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THE STUDIES OF PROTEOMIC PROFILE OF MUTTON WITH CONSIDERATION TO THE INFLUENCE OF FEED COMBINATORICS

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Abstract
The present article is devoted to the search for markers that attest the changes in the protein profile when fattening the lambs with enriched diets, taking into account tissue specificity. The purpose of scientific research was to expand scientific knowledge about the influence of essential trace elements of organic origin in the diet of lambs on the marker-proteins of skeletal muscle tissue, of myocardium and renal cortical substance. The objects of research were the tissues of the m.L.dorsi skeletal muscle, myocardium and cortical substance of the kidneys in lambs of the Edilbaev breed. These lambs received a diet with organic trace elements (iodine, selenium, silicon). To study the effect of microelements in the diets of lambs on change in marker-proteins, we ran the studies of the skeletal muscle, myocardium and cortical substance of the kidneys of lambs, using the method of two-dimensional electrophoresis. When searching for proteins which mark the action of organic additives in the fattening diet of lambs within the tissue of the myocardium and the cortical substance of the kidneys, a number of differences were noted; no pronounced effects were observed in the skeletal muscle. Enrichment of the diet of lambs with selenium derivatives led to a change in the protein composition in the kidneys cortical substance for some isoforms of selenium-containing proteins and related enzymes of the glutathione cycle. Reaction in this process of thermal shock proteins, utilization of aflatoxins and changes in proteins of energy metabolism within mitochondria of kidney cells was also revealed. The determination of functional activity, or immunofermental analysis of glutathione peroxidase 3 count in the blood, can be a promising biomarker for controlling the level of selenium digestion and the count of selenium in blood as a more rapid and cheap method in comparison with the proteomic technologies. And for assessing the rate of digestion and fixation of iodine, it seems promising to determine it via the activity of mitochondrial superoxide dismutase [Mn]. In the myocardium, the effect of digested feed additives mainly affected the mitochondrial apparatus, which changed its functional orientation, with the activation of several other metabolic cycles. These results are prerequisites for obtaining more fundamental data on the effect of diets enriched with essential micronutrients on the molecular mechanisms that take place within the myocardial and kidney tissue of lambs.
New data have been obtained on the effect of diets enriched with essential microelements on the molecular mechanisms occurring in the myocardial tissue and cortical substance of the kidneys of goats and sheep. The research results obtained by proteomics methods will be used for modeling and targeted adjustment of diets in order to obtain raw materials with the necessary technological characteristics.

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Introduction

The quality of meat raw materials is influenced by the factors like genetics, management conditions, feeding diets, slaughter methods, method of packaging and refrigeration [1]. It is important to note that the protein composition of raw materials is quite dynamic and changes depending on the factors affecting synthesis or degradation of proteins [2]. The study of the proteome makes it possible not only to study the molecular mechanisms that take place in tissues at a deeper level, but also to predict the functional and technological parameters of meat raw materials. Therefore, nowadays the studies of the molecular mechanisms underlying the modification of proteins are relevant [3].

Proteomics methods are used to separate proteins, mainly in two directions: either chromatographic or electrophoretic methods with subsequent identification of point markers with the help of mass spectrometric detectors [4]. Proteomic analysis expands the technical possibilities for a more detailed study of the muscle tissue of productive cattle; therefore, in recent decades, omics technologies have been actively used in the meat-process-

ing industry, which significantly changed experimental approaches in food sciences [5]. Proteomic technologies make it possible to evaluate the composition and ongoing processes at the formation stages of the meat raw materials and in the commercial products also. First of all, the most common types of meat raw materials — beef, pork and poultry — got investigated. The processes of formation of meat tenderness, water-holding capacity, pre-slaughter conditions, the role of immune castration (immunization against gonadotropin-releasing hormone) as the mild alternative to surgical castration, which is used to reduce the risk of boar flavor, the effect of diets on the growth of broilers and the final quality of meat, gender features and differences in the composition of different muscle types, as well as age-related changes and breed differences in taste, identification of biomarkers of meat quality and adulteration of meat products [6,7].

The proteomic approach was used to study the effect of heat treatment on proteins modification in lamb [8], the degradation of myofibrillar proteins during the meat maturation [9], used for the search for species-specific markers of animal meat, including lamb [10], seasonal weight loss [11], changes in the protein composition depending on the method of slaughter [12], the search for protein markers predicting changes in the meat color during its post-slaughter storage [13,14].

The diet of animals brings on the qualitative changes in weight gain and the final quality of meat [15,16].

The important role of selenium for the functional activity of such important organs as a heart, liver, kidneys, and others is known, and it is closely related to the content of iodine [17,18]. Endemic spots of selenium deficiency are known in some regions of Siberia, and the use of appropriate meat products can help prevent dysmicroelementosis (microelements deficit syndrome) among the population [3,19]. The results obtained in this work reflect the main trends that have developed to date in the global meat industry. The results can be regarded to as another important step that contributes to the modernization of domestic developments aimed at improving the quality of raw materials and meat products.

The purpose of scientific research was to expand scientific knowledge about the effect of essential microelements of organic origin in fattening lambs on the proteomic profile of their skeletal muscle, myocardium and cortical substance of the kidneys.

