ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Proteomic Protein Research In The Samples Of Poultry Grown Under Different Conditions.

DY Ismailova[1]*, VG Volik [1], VS Lukashenko[2], IP Saleeva[2], and VY Morozov[3].

[1]All-Russian Scientific Research Institute of Poultry Processing Industry - branch of the Federal State Budgetary Scientific Institution, Federal Scientific Center "All-Russian Research and Technological Institute of Poultry Farming" of Russian Academy of Science. Rzhavki sett., Moscow region., Russia.
[2]Federal State Budget Scientific Institution Federal Scientific Center "All-Russian Research and Technological PoultryInstitute" of Russian Academy of Sciences. 141311, Moscow region, Sergiev Posad, st.Pticegradskaya, 10, Russia
[3]Stavropol State Agrarian University, Zootekhnicheskiy lane 12, Stavropol 355017, Russia.

ABSTRACT

For proteomic analysis were used samples of breast and thigh tissues of cell and floor cultivatied broiler chickens of 38 and 49 days of age. During the study, proteomic maps of 8 broiler meat samples were obtained. In birds of 49 days of growth (compared to 38 days) no significant changes in protein profiles were observed, but the samples of femoral tissues were characterized by increased production of myosin proteins. Proteins that are the biomarkers of meat quality were established, as well as unique proteins present in the meat based on different technologies of cultivation. Proteomic data showed that proteins associated with glucose metabolism (glycolysis), signal transmission (including calcium), as well as molecular chaperones, can play a crucial role in the formation of taste of poultry meat products. The tender structure of meat is characterized by the presence of a large number of additional fragments of structural proteins, such as actin, troponin and myosin – heavy and light chains. The largest number of additional fragments of these proteins was found in breast meat samples with cell cultivation.

Keywords: broiler chickens, cell cultivation, floor cultivation, meat quality, proteomic proteins, actin, troponin, myosin.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The emergence of new proteomic methods in recent years significantly contributes to the understanding of biological mechanisms that affect the taste of food products, including meat, and the detection of various biomarkers of meat quality. For example, previously pigs protein profiles were analyzed and have identified the proteins associated with low water-holding capacity of meat, which worsened its functional properties. For poultry, various researchers have noted calcium-binding proteins, glycolytic enzymes, and heat shock proteins as potential biomarkers of the quality of meat and products derived from it.

Broiler meat, obtained with widely used accelerated intensive fattening, has a number of significant drawbacks. About 15% of broiler meat has pronounced signs of pale, soft and exudative meat (PSE-like syndrome), insufficiently formed muscle and connective tissues, unformed bone tissue, reduced antioxidant capacity, weakly expressed smell and taste. To solve the foregoing problems, a comprehensive approach is necessary, including the study of poultry proteome (qualitative and quantitative composition of marker proteins responsible for the quality of meat products), antioxidant capacity and organoleptic characteristics.

On the basis of the data obtained, it will be possible to establish the relationship between molecular markers, the quality of raw meat and technological approaches (feed rations and cultivation technologies).

Analysis of literary sources from the position of contribution to the formation of the given properties of raw meat gives reason to believe that the technology of cultivation is one of the main determining factors of influence on the quality of raw meat. A large number of studies on the exposure and identification of proteins and peptides in all raw sources of animal and plant origin are carried out worldwide [1]. At present, a large number of polypeptide substances containing approximately 2-30 amino acids and participating in the mechanisms of formation of quality characteristics of raw meat and, indirectly, the finished product are isolated from raw meat materials, especially from muscle proteins of beef, poultry, pork. Muscle proteins associated with meat tenderness and water-holding capacity (WHS) have been extensively studied [2, 3].

It is proved that inhibition of lipid oxidation improves the taste, texture and nutritional value of meat products. Hydrolysis and oxidation of dietary fat have long been a subject of comprehensive studies from the point of view of studying the mechanism and dynamics of the process itself, as well as to identify ways of active deceleration of oxidation that affect the quality of the product [4].

In connection with the foresaid, the aim of this research was to study the effect of the cultivating conditions of broiler chickens (cell and floor cultivation) on the proteomic profile of poultry.

