \times 10^7$	$5.16 \pm 0.83 \times 10^7$	$1.75 \pm 1.52 \times 10^7$
9	$4.90 \pm 2.28 \times 10^6$	$1.86 \pm 1.21 \times 10^7$	$2.08 \pm 0.16 \times 10^6$
10	$3.33 \pm 0.48 \times 10^7$	$1.27 \pm 0.92 \times 10^7$	$8.07 \pm 5.80 \times 10^6$
11	$5.93 \pm 1.10 \times 10^6$	$5.78 \pm 1.72 \times 10^6$	$2.03 \pm 0.49 \times 10^6$
12	$1.77 \pm 0.45 \times 10^7$	$8.42 \pm 2.46 \times 10^6$	$1.69 \pm 0.54 \times 10^6$
13	$4.37 \pm 0.32 \times 10^7$	$3.38 \pm 1.30 \times 10^7$	$1.24 \pm 0.34 \times 10^7$
14	$5.99 \pm 0.19 \times 10^7$	$5.19 \pm 1.02 \times 10^7$	$1.84 \pm 1.53 \times 10^7$
15	$5.31 \pm 0.88 \times 10^7$	$4.18 \pm 0.62 \times 10^7$	$2.52 \pm 0.99 \times 10^7$
16	$1.56 \pm 0.28 \times 10^7$	$7.86 \pm 2.20 \times 10^6$	$3.90 \pm 2.56 \times 10^6$
17	$1.40 \pm 0.12 \times 10^8$	$1.16 \pm 0.05 \times 10^8$	$6.86 \pm 1.51 \times 10^7$
18	$1.07 \pm 0.23 \times 10^7$	$1.21 \pm 0.17 \times 10^7$	$3.71 \pm 1.17 \times 10^6$

Note. The spot volume (Vol) was normalized to the total actual volume of the spot and to the mean for duplicate gels, in triplicate. The presented data are mean ± SD of three independent experiments.
*** Spot volume is the sum of the gray values minus the background of all pixels limited by the spot.**

No. 9 and No. 10. Thus, in *b. femoris* only protein No. 9 was detected, but the fraction No. 10 (adenylate kinase) was present in a small amount. Moreover, in the longest muscle of the back *longissimus dorsi*, its intensity was stronger than in *m. brachiocephalicus*. The volume of stain No. 11 in *m. brachiocephalicus* was three times smaller than that of *l. dorsi* and *b. femoris*. The highest value of the Fold index was observed in the fraction No. 12, which is probably phosphoglycerate mutase, and was equal to 10.5; also it was maximally expressed in *l. dorsi*. Protein No. 18 was more strongly expressed in *b. femoris*, less expressed in *l. dorsi* and was found in small quantities in *m. brachiocephalicus* [26].

The changes in the above described intensity of staining in the selected protein fractions may reflect the intensity of the growth of muscle tissue in growing animals. For example, for *l. dorsi*, the strongest muscle of the spinal column, which determines the movement of the trunk and

head, the maximum amount of intensely stained protein fractions was revealed. The fractions 1, 3, 6, 8, 10–17 can be candidate markers for *l. dorsi* of piglets (Figure 5, Table 1). For muscle *b. femoris*, which is functionally active: hip extensor and hock and knee flexor, muscle fractions 2, 4, 7, 9, and 18 can serve as markers (Figure 5, Table 1). The hip muscle and its protein profile have been researched quite well, since various types of dried ham (jamon, prosciutto, prosciutto) are made from pork ham. It is already known that peptides produced in this muscle have biological functionality (some peptides from proteins MLC1, CK, MYO, TNT and MHC7 showed the highest functionality).

Thus in the composition of *l. dorsi* 12 proteins were identified, *b. femoris* — 5 proteins, and for *m. brachiocephalicus* — 1 protein. In addition, the latter revealed an inclination for a lower intensity of protein fractions, some of which are smaller by one order of magnitude in comparison with *l. dorsi*. The lowest intensity of staining of protein fractions was noted in the tissues of *m. brachiocephalicus*, which is possibly related to low metabolic processes in this muscle due to low functional load.