Materials and methods

As objects of study the samples of skeletal muscle (*m. Longissimus dorsi*), myocardium and kidneys of lambs were used. The study was carried out on 4 groups with different fattening options: normal diet (control group), group 1 (with the addition of selenmethionine into their diet), group 2 (with the addition of iodotyrosines in the form mono- and di-iodothyrosine) and group 3 (with the combination of the above-mentioned additives).

For the preparation of the sample for proteomic analysis, 100 mg of the ground sample was finely homogenized in 2 ml in a Teflon-glass system in a lysing solution of the following composition: 9 M urea, 5% mercaptoethanol, 2% triton X-100, 2% ampholines pH 3.5–10. The obtained homogenate was clarified by centrifugation at 800g for 5 min, and the supernatant fraction containing solubilized proteins (extract) was used for fractionation.

Molecular weights (Mw) of protein fractions were determined with a set of highly purified recombinant proteins with molecular weights: 10–170 kDa "PageRuler™ Prestained Protein Ladder" (No.SM0671–10 proteins) from "Fermentas" company (USA).

Two-dimensional electrophoresis (TDE) according to the method of O'Farrell with ampholine isoelectric focusing (IEF-PAGE) was used as the main proteomic technology. Detection of proteins on two-dimensional electrophoregrams was performed by staining the mass with Coomassie blue R-250 dye (CBB R-250) and then sequentially with silver nitrate. For computer densitometry, two-dimensional electrophoregrams in a wet state were used. Their complete digital images and/or images of some individual fragments were obtained by scanning in the Epson Expression 1680 scanner. The sample was scanned in the following mode: resolution 300 dpi, 48 bit Color, the results were saved in TIFF format. The obtained digital images were edited in a graphics editor and the quantitative content of proteins was calculated using the software package ImageMaster 2D Platinum, version 7 ("GE Healthcare", Switzerland). At least 3 electrophoregrams with equal coverage were used for determination of protein count.

To identify proteins, individual fractions were excised from DE, the excised fragments were finely crushed and trypsinolyzed. Further, the corresponding sets of peptides were studied by the methods of MALDI-TOF MS and MS/MS mass spectrometry on a MALDI — Ultraflex time-of-flight mass spectrometer ("Bruker", Germany) with a UV laser (336 nm) in the positive ion mode within the mass range 500–8,000 Da with their calibration according to the known peaks of trypsin autolysis. The obtained mass spectra ("peptide fingerprints") were deciphered by traditional bioinformatic technologies. Bioinformatic analysis of mass spectrometric "peptide fingerprints" was carried out by the "Mascot" software and other bioinformatic technologies [20].

The mass spectra of tryptic peptides were analyzed using the "Mascot" software, the option Peptide Fingerprint ("Matrix Science", USA), with an accuracy of MH+ mass determination equal to 0.01%, by searching the databases of the US National Center for Biotechnology Information (NCBI). During the research, the equipment of the Center for Collective Use "Industrial Biotechnologies" of the Federal State Institution "Federal Research Center "Fundamental Foundations of Biotechnology of the Russian Academy of Sciences" was used. In a comparative analysis of the proteomic profiles of the studied samples, the data

modules of the database "Proteomics of Muscular Organs" http://mp.inbi.ras.ru/ [21] were also used.

Statistical processing of the results

The obtained results were statistically processed with the help of software packages: Statistica 6.1; BIOSTAT, MS Office Excel 2003 [Microsoft]. Comparison of quantitative characteristics in two independent groups was performed using Student's t-test. Results with a significance level of less than 0.05 were considered as statistically significant.

Results and discussion

Studies have shown that no pronounced effects of the fattening diet in the skeletal muscle (*m. Longissimus dorsi*) of lambs were found. Therefore, the results of protein fractionation in organs, more sensitive to the action of essential microelements, were taken and analyzed. Those organs tissues were the cortical substance of the kidney of lambs (Figure 1) and the tissue of the left ventricle of the heart (Figure 2).

In the control group, in the cortical substance tissue, over the albumin fraction there was a trace amount of a minor fraction (No. 1), which turned out to be a mixture of the dominant 70 kDa mitochondrial thermal shock protein (HSPA9) and the oligomer of glutathione peroxidase 3 (GPX3). The latter element is a tetrameric selenoenzyme in its native state. Obviously, some of its molecules did not decompose into subunits even being exposed to the used detergents, and there was no complete rupture of S-S bonds. All detected peptides in its amino acid sequence started from positions 121 to 197 out of 226 in a. p. of this protein.