MATERIALS AND METHODS

The following drugs were used for proteomic analysis:

- Samples of breast and thigh tissue of broilers, cell cultivation ("Cell", 38 and 49 days);
- Samples of breast and thigh tissue of broilers, floor cultivation ("Floor", 38 and 49 days).

Protein extraction was performed in three stages:

1) Blender homogenization;
2) Lysis buffer homogenization with Potter homogenizer: 100mg tissue + 400 µl LB (1% Dithiotreitol (DTT); 4% CHAPS; 7M urea; 2M thiourea; 5% ampholites 3/10);
3) CF 800g, 5 minutes

Two-dimensional electrophoresis (2-DE) followed by MALDI –TOF/TOF mass spectrometry was used as the main proteomic technologies.

Two-dimensional electrophoresis (2-DE)

2-DE electrophoresis was performed by the method described in [O'farrell, 1975] on the PROTEAN II xi 2-D Cell system (Bio-Rad, USA).

When performing isoelectric focusing, the pH gradient was: from 3 to 10 (or 5-7, servalite, Serva Electrophoresis). The sample quantity was 70-100 µg of protein per tube. Isofocusing was performed for 16 hours in the following modes: 100V - 45 min, 200V - 45 min, 300V - 45 min, 400V - 45 min, 500V - 45 min, 600V - 45 min, 700V - 10 h, 900V - 1.5 h.

The subsequent electrophoresis of the samples obtained after isoelectric focusing was carried out in a gradient acrylamide DDS gel (7.5-25%) at a voltage of 300V. Before applying to the second direction, the samples were incubated for 20 min in a solution containing dithiotreitol (6M urea, 2% DDS-Na, 10mM DTT, 0.5 M TRIS-HCl, pH=6.8) to prevent oxidation of sulfhydryl groups in proteins. For visual analysis of protein components distribution gels were stained with silver nitrate and for mass spectrometric analysis with Brilliant Blue R Staining Solution (Sigma, USA).

To produce protein maps, a system of gel documentation Infinity1000/26MX (Vilber Lourmat) was used. Protein maps were analyzed using ImageMaster 2D Platinum, v.7 ("GE Healthcare").

Mass spectrometry analysis of proteins

For mass spectrometry analysis, gel pieces of 3-4 mm^3 in size corresponding to protein spots were cut out and washed twice to remove dye in 100 µl of 40% acetonitrile solution in 0.1 M NH4HCO3 for 20 minutes at 37 °C. After removing the solution, 100 µl of acetonitrile was added to dehydrate the gel. After removing the acetonitrile and drying the gel, a solution of modified trypsin (Promega) in 0.05 M NH4HCO3 with a concentration of 15 µg/ml was added. Hydrolysis was carried out for 8 h at 37 °C, then 0.5% TFA in 10% aqueous acetonitrile was added to the solution. The overgel solution containing the protein hydrolyzate was used for mass spectrometry analysis. The matrix used was a solution of 2.5-dihydroxybenzoic acid (Aldrich, 10 mg/ml in 20% aqueous acetonitrile, 0.5% TFA).