The electrophoretic study of muscles tissues of different localization taken from mature pigs (Figure 6, Table 2), the differences were mostly found in minor protein fractions.

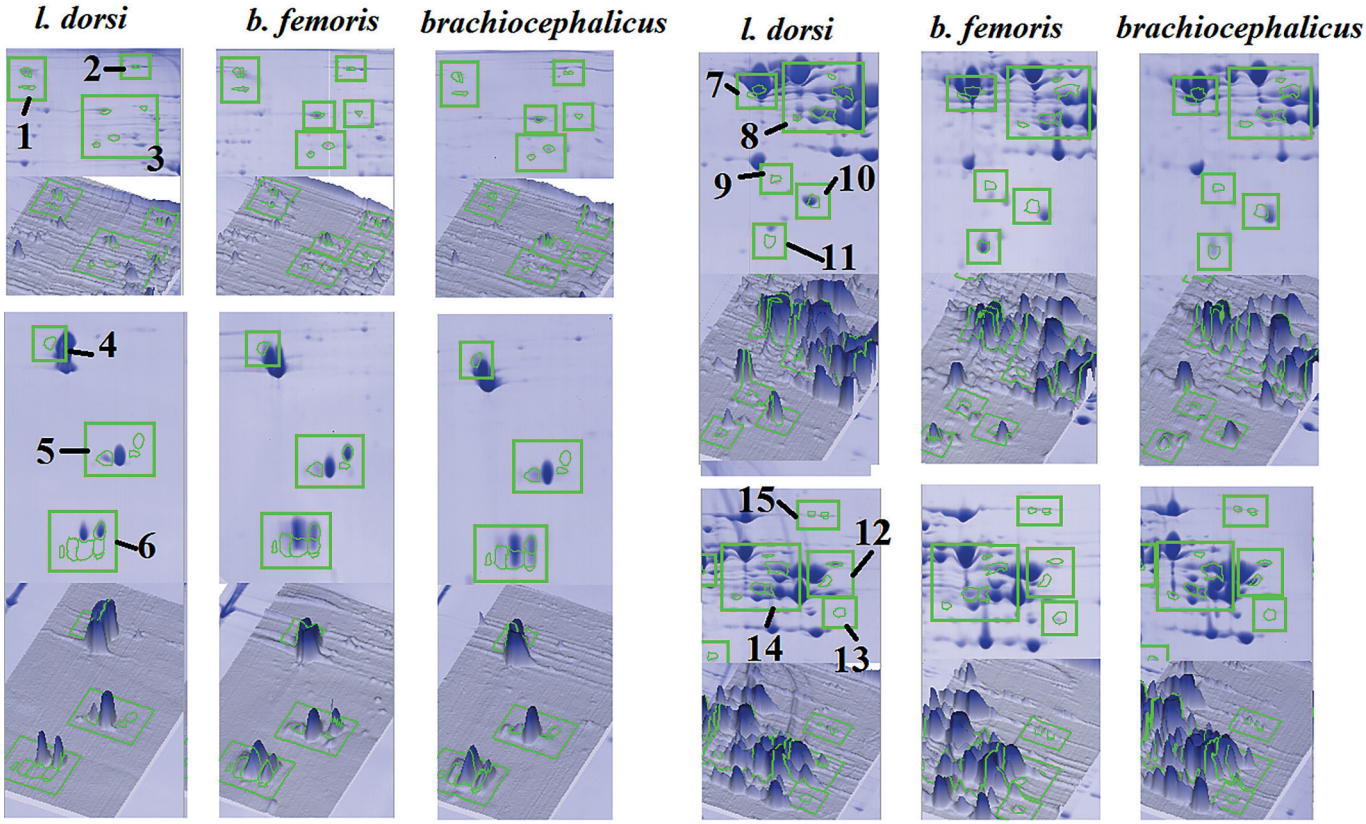

Figure 6. Fragments of 2-DE gels of muscle tissue taken from the mature pigs

Table 2. Results of densitometric analysis of protein fractions of muscle tissue taken from the mature pigs (volume ± SD)