Presumably, the presence of selenium can hinder the trypsinolysis of the protein in the oligomeric form. This protein was also identified as a monomer (nos. 4, 5) as two electrophoretic isoforms, with different pI. Peptides from positions 30 to 197/201 were identified. The homologue of this protein (No. 4) was found in all diets and in control group too. And fraction No. 5 (more acidic in pI) was found only in group 1. Here, another representative of

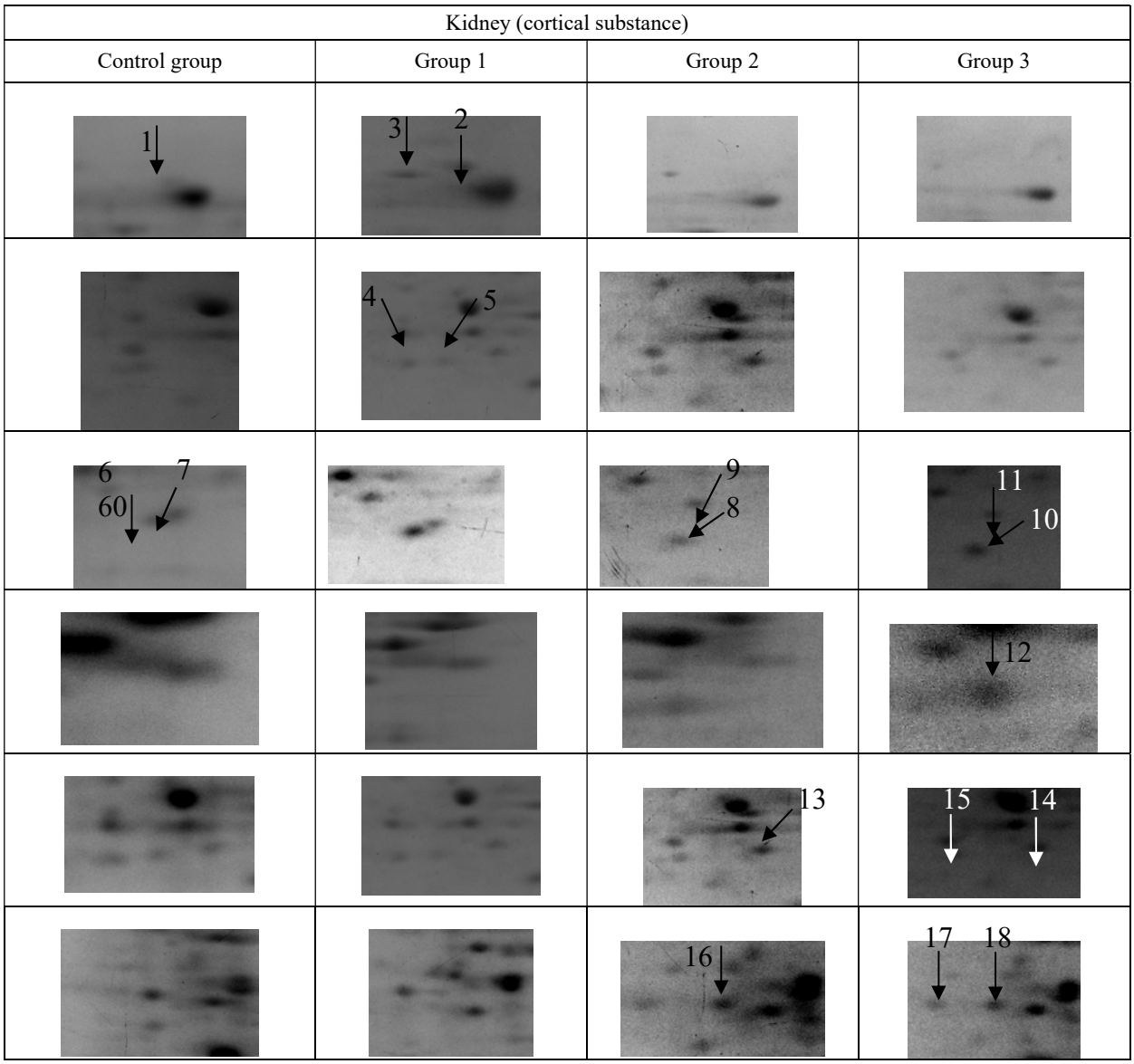

Figure 1. Fragments of DE zones of proteins, showing changes depending on the fattening diet (cortical substance of the kidney). Identified fractions are shown by arrows with numbers in accordance with the Table 1