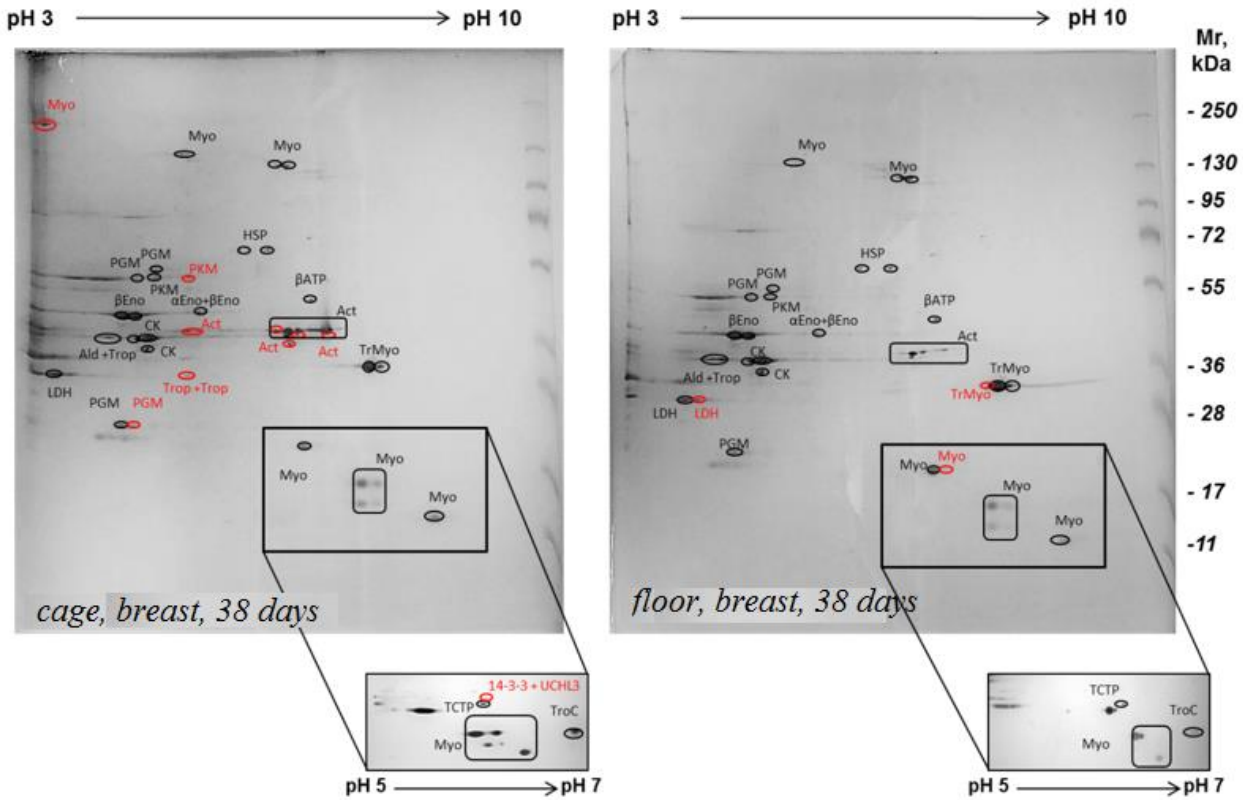
Mass spectra were obtained on an MALDI time of flight mass spectrometer Ultraflex II BRUKER (Germany) equipped with a UV laser (Nd) in the positive ion mode using a reflectron; the accuracy of the measured monoisotopic masses after the calibration over the peaks of trypsin autolysis was 0.005% (50 ppm). Spectra were obtained in the mass range of 700–4500 m/z, choosing the laser power optimal for achieving the best resolution. To obtain fragmentation spectra, the tandem mode of the instrument was used; the measurement accuracy of fragment ions was not lower than 1 Da.

Mass spectra were processed using the FlexAnalysis 3.3 software package (Bruker Daltonics, Germany). Protein identification was performed using the Mascot program (www.matrixscience.com). To do this, using the peptide fingerprint option, we searched the NCBI database (www.ncbi.nlm.nih.gov) among the proteins of all organisms with the above accuracy, taking into account the possible oxidation of methionines with atmospheric oxygen and the modification of cysteines with acrylamide gel. Candidate proteins having a confidence parameter of score > 76 in the NCBI database were considered to be reliably identified ($p < 0.05$), proteins having a confidence parameter of score > 50 were considered probable. Using the software Biotools 3.2 (Bruker Daltonics, Germany), a search was performed for the combined MS + MS / MS results.

RESULTS AND DISCUSSION

During the study, proteomic maps of 8 samples of broiler meat were obtained (Fig. 1). After 49 days of growth (as compared to 38 days), no significant changes in protein profiles were observed in birds, but increased protein production of the myosin fraction was characteristic of thigh tissue samples. Therefore, further, the results obtained for birds of 38 days of growth are mainly discussed.

ISSN: 0975-8585



A low molecular weight region was isolated, for which additional research was carried out (silver staining). Proteins unique to the sample are marked red.

Figure 1: Two-dimensional electrophoregrams of broiler meat samples.

The protein fractions were located in a wide range of pH and molecular weights. Fractionation of samples by two-dimensional electrophoresis allowed us to obtain up to 100 protein fractions, stained with a Kumasi Brilliant Blue R solution (Table 1).