No. of a spot	l. dorsi	b. femoris	m. brachiocephalicus	Fold
1	$9.03 \pm 0.24 \times 10^5$	$6.54 \pm 0.10 \times 10^5$	$2.00 \pm 0.11 \times 10^6$	3.05
2	$7.29 \pm 0.27 \times 10^5$	$0.97 \pm 0.06 \times 10^5$	$3.65 \pm 0.18 \times 10^5$	7.45
3	$3.95 \pm 0.42 \times 10^5$	$3.20 \pm 0.22 \times 10^5$	$1.10 \pm 0.17 \times 10^6$	3.43
4	$2.78 \pm 0.69 \times 10^6$	$1.06 \pm 0.14 \times 10^7$	$7.11 \pm 1.28 \times 10^6$	3.82
5	$8.03 \pm 2.50 \times 10^6$	$2.20 \pm 0.41 \times 10^7$	$7.73 \pm 0.91 \times 10^6$	2.85
6	$2.76 \pm 0.09 \times 10^7$	$1.24 \pm 0.02 \times 10^7$	$1.35 \pm 0.42 \times 10^7$	2.23
7	$1.86 \pm 0.07 \times 10^7$	$4.80 \pm 0.11 \times 10^7$	$4.70 \pm 0.15 \times 10^7$	2.58
8	$2.69 \pm 0.29 \times 10^7$	$2.35 \pm 0.23 \times 10^7$	$1.35 \pm 0.15 \times 10^7$	2.13
9	$8.32 \pm 0.86 \times 10^5$	$2.24 \pm 0.46 \times 10^6$	$1.86 \pm 0.46 \times 10^6$	2.68
10	$2.31 \pm 0.18 \times 10^7$	$1.17 \pm 0.05 \times 10^7$	$5.76 \pm 2.44 \times 10^6$	4.01
11	$3.84 \pm 1.57 \times 10^6$	$1.02 \pm 0.70 \times 10^7$	$3.75 \pm 1.38 \times 10^6$	2.70
12	$5.13 \pm 1.25 \times 10^6$	$1.93 \pm 0.81 \times 10^6$	$2.53 \pm 0.12 \times 10^6$	2.66
13	$1.03 \pm 0.06 \times 10^6$	$1.36 \pm 0.01 \times 10^6$	$3.22 \pm 0.51 \times 10^6$	3.13
14	$4.55 \pm 0.57 \times 10^7$	$4.25 \pm 0.06 \times 10^7$	$1.80 \pm 0.17 \times 10^7$	2.52
15	$8.50 \pm 0.16 \times 10^5$	$1.57 \pm 0.16 \times 10^6$	$1.87 \pm 0.25 \times 10^6$	2.20

A group of proteins (No. 1–3, figure 6) within the range of more than 70 kDa, such as alpha-1.4-glucan phosphorylase, amine oxidase, actin-depolymerizing factor and heat shock protein HSP 90-alpha in significantly larger quantities (Table 2, Fold > 3.05) was found in *m. brachiocephalicus*. Also the we noted the predominance of *m. brachiocephalicus* proteins CFL2b variant 1 and troponins C of fast and slow skeletal muscles, which proteins are responsible for the binding of actin filaments. As far as the structural muscle proteins are concerned, the significant differences there were found in fragments of tropomyosin, myosin light chains 1 and αβ-crystallin (No. 4, No. 5, No. 11 in Figure 6), which were at least twice as intense in tissues of *b. femoris*. For *l. dorsi* the fractions No. 6, No. 10 and No. 12 are predominant, which are myosin light chains 2, acireductone dioxygenase and gamma actin-2 in accordance with mass spectrometric identification. It is worth and interesting to note, that creatine kinase (subunit M) and long-chain 3-ketoacyl-CoA thiolase were found there only in minimal quantities compared to *b. femoris* and *m. brachiocephalicus*. At the same time, L-lactate dehydrogenase A chain and porin are less pronounced in *b. femoris*.

Mass spectrometric analysis of the cut out bands with expressed differences, taken from the one-dimensional electrophoretogram (Figure 7), revealed 214 proteins, involved to a greater extent in the cellular and metabolic processes, motor activity and localization.