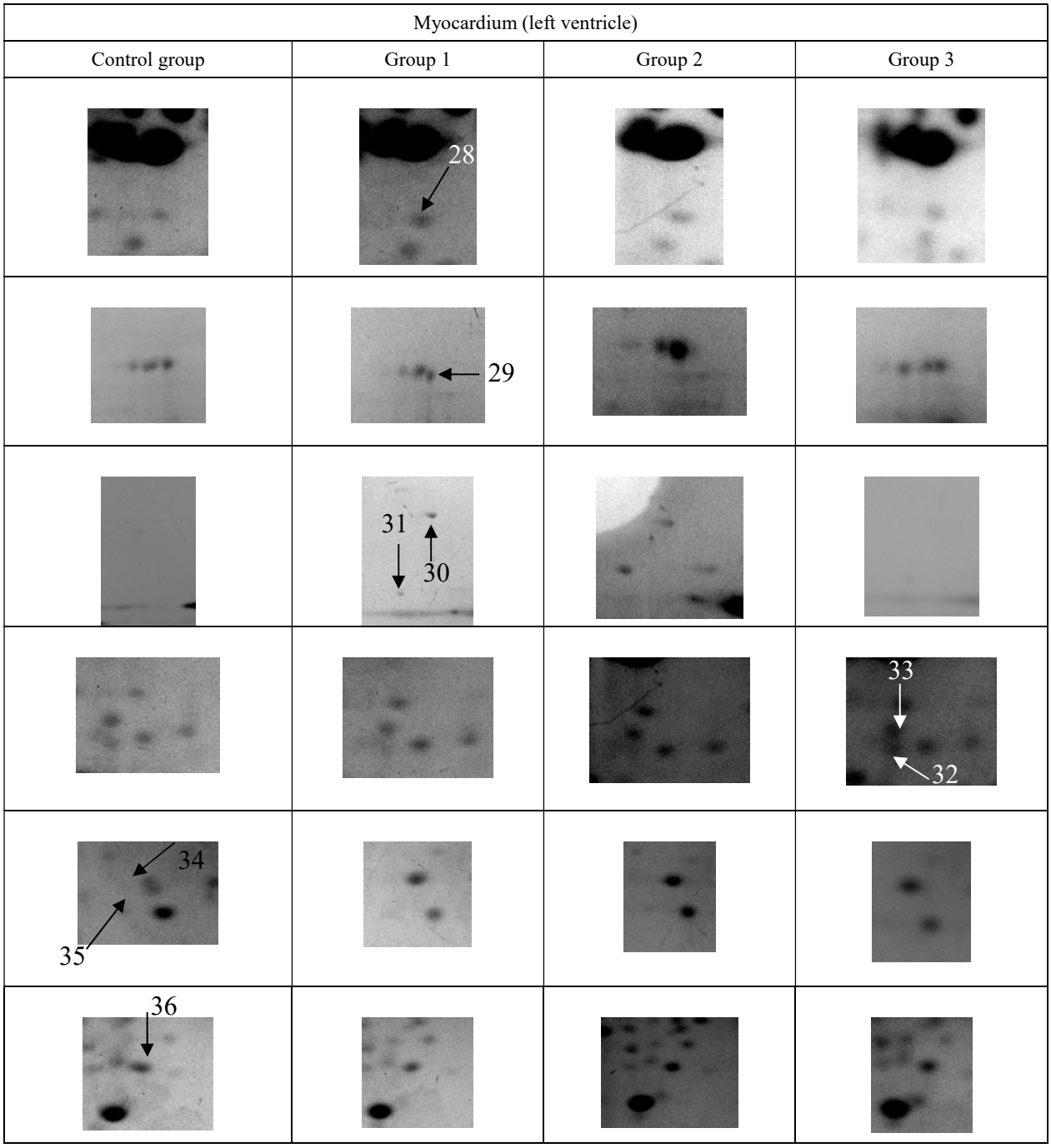

Figure 2. Fragments of DE zones of proteins showing changes depending on the fattening diet (Myocardium (left ventricle). The identified fractions are shown by arrows with numbers in accordance with the Table 1

selenium-containing proteins family — glutathione peroxidase 1 (GPX1) — was also found (Figure 1).

It is obvious that the figures below were taken from the materials of some report. Therefore the numbering of fractions is not clear. The authors present the experiment in the form of a publication. It is necessary to give a continuous numbering of fractions, or give a complete table of identified fractions.

It should be noted that in general these enzymes are associated with glutathione metabolism too, while the glutathione S-transferase P-like protein was found in the same zone also. Glutathione peroxidase isoforms protect cells from oxidative stress, and in case of increased amount of selenium (which is toxic to cells) which can activate the strength of protective mechanisms, is not consistent with the beginning of the proposal. In group 1, two fractions showed up (No. 2 and 3 — the latter is also present in other types of fattening diets, but in a smaller amount. These fractions were identified (No. 2) as a mixture of 6 proteins that do not correspond in weight and pI, meanwhile no glutathione peroxidase oligomer 3 was found; but, for example, an aflatoxin utilization enzyme was identified. These fractions is a variant of the artifact, the formation of an oligomeric complex of a number of proteins with different functions in the group of lambs fattened with selenium-enriched diet, although a fraction of thermal shock protein, HSPA9 gene product, is habitually present in muscle tissues.

Table 1. The results of mass spectrometric identification (MALDI-TOF MS and MS/MS) of fractions extracted from preparations of cortical substance of the lambs kidney and myocardium proteins