Table 1: The number of protein components present on electrophoregrams

	Sample code	Number of detected proteins	Number of unique proteins
Cage, Breast, 38 days	K1	90	15
Floor, Breast, 38 days	K2	67	5
Cage, Thigh, 38 days	K3	95	9
Floor, Thigh, 38 days	K4	98	5

Meanwhile, from 5 to 15 proteins were unique for a specific sample, some of them were in quantities sufficient for mass spectrometric identification. As a result of a comparative analysis of protein maps of the proteome, it was found that the overlap of protein components obtained by separation in electrophoregrams is 85-95%. Analysis of the gels showed no significant differences in the thigh muscles of birds with cage and floor rearing, and slightly more pronounced differences in the breast muscle samples.

Protein components have been identified. Most of the tryptic protein spectra corresponded to the sequences found in the chicken database (Gallus gallus), but for some homologs were found in other species. Among the identified proteins, more than half (61%) comprised proteins of structural function (including fibrillary): actin, myosin, tropomyosin, troponin; type 1 collagen, tectin-2, and parvalbumin were also identified.

The conducted proteomic analysis revealed the localization zones of important structural muscle proteins: actin, myosin, tropomyosin, troponin (Fig. 1). For the main participants of muscle contraction

(myosin, actin, tropomyosin, troponin), significant changes in product profiles depending on the sample are shown. In each group of proteins there are "stable" forms, the production of which is unchanged from sample to sample. However, individual protein components were identified, varying in both quantity and quality. For example, the sample **K1** (Cage, Breast) is characterized by the production of a number of forms of α-actin, which are absent in other samples. Also for **K1** there is an increased production of myosin light chains, and two unique forms of troponin T (Troponin T, fast and slow skeletal muscle isoforms). Also in sample **K1**, increased production of Troponin C (9.6 times as compared to **K2**) – a protein of slowly contracting skeletal fibers – was observed. Troponin C is a key protein that transmits the calcium signal to the contractile complex and is responsible for the contraction of the striated muscles, while its activity is regulated by the intracellular concentration of Ca^{2+} ions. Changes in its products may indicate a transformation of the type of muscle fibers [5].

Sample **K2** (Floor, Breast) is characterized by changes in the spectrum of the heavy and light chains of myosin and the α-chains of tropomyosin, apparently due to amino acid substitutions or modifications of these forms.

The second widely represented group consists of enzymes, mainly the pathways of glycolysis and glucose metabolism. The main soluble protein components of the proteome are aldolase, glyceraldehyde-3-phosphate dehydrogenase, α- and β-enolase, pyruvate kinase, phosphoglycerate mutase, phosphoglucomutase, L-lactate dehydrogenase and creatine kinase.

In the pectoral muscle of birds, glycolysis is one of the main ways of obtaining energy for muscle contraction and to meet energy needs during growth. To maintain muscle mass, as well as to meet the needs of the contractile muscles of the bird need a significant amount of energy. Therefore, it is not surprising that glycolytic enzymes predominate in the fraction of soluble proteins. For all samples in this category, there is also a "minimal" conservative set of proteins that do not change their products. For example, the enolase family is equally represented in K1-K4. Previously, it has been shown that the β-isoform of the enzyme prevails in adult birds, while α-enolase is more characteristic of chickens and growing birds [Doherty, 2004]. Moreover, the ratio α: β can reach 1:93 in adults. In our case, the predominance of β-forms is also observed.

In sample **K1**, additional forms of phosphoglyceramutmutase and pyruvate kinase were detected. Enzymes of the glycolytic pathway are capable of catalyzing the formation of intermediate products - glycolysis regulators. Thus, we were able to identify changes in the production of enzymes that directly limit the rate of glycolysis (pyruvate kinase), as well as enzymes that play an important role in regulating the production of metabolic intermediates (phosphoglycerate mutations). Previous studies have shown that an increase in the rate of glycolysis leads to a rapid decrease in the pH of the muscles, which ultimately affects the quality of the meat.

Animal studies have shown that plasma creatine kinase and L-lactate dehydrogenase (LDH) activity can be used to assess stress conditions. The creatine kinase fraction is unchanged for all 4 samples, while an additional LDH isoform appears on **K2**. An increase in LDH levels reflects changes in the permeability of muscle cell membranes with consequent muscle damage, which can lead to a decrease in meat quality.