In the tissue of *l. dorsi* muscle, the protein responsible for growth and development was identified by mass spectrometry through binding of the semaphorin receptor and chemorepellant activity — semaphorin-6B (96.78 kDa). Development proteins were also identified in *l. dorsi* and *b. femoris* — cadherin-13 (78.23 kDa), cadherin-7 (87.01 kDa), the F-actin-capping protein *beta* subunit (30.66 kDa), and two uncharacterized proteins at the band of 65.60 kDa and 63.88 kDa.

Conclusion

The data obtained in result of comparative analysis of piglets' and mature pigs' muscles showed a high degree of tissue differentiation in the young animals, with subsequent change in the fractional composition of proteins during the growth and development of the animals. In case of *longissimus dorsi* the most significant changes mainly affect the proteins fractions with a molecular weight of less than 31 kDa; and in the samples of *brachiocephalicus* and *biceps femoris*, the entire presented range of molecular weights was also affected with this change. Using the method of two-dimensional electrophoresis, it was shown that in the process of pig's growth and development, the number of fractions of troponins, light myosin chains and proteins of the actomyosin complex increases, but their quantitative content decreases. The changes revealed in protein compounds reflect the intensity of processes of muscle tissue development in animals, which changes contribute to capability to monitor the patterns of formation of the above-specified parameters of meat and meat products quality.

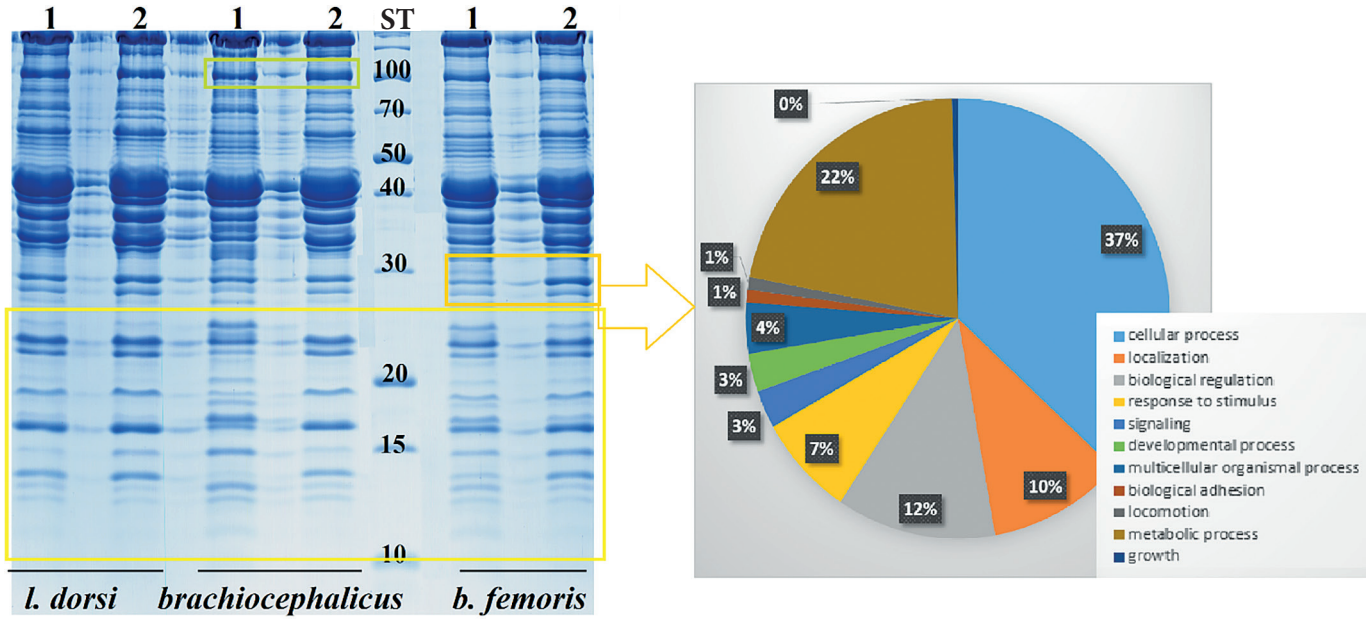

Figure 7. One-dimensional electrophoregram of pig muscles during growth (1 — piglets, 2 — mature pigs)

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