No.	Protein (*symbol of gene*)	Numbers in the Protein NCBI or SwissProt	S / M/ C *	Mw/pI (experimental)**	Mw/pI (calculated)**
1	Mix of stress-70 protein, mitochondrial (*HSPA9*)*** (1) with admixture of oligomer of glutathione peroxidase 3 (*GPX3*)***(1)	XP_004008889.1 XP_014951639.1	232/34/46 70/9/24	70,0/5,50	73,7/5,97 25,8/8,26
2	Mix of thermal shock protein beta-1 (*HSPB1*), acyl-protein thioesterase 1 (*LYPLA1*)***(1) + Acetyl (Protein N-term) 7S, protein ABHD14B (*ABHD14B*)***(1) and glutathione peroxidase 3 (*GPX3*)***(1)	XP_027817273.1 NP_001293032.1 XP_004018465.1 XP_014951639.1	100/9/61 84/9/51 191/9/52 112/12/42	24,0/6,30	22,4/6,22 24,6/6,28 22,5/6,29 25,8/8,26
4	Mix of glutathione peroxidase 3 (*GPX3*), glutathione peroxidase 1 (*GPX1*)***(1), acyl-protein thioesterase 1 (*LYPLA1*)***(1), glutathione S-transferase P-like (–) and protein/nucleic acid deglycase DJ-1 (*PARK7*)***(3)	XP_014951639.1 XP_004018511.2 NP_001293032.1 XP_027828341.1 XP_004013798.1	90/14/61 137/6/37 120/6/49 97/9/53 302/24/78	25,0/6,60	25,8/8,26 22,6/6,82 24,6/6,28 24,0/6,89 20,3/6,84
5	phosphatidylethanolamine-binding protein 1 (*PEBP1*)***(1)	XP_004017427.1	248/20/89	21,0/7,10	21,1/6,96
6	superoxide dismutase [Mn], mitochondrial (*SOD2*)***(1)	NP_001267632.1	154/32/70	21,5/7,40	24,6/8,89
7	phosphatidylethanolamine-binding protein 1 (*PEBP1*)***(3)	XP_004017427.1	285/26/77	21,0/7,10	21,1/6,96
8	superoxide dismutase [Mn], mitochondrial (*SOD2*)	NP_001267632.1	171/31/71	21,5/7,40	24,6/8,89
9	Mix of adenylate kinase 2, mitochondrial isoform X2 (*AK2*)***(1) and dehydrogenase/reductase SDR family member 4 (–) ***(2)	XP_004005056.2 XP_004010370.1	175/18/70 186/31/66	26,0/8,40	25,5/8,35 29,6/9,17
10	glutathione S-transferase P-like (–)***(3)	XP_027828341.1	142/26/74	24,0/6,90	23,7/6,89
11	thermal shock protein beta-1 (*HSPB1*)***(2)	XP_027817273.1	347/23/70	24,0/6,40	22,3/6,22
12	Mix of aspartoacylase (*ASPA*)***(1), lambda-crystallin homolog (*CRYL1*)+ Acetyl (Protein N-term and гомолог Malate dehydrogenase, cytoplasmic (–)	XP_004012613.1 XP_027829478.1 ELR52421.1	166/20/73 99/13/47 83/13/35	33,0/5,90	35,6/6,19 35,1/6,46 36,4/6,18
13	Mix of aspartoacylase (*ASPA*), фрагмент isocitrate dehydrogenase [NADP] cytoplasmic isoform X1 (*IDH1*)***(1), lambda-crystallin homolog (*CRYL1*)***(1) and nicotinate-nucleotide pyrophosphorylase [carboxylating] isoform X1 (*QPRT*)***(1)	XP_004012613.1 XP_027819687.1 XP_027829478.1 XP_027817331.1	201/19/70 178/6/20 162/9 /43 111/11/47	33,0/6,50	35,6/6,19 46,7/6,34 35,1/6,46 35,9/8,67
14	Mix of isocitrate dehydrogenase [NADP] cytoplasmic isoform X1 (*IDH1*)***(1), 4-hydroxyphenylpyruvate dioxygenase (*HPD*)***(1) and long-chain specific acyl-CoA dehydrogenase, mitochondrial isoform X1 (*ACADL*)***(1)	XP_027819687.1 XP_004017393.1 XP_027820950.1	353/41/75 194/12/48 183/11/34	45,0/6,40	46,7/6,34 44,9/6,25 47,8/6,92
15	Mix of argininosuccinate synthase (*ASS1*)***(1), citrate synthase, mitochondrial (*CS*)**(1), гомолог pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial (*PDHA1*)***(1) and methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial isoform X1 (*ALDH6A1*) ***(1)	XP_027822083.1 XP_004006633.1 XP_014703413.1 XP_027827815.1	347/51/79 224/14/46 162/10 /21 167/13 /40	44,0/6,90	46,7/7,17 52,1/8,12 52,0/8,87 58,5/8,47
16	phosphatidylethanolamine-binding protein 1 (*PEBP1*)***(1)	XP_004017427.1	231/32/85	21,0/7,10	21,1/6,96
17	superoxide dismutase [Mn], mitochondrial (*SOD2*)***(1)	NP_001267632.1	141/35 /71	21,5/7,40	24,6/8,89
18	Mix of enoyl-CoA hydratase, mitochondrial (*ECHS1*)***(2), alcohol dehydrogenase [NADP(+)] isoform X2 (*AKR1A1*), carbonic anhydrase 2 (*CA2*)***(1)	XP_027815979.1 XP_011982310.2 XP_027829052.1	305/38/73 118/16/51 146/4/21	29,0/6,80	31,1/8,76 36,6/6,80 29,1/6,41
19	Mix of aspartoacylase (*ASPA*)***(1), lambda-crystallin homolog (*CRYL1*), гомолог isocitrate dehydrogenase [NADP] cytoplasmic (*IDH1*)	XP_004012613.1 XP_027829478.1 XP_020749303.1	270/28/84 179/19/78 64/10/37	33,0/6,50	36,0/6,19 35,6/6,46 37,1/7,05
20	Mix of S-formylglutathione hydrolase (*ESD*), фрагмент aflatoxin B1 aldehyde reductase member 2 (*AKR7A2*)***(1), serine protease HTRA2, mitochondrial (*HTRA2*)***(1)	XP_027829356.1 XP_027821430.1 XP_004006156.2	187/19/82 86/14/40 116/84/28	35,0/6,70	32,2/6,30 41,1/8.37 49,0/9,87
21	Mix of фрагмента albumin (*ALB*), isocitrate dehydrogenase [NADP] cytoplasmic isoform X1 (*IDH1*)***(1), secernin-2 isoform X1 (*SCRN2*)***(1)	NP_001009376.1 XP_027819687.1 XP_027830441.1	249/42/50 120/9/19 86/7/20	43,0/5,80	69,1/5,80 46,7/6,34 53,4/7,92