Among other identified proteins, homologues of heat shock proteins (chaperones of the HSP70 family), apolipoprotein A1, translationally controlled tumor protein (TCTP), protein of the 14-3-3 family, and ubiquitin-carboxy-terminal hydrolase L3 were presented. Moreover, the last 2 proteins are unique for sample **K1**, and TCTP production in this sample was increased compared to **K2** (5 times). Recent studies have established the relationship between heat shock proteins and meat quality, such as tenderness and moisture-holding capacity of meat [6, 7].

So in [8] it was shown that the level of heat shock protein HSP70 in meat of chickens with PSE syndrome was significantly lower than in the fowl of a normal group. At the same time, HSP70 is associated with increased production of glycolysis proteins, structural proteins and molecular chaperones, which, in turn, is associated with an unknown mechanism leading to deterioration in meat quality. A similar picture is observed in the protein profile of sample **K1** (cellular content), which may indicate a lower quality of meat with signs of the PSE syndrome of this sample of meat compared to sample **K2** (floor housing of poultry).

The proteins unique to **K1** can be classified as signal transmission proteins (Signal transduction), their homologs were previously found in muscle samples of pigs and cattle [Xing et al., 2017], where the authors also associate their increased production with stressful conditions. TCTP protein is highly specific in various species, is expressed everywhere, but its mRNA level varies depending on cell type and developmental stage. The spectrum of its biological activity is very wide, and expression is assumed to be strictly regulated at different levels and different mechanisms [9, 10].

Also for sample **K3** (Cell, thigh) compared with **K4**, decreased production of HSPB1 (2.5 times) – a small heat shock protein, which plays a role in the body's resistance to stress and actin organization. Researchers associate a decrease in HSPB1 protein expression with the development of PSE syndrome, manifested as pale, soft, and exudative meat [11].

For all samples, the presence of apolipoprotein A1, the main carrier of high-density cholesterol, is shown. In humans, apolipoprotein A1 is associated with lipid absorption and modulation of body weight, however, for chicken, a different role of this protein has been established, associated with the formation of triglyceride-rich lipoproteins [12].

In floor and cage broilers, the protein profile of meat samples of breasts and thighs is different. For the main participants of muscle contraction (myosin, actin, tropomyosin, troponin), significant changes in product profiles depending on the sample are shown. In each group of proteins there are "stable" forms, the production of which is unchanged from sample to sample. However, individual protein components were identified, varying in both quantity and quality. At the same time, proteins that are markers of PSE-syndrome, manifested in the form of pale, soft and exudative meat, were found in samples of broiler meat at the cellular content.

In this regard, presumably, the meat of the breast and thigh of broilers of floor content, as compared with broiler meat of cellular content, has a higher quality.

The tasting assessment on a 5-point scale showed that the taste and aromatic advantages of the broth, with outdoor broiler rearing, had the highest scores - 4.68 points, whereas with cell-growing - 4.55 points.

The taste of meat with outdoor cultivation was also higher than with cellular. Pectoral muscles were estimated at 4.55 points, leg muscles - at 4.40 points. With cell cultivation - 4.47 and 4.37 points, respectively.

These proteomic studies have shown that proteins associated with glucose metabolism (glycolysis), signal transmission (including calcium), as well as molecular chaperones, can play a crucial role in shaping the taste of meat products from poultry. Table 2 presents proteins that are biomarkers of meat quality and are unique for the studied samples of poultry meat. The tender structure of meat is characterized by the presence of a large number of additional fragments of structural proteins, such as actin, troponin and myosin - heavy and light chains. The largest number of additional fragments of these proteins was found in samples of breast meat with cellular content.

