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The journal "Theory and practice of meat processing" is an international peer-reviewed scientific journal covering a wide range of meat science issues.

We publish original results of the fundamental and applied research in the field of:

- regulation of feeding rations and keeping conditions of animals (including poultry);
- targeted modification (selection, hybridization, operative manipulation);
- processing of meat raw materials;
- improvement of technologies for meat product manufacture;
- study of effects of meat and meat product consumption on human health;
- rational use of secondary resources of the meat industry and the ecological problems of the industry

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FOOD ALLERGEN CONTROL AT MEAT PROCESSING ENTERPRISE: SCIENTIFIC RATIONALE AND PRELIMINARY HAZARD ANALYSIS

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Keywords: *allergens, allergen control, meat products, questionnaire survey, PCR*

Abstract
From a public health point of view, the control of food allergens in enterprises is one of the main methods of food safety management required by national and international standards. The implementation results of measures for allergen control and identification of noncompliance are presented using the developed checklist, which includes 41 questions. The survey was conducted at meat processing plant in the Moscow region, which is certified for compliance with the requirements of ISO 22000:2018 "Food safety management systems — Requirements for any organization in the food chain" and FSSC22000 certification scheme. Compliance with the criteria included in the checklist was assessed by the method of interviewing employees at the enterprise and direct on-site observation. The highest level of noncompliance according to 7 groups of criteria established in the checklist was identified in the following sections; "Cleaning", "Transport and storage" and "Hazard awareness". Factors complicating the implementation of allergen control activities include available methods to assess cleaning effectiveness when removing specific allergens, experience in separating allergen-containing and allergen-free products and raw materials during transport and storage, and staff training in allergen control. At the same time, the PCR method was used to study 15 samples of meat products manufactured at the selected enterprise for the presence of legumes (soybeans), gluten, mustard, and peanuts. In six samples, undeclared allergens were detected in quantities hazardous to the health of the consumer. The results obtained indicated the need to develop and implement measures aimed at minimizing the risk of allergen transfer to the meat products during their production. Based on the results of the research, a procedure for allergen control has been developed, including additional measures for the control of food allergens.

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Introduction

Food allergens have a negative impact on health and quality of life of people with hypersensitivity to certain food ingredients.

The reaction of an allergic person consumed soy-containing food products may be different. Examples include loose stools, abdominal pain, asthma attack, exacerbation of eczema, difficulty in breathing and anaphylactic shock [1].

Gluten allergy is usually associated with gastrointestinal dysfunction (cheilitis, gastritis, colitis, gastroenteritis, irritable bowel syndrome), skin manifestations (atopic dermatitis, urticaria, angioedema) and, less commonly, with respiratory dysfunction and systemic manifestations such as anaphylactic shock [2].

Symptoms of a true food allergy to mustard most often develop within a few minutes or, less often, in a couple of hours after contact with it. Mild symptoms may include tingling or itching in the mouth, nausea and abdominal discomfort, and rashes in various places (also similar to urticaria). More severe symptoms include face, throat and/or mouth swelling, difficulty in breathing, and status asthmaticus. In some cases, there is a severe decrease in blood pressure (anaphylactic shock). Most often, this is accompanied by severe weakness, dizziness, and rapid heartbeat [3].

Peanut is a strong allergen containing up to 32 different proteins, of which at least 18 are capable of causing an allergic reaction. Among them, vicilin, a reserve protein of seeds, a heat-resistant main allergen (amounts up to 12–16% of the total protein in peanuts); conglutin; profilin; albumin, a heat-resistant protein that does not break down during digestion; lipid transfer protein. From a biological point of view, it is not a nut, but a seed of a plant from the legume family. It is widely used in the food industry and is often a "hidden" allergen in products that, at first glance, do not contain it. Among all legumes, it has the most allergenic properties and may cause life-threatening allergic reactions, allergic shock, angioedema, urticaria, exacerbation of respiratory allergies and atopic dermatitis. With a

certain heat treatment, the allergenic properties of peanuts are enhanced. The allergenicity of peanuts depends on the degree of heat treatment: dry roasting increases the allergenicity, although it decreases during the cooking process as, presumably, part of the protein transfers into the water [3].

Time period of the peanuts introduction into the diet significantly affected the prevalence of peanut allergy among Israeli schoolchildren [4]. Israeli children consumed more peanuts during their first year of life than the British children, and the prevalence of peanut allergy was 0.17% in Israel versus 1.85% in the UK; while changes in atopy, social class, or genetic background had no significant effect [4]. In the US, in just 4 years (2006–2010), the number of people with peanut allergies doubled. At the same time, the number of cases of anaphylactic shock caused by peanuts doubled over a five-year observation period [5]. In addition, the form in which peanuts are consumed may determine whether an allergic response occurs. The stability and allergenicity of allergenic proteins may be altered during food processing. For example, roasting of peanuts affects the stability of peanut allergens through the Maillard reaction, and modified peanut allergens have an increased ability to bind IgE [6]. However, there is no conclusive evidence to link changes in eating habits or in the food industry with an increase in the prevalence of food allergies [4].

In the US, milk, eggs, and peanuts are the most common allergenic foods in children, while adults are more likely to be allergic to shellfish, peanuts, and tree nuts [5]. Many children will get through food allergies and become more tolerant to milk, eggs, soy, and wheat. Allergies to peanuts, tree nuts, and shellfish rarely decrease with age [5].

In the Russian Federation, the mechanisms for controlling the content of allergens (including gluten) in food products, unfortunately, still need to be addressed.