End of Table 1

No.	Protein (*symbol of gene*)	Numbers in the Protein NCBI or SwissProt	S / M/ C *	Mw/pI (experimental)**	Mw/pI (calculated)**
22	Mix of hydroxyacyl-coenzyme A dehydrogenase, mitochondrial (*HADH*)***(2), malate dehydrogenase, mitochondrial (*MDH2*)	XP_004009686.1 XP_004021309.2	233/25/68 179/21/65	34,0/8,90	34,5/9,04 36,1/8,82
23	Mix of isocitrate dehydrogenase [NADP] cytoplasmic (*IDH1*), гомолог argininosuccinate synthase (*ASS1*), 3-ketoacyl-CoA thiolase, peroxisomal (*ACAA1*)***(1), 2-amino-3-ketobutyrate coenzyme A ligase, mitochondrial isoform X4 (*GCAT*)***(1), mannose-1-phosphate guanyltransferase alpha isoform X2 (*GMPPA*)***(1), citrate synthase, mitochondrial (*CS*)	NP_001009276.1 XP_019826582.1 XP_004018276.4 XP_004007030.2 XP_027821026.1 XP_004006633.1	234/45/80 131/27/66 182/17/60 139/13 /54 105/8/44 189/12/36	41,0/6,70	47,3/6,34 46,8/6,82 44,8/8,68 45,6/6,76 47,1/6,38 52,1/8,12
24	Mix of delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial (*ALDH4A1*)***(3), acyl-coenzyme A synthetase ACSM1, mitochondrial (*ACSM1*), phosphoenolpyruvate carboxykinase, cytosolic [GTP] isoform X2 (*PCK1*)	XP_027821443.1 XP_004020864.2 XP_004014490.2	312/44/82 119/23/55 113/30/52	65,0/6,70	62,2/8,22 65,9/8,12 70,3/6,46
25	Mix of serotransferrin (TF) and ezrin (EZR)	XP_027816111.1 XP_027828426.1	265/47/66 240/54/64	70,0/6,50	80,1/6,31 68,0/6,06
26	60 kDa thermal shock protein, mitochondrial (*HSPD1*)***(2) с признаками + Deamidated (NQ)	XP_027820861.1	346/57/75	60/0/5,30	61,1/5,71
27	Mix of aspartoacylase (*ASPA*), lambda-crystallin homolog (*CRYL1*), 3-mercaptopyruvate sulfurtransferase isoform X1 (*MPST*)***(1), fragments of homologue of eukaryotic translation elongation factor 2 (*EEF2*)***(1), фрагмента isocitrate dehydrogenase [NADP] cytoplasmic (*IDH1*)***(1), aflatoxin B1 aldehyde reductase member 3 (–)***(1), гомолог voltage-dependent anion-selective channel protein 2 (*VDAC2*)***(1)	XP_004012613.1 XP_027829478.1 XP_004006777.1 KAF6348325.1 NP_001009276.1 XP_027821432.1 XP_027821432.1	185/27/73 141/25/69 112/19/72 144/14 /38 118/5/16 117/10/38 115/9/42	69,0/5,60	35,6/6,19 35,1/6,46 33,2/6,16 52,4/6,84 47,3/6,34 40,6/6,88 32,2/7,48
28	thermal shock cognate 71 kDa protein isoform X1 (*HSPA8*)***(1)	XP_011951023.2	115/48 /42	69,5/5,30	71,5/5,37
29	Homologue of the fragment of structure of caprine serum albumin in orthorhombic crystal system (–)	5ORI_A	101/27/39	30,0/7,50	68,7/5,58
30	Serotransferrin (*TF*)	XP_027816111.1	326/51/61	70,0/6,50	77,4/6,31
31	C-terminal fragment of myosin-7 (–)	XP_004010374.1	447/94/42	140,0/5,20	224,1/5,59
32	endoplasmic reticulum chaperone BiP (*HSPA5*)	XP_004005686.1	297/27/45	71,0/5,00	72,5/5,07
33	Mix of D-beta-hydroxybutyrate dehydrogenase, mitochondrial (*BDH1*), voltage-dependent anion-selective channel protein 1 (*VDAC1*)***(1), fragment of ATP synthase subunit alpha, mitochondrial (*ATP5F1A*), electron transfer flavoprotein subunit alpha, mitochondrial (*ETFA*)***(1)	XP_004003074.1 NP_001119824.1 XP_004020569.1 XP_004017875.1	104/16/42 88/9/51 128/8/21 155/5/22	30,0/7,10	38,7/8,76 30,8/8,62 59.8/9.21 35,4/8,77
34	C-terminal fragment of creatine kinase M-type (*CKM*)	XP_012045938.1	284/28/63	34,0/7,20	43,3/6,66
35	Mix of NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial (*NDUFV2*)***(2), thioredoxin-dependent peroxide reductase, mitochondrial (*PRDX3*)***(1)	XP_014959141.2 XP_004020276.3	207/25/60 230/13/69	25,0/5,30	27,3/8,21 28,2/7,75
36	thermal shock protein beta-1 (*HSPB1*)***(1)+ Phospho (78S)	XP_027817273.1	236/24/91	24,0/5,35	22,4/6,22
37	Mix of glutamine amidotransferase-like class 1 domain-containing protein 3A, mitochondrial (*GATD3*), fragment of cytochrome b-c1 complex subunit 2, mitochondrial (–), fragment of four and a half LIM domains protein 2 (*FHL2*)***(1)	NP_001156032.1 XP_004020879.1 XP_004006164.1	127/18/59 95/11/43 145/4/15	25,0/7,70	29,0/8,76 48,4/8,89 32,0/7,80