Table 2: Proteins unique to poultry meat samples

Symbol	Protein homotypes	Number in NCBI Database	Observed mM, kDa	Covering, %	Estimated IEP	Score	Organism
Structural Proteins							
Actin							
	Actin, alpha skeletal muscle, partial	KFV05090.1	37	67	5,66	225	Tauracoerythrolophus
	Actin, alpha skeletal muscle, partial	KFQ14345.1	38	78	5,66	341	Leptosomus discolor
	Actin, alpha skeletal muscle, partial	KFQ14345.1	35	81	5,66	358	Tauracoerythrolophus

	Actin, alpha skeletal muscle, partial	KFV05090.1	38	74	5,66	259	Tauracoerythrolophus
	Actin, alpha skeletal muscle, partial	KFQ14345.1	38	83	5,66	308	Leptosomus discolor
Myosin							
	Myosin, heavy chain 1E, skeletal muscle	NP_001013415.1	165	31	5,63	272	Gallus gallus
	Myosin light chain 1, skeletal muscle isoform X2	XP_015144628.1	19	89	4,55	287	Gallus gallus
Troponins							
Trop	Troponin T, fast skeletal muscle isoform	XP_010709329.1	32	35	6,4	143	Gallus gallus
	Troponin T, fast skeletal muscle isoform	XP_010709326.1	29	29	6,09	179	Meleagrisgallopavo
	Troponin T, slow skeletal muscle	NP_990445.1	29	62	5,95	251	Gallus gallus
Tropomyosins							
	Tropomyosin alpha-1 chain isoform X1	XP_015134260.1	31	75	4,7	437	Gallus gallus
Enzymes							
Creatine Kinase							
	L-lactate dehydrogenase A chain	NP_990615.1	29	83	7,75	264	Gallus gallus
Glycolysis							
	Phosphoglycerate mutase 1	NP_001026727.1	22	85	7,03	217	Gallus gallus
	Pyruvate kinase PKM	NP_990800.1	51	63	7,29	282	Gallus gallus
Other							
UCHL3	Ubiquitin carboxyl-terminal hydrolase isozyme L3, partial	XP_010204976.1	20	18	4,94	144	Coliusstriatus
14-3-3	14-3-3 protein epsilon isoform X2	XP_015151225.1	20	89	4,77	290	Gallus gallus

CONCLUSION

For a more accurate determination of the biomarkers of broiler chicken meat quality, it is necessary to conduct a correlation analysis between the identified unique proteins in the meat samples and the quality of this meat.

ACKNOWLEDGMENTS

The work was prepared with the support of the Russian Science Foundation, Agreement No. 17-16-01028.

REFERENCES

[1] Ohlendieck K. Skeletal muscle proteomics: current approaches, technical challenges and emerging techniques. Skeletal Muscle, 2011; 1(6).

[2] Wu W., Fu Y., Therkildsen M., Li X.-M., Dai R.-T., Molecular understanding of meat quality through application of proteomics. Food Rev. Int, 2015; 31: 13-28.

[3] Xing T., Wang C.,Zhao X.,Dan C.,Zhou G.,Xu X. Proteome analysis using iTRAQ reveals the alterations in stress-induced dysfunctional chicken muscle.J.Agrical.a. Food Chem, 2017.

[4] Faustman C., Sun Q., Mancini R., Suman S.P. Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Science, 2010; 86: 86-94.

[5] Lomiwes D., Farouk M. Wiklund E., Young O. Small heat shock proteins and their role in meat tenderness: A review. Meat Sci, 2014; 96: 26–40.

[6] Zhang M., Wang D., Geng Z., Bian H., Liu F., Zhu Y., Xu W. The level of heat shock protein 90 in pig Longissimus dorsi muscle and its relationship with meat pH and quality. Food Chem, 2014; 165: 337–341.

[7] Xing T., Wang P., Zhao L.; Liu R.; Zhao X.; Xu X., Zhou G., A comparative study of heat shock protein 70 in normal and PSE (pale, soft, exudative)-like muscle from broiler chickens. Poultry Science, 2016; 95(10): 2391-2396.
[8] Guillaume E. et al. Cellular distribution of translationally controlled tumor protein in rat and human testes. Proteomics, 2001; 1(7): 880-889.
[9] Chen Z. et al. The expression of Amphi TCTP, a TCTP orthologous gene in amphioxus related to the development of notochord and somites. Comparative Biochemistry and Physiology, 2007; 147(3): 460-465.
[10] Laville E. et al. Meat Sci, 2005 ; 70(360) : 167-172.
[11] Weinberg R. B., et al., J. Lipid Res, 2000 ; 41 : 1410-1418.
[12] Steinmetz A. et al, Trends Cardiovasc. Med, 1999; 9: 153-157.