* S / M/ C — traditional indicators of identification, adopted in the English literature: Score — indicator of compliance or "scoring"; Match peptides — the number of matched peptides; Coverage —% coverage of the complete amino acid sequence of the protein by the identified peptides.

** Mm/pI (exp.) are the estimates obtained from the results of electrophoretic mobility on DE, and Mm/pI (calc.) are calculated estimates obtained from amino acid sequence data, taking into account the removal of the signal peptide, but without taking into account other postsynthetic modifications using the ExPASy Compute pI / Mw tool.

*** msms is an indication of confirmatory identification by tandem mass spectrometry, the number of sequenced tryptic peptides is shown in brackets.

**** Due to the pandemic the use of the resources of the Mascot search software package (which was engaged in transferring information resources mainly to COVID-19 research) limited the possibilities of using the NCBI protein database (USA), and in some cases the SwissProt/ database was used for identification. Uniprot (European Union).

Fraction No. 3 turned out to be the product of HSPA8, which is also standard substance for the farm animals. The HSPB8–9 family is thermal shock proteins, chaperones responsible for folding and the response to unfavorable conditions which affect the cells, so their number should increase. It is obvious that the role of HSPA8 in this process is more significant.

In general, it can be noted that along with an increase in the count and activity of glutathione peroxidase 3 in the blood, these processes may indicate an increased count of selenium in an animal's body.

In addition the changes were also noted in other proteins in the animals of the group 1. Fractions No. 20 (a complex mixture of argininosuccinate synthase, mitochondrial citrate synthase, a homologue of the α-subunit component of pyruvate dehydrogenase E1 and methylmalonate-semialdehyde dehydrogenase) and No. 22 (a mixture of isocitrate dehydrogenase [nicotinamide adenine dinucleotide phosphate — NADP] and serine peptidase secernin-2, which is still hardly studied, but which is actively expressed in the kidney) decreased in their count, although they were profoundly present in the group 4.

Fractions No. 21 (a mixture of S-formylglutathione hydrolase, fragment of aflatoxin B1 of aldehyde reductase (another isoform), mitochondrial serine protease HTRA2), No. 24 (a complex mixture of cytoplasmic isocitrate dehydrogenase [NADP] — the major component, arginine succinate synthase homologue, 3-ketoacyl-CoA thiolase peroxisome, mitochondrial 2-amino-3-ketobutyrate coenzyme A ligase, α-mannose-1-phosphate guanyltransferase, and mitochondrial citrate synthase), No.25 (mixture of mitochondrial delta-1-pyrroline-5-carboxylate dehydrogenase, acyl-coenzyme A synthase ACSM1 and cytosolic phosphoenolpyruvate [GTP] carboxykinase), No. 26 (a mixture of serum transferrin and ezrin (EZR) increased their quantitative count.

Two-dimensional electrophoresis (TDE) according to O'Farrell was used as the main proteomic technology. To identify proteins, individual fractions were excised from DE, the excised fragments were crushed, and trypsinolyzed. Next, the corresponding sets of peptides were studied by MALDI-TOF MS and MS/MS mass spectrometry on MALDI.

The contours of fractions No. 23 (a mixture of mitochondrial hydroxyacyl-coenzyme A dehydrogenase and malate dehydrogenase) and No. 27 (mitochondrial protein 60 kDa with signs of deamination by a. o.NQ) changed, which proved the loss or blocking of some of the charged groups in the amino acid sequence of proteins.

As a result, it can be concluded that the introduction of selenium derivatives into the diet of lambs brings on certain change in the protein composition in the cortical substance of the kidneys (at the first stage of blood filtration) — i. e. the count of some isoforms of selenium-containing proteins, and the enzymes of the glutathione cycle associated with them, as well as how reactions to a possible toxic effect — participation in this process of thermal shock proteins, utilization of aflatoxin homologues and changes in energy metabolism within the kidney cells mitochondria.

Group 2 (the group that consumed the diet supplemented with iodine derivatives). Fractions No. 6, 10 and 8 (phosphate diethanolamine-binding protein) in all groups were found in equal amounts and at standard positions. During fattening in the groups 3 and 4, fraction No. 7 (mitochondrial superoxide dismutase [Mn]) was no longer detected, but fractions No. 11 and No. 9 appeared there. Those fractions were identified as mitochondrial superoxide dismutase [Mn]), but their electrophoretic properties were altered towards an increase in molecular weight of 1–2 kDa (no differences in the fragmentation spectra were found). The changed properties may affect the functional activity of the enzyme under the given conditions of fattening. It can be considered as a potential biomarker to determine the functional activity of the enzyme for the proper management of the fattening conditions. It is quite possible that primary structure of the enzyme molecule was not completely unfolded and deployed because of the interaction of iodine ions with certain amino acid residues, due to which failure the electrophoretic mobility of SOD2 molecules changed.

The count of fraction No.13 (mixture of mitochondrial enoyl-CoA hydratase, alcohol dehydrogenase [NADP(+) and carbonic anhydrase 2) also grew up. It was difficult to define which component was the leading one, but the mitochondrial protein was highly likely the predominant one.

In group 2, fraction No. 16 appeared (mixture of aspartoacylase — zinc dependent hydrolase, lambda-crystallin homologue, cytoplasm isocitrate dehydrogen [NADP] homolog (IDH1). The same appeared in the fraction No. 18, but in a slightly smaller count, and in case of diet 3 it was found almost in the same composition (mixture of aspartoacylase — zinc dependent hydrolase, lambda-crystallin homologue, cytoplasm isocitrate dehydrogenase [NADP] homologue and nicotinate nucleotide phosphorylase appeared additionally. The predominant component was aspartoacylase.

The number of changes was also found in the myocardial tissue. In group 1 there was an increase in the count of such proteins (No. 30) as the C-terminal fragment of the heavy chain of myosin 7, No. 31 — the BiP chaperone of the endoplasmic reticulum, No. 28 — an albumin fragment, and No. 37 — a mixture of mitochondrial glutamine aminotransferase-like class 1 domain — including protein 3A (*GATD3*), a fragment of the mitochondrial subunit 2 of the cytochrome b-c1 complex and a fragment of protein 2 containing a 4.5 LIM domain. The GATD3 gene product is dominant. The amount of fraction No. 36, which is a phosphorylated form of thermal shock protein β-1, decreased. In group 2, like in group 1, the number of fractions of homologues No. 30, 31 increased and the amount of transferrin homologue was repeatedly increased, which

proved a certain relationship between excess iodine ions and iron metabolism in the animal body, which may indicate increased iodine digestion and fixation. In group 3 a certain increase was noted in the amount of fraction No. 32 (a complex mixture of mitochondrial D-β-hydroxybutyrate dehydrogenase, mitochondrial porin 1, a fragment of mitochondrial ATP synthase and mitochondrial electron-carrying flavoprotein), which once again showed the specificity of participation in the digestion and fixation of the additives used by mitochondria from different organs and tissues. And in the control group (no. 35) there was a fraction containing a mixture of mitochondrial electron-transporting flavoprotein 2 NADH of dehydrogenase and mitochondrial thioredoxin-dependent peroxide-reductase, which disappeared in all fattening diets.

As a result, it can be noted that under the conditions of fattening diet change, the mitochondrial apparatus of some tissues and organs of sheep also seriously changes its functional orientation in order to adapt to the changed conditions, bringing on the activation of other metabolic cycles (Figure 2).

The role of feed additives in the diet of lambs has been hardly studied by the proteomic technologies. There is only one publication on the results of flaxseed and/or quinoa introduction into the sheep diet. It was found that in one group of tested sheep the protein complex of desmin and troponin T degraded, also a large number of fractions and phosphorylated isoforms of fast light chains of myosin 2 was detected, obviously under the influence of herbal supplements [14].

In other types of farm animals, the effect of selenium has been studied in a quite detailed way, but with the help of other methods [17]. In general, the authors of the studies note the dominant role of glutathione peroxidases, which is confirmed by our studies as well.

Conclusion

68 two-dimensional electrophoregrams were obtained (108 proteomic maps in various variants of staining/detection). Time-of-flight mass spectrometric identification of 91 fractions from DE was carried out, 175 proteins or their large fragments were identified.

When searching for proteins-markers of the effect of various diets for fattening lambs in the tissue of their myocardium and cortical substance of the kidneys, a number of differences were found.

The introduction of selenium derivatives into the diet of lambs brings on a change in the protein composition in the cortical substance of the kidneys in some isoforms of selenium-containing proteins and related enzymes of the glutathione cycle, as well as it is reaction to a possible toxic effect — the participation of thermal shock proteins in this process, utilization of aflatoxins and changes in energy metabolism in the mitochondria of the kidney cells. The determination of the activity or count of protein in the blood of glutathione peroxidase 3 can be a promising biomarker for controlling the level of digestion, fixation and the amount of selenium in tissues. To assess the role of iodine in the fattening diet, it seems promising to determine the activity of mitochondrial superoxide dismutase [Mn]. In the myocardium, the introduction of feed additives mainly influenced on the mitochondrial apparatus, which seriously changed its functional orientation together with activation of several other metabolic cycles.

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