Clause 13 of part 4.4 of article 4 of CU TR 022/2011 "Food products in terms of their labeling"[1] contains a requirement to indicate in the composition of food products components (including food additives, flavors), biologically active additives, the use of which may cause allergic reactions or is contraindicated in certain types of diseases and which are specified in clause 14 of part 4.4 of article 4 of CU TR 022/2011, regardless of their amount.

In addition, in accordance with the Technical Regulation of the Customs Union CU TR 027/2012 "On the safety of certain types of specialized food products, including therapeutic and preventive nutrition"[2], gluten-free food products must be made from one or more components that do not contain wheat, rye, barley, oats or their cross-bred variants and/or must be made in a special way (to reduce gluten levels) from one or more components that are derived from wheat, rye, barley, oats or their cross-bred variants, in which the level of gluten in ready-to-eat products is not more than 20 mg/kg.

A number of publications by foreign authors contain the results of studies to establish the concentrations of food allergens that may cause an allergic reaction [7,8,9,10].

Voluntary Incidental Trace Allergen Labeling (VITAL®) is a scientifically based standardized allergen risk assessment process used by the food industry in Australia, New Zealand and a large number of companies in other countries. The VITAL® program is based on scientific research by a group of scientists from New Zealand and the USA to establish threshold doses of allergens that may cause an allergic reaction [7,8,9,10]. The goal of the VITAL® program is to ensure that manufactured foods are safe for the majority of consumers suffering from food allergies by providing precautionary labeling criteria that enable consumers with allergies and their caregivers to avoid purchasing foods that may be hazardous for them.

To date, an updated version of the VITAL® program is in use, i. e. VITAL® 3.0. Changes in allergen doses that may cause an allergic reaction in sensitive people in the new version 3.0 of the VITAL® program compared to version 2.0 are shown in Table 1.

Table 1. Changes in allergen doses that may cause an allergic reaction in sensitive people in the new 3.0 VITAL® program compared to version 2.0 [11]

Allergen	Reference Dose (mg of protein)		Comment
	VITAL 2.0	VITAL 3.0	
Reference Dose has decreased			
Cereals containing gluten (including wheat)	1.0	0.7	Labelling outcomes may have shifted from Action Level 1 to Action Level 2 — check for affected recipes. *Refer to information below about changes to tree nuts.
Soy	1.0	0.5	
Sesame	0.2	0.1	
Lupin	4.0	2.6	
Cashews & Pistachio nuts	0.1	0.05	
Pecan & Walnut	0.1	0.03	
Reference Dose has increased			
Egg	0.03	0.2	Labelling outcomes may have shifted from Action Level 2 to Action Level 1.
Milk	0.1	0.2	
Fish	0.1	1.3	
Crustacea	10	25	
New Reference Dose			
Celery/Celeriac	None	0.05	
Reference Dose is unchanged			
Peanuts	0.2	0.2	
Other tree nuts (Almonds, Brazil nuts, Hazelnuts, Macadamia or Queensland nuts)	0.1	0.1	
Mustard	0.05	0.05	

[1] Technical regulations of the Customs Union CU TR 022/2011 "Food products in terms of its labeling" (Adopted by The decision of the Council of the Eurasian economic Commission of December 9, 2011 № 881). Moscow, 2011. Retrieved from https://docs.cntd.ru/document/902320347. Accessed May 24, 2021 (In Russian)

[2] Technical regulation of the Customs Union CU TR 027/2012 "On safety of certain types of specialized food products, including dietary therapeutic and dietary preventive nutrition" (Adopted by The decision of the Council of the Eurasian economic Commission of June 15, 2012 № 34). Moscow, 2012. Retrieved from https://docs.cntd.ru/document/902352823. Accessed May 24, 2021 (In Russian)

It is important to note that some reference doses have increased, some reference doses have decreased, and some have remained the same. So, for example, for pecans and walnuts, the reference dose decreased by 3 times from 0.1 to 0.03 mg of protein. The reference dose for fish increased 13-fold from 0.1 to 1.3 mg of protein; for eggs, the reference dose increased 6-fold from 0.03 to 0.2 mg of protein. Reference doses for peanuts, other nuts and mustard have not changed.

To reduce the risk of adverse allergic reactions in consumers with hypersensitivity, it is necessary to eliminate certain food allergens from the diet [12]. Such an elimination diet will not be effective unless the person with a food allergy is reliably informed by food manufacturers about the allergens present in food [13]. In this regard, the legislation of a number of countries establishes a list of components, the use of which may cause allergic reactions or is contraindicated in certain types of diseases, as well as the requirement for mandatory information on the presence of such components in the labeling of food products [14].

Such food allergens have been identified as a major food safety hazard and their control is one of the main areas of food safety management systems [15, 16, 17, 18, 19]. Regardless of mandatory food law provisions, food allergen surveillance is required by all voluntary standards that set requirements for food safety management systems such as ISO 22000[3], FSSC 22000[4], BRC[5], IFS[6] и SQF[7]. However, the scope of control measures applied to the control of food allergens arising from the requirements of these standards is much wider than just food labeling required by food regulations [17, 20].

Control measures include such issues as identification of food allergens, prevention of cross-contamination with allergens during transportation and storage of raw materials, during production, and also during storage of finished products [17, 18, 20, 21]. Important issues are the separation of food products containing allergens from those that do not contain them, as well as the removal or reduction of allergen residues from food contact surfaces by cleaning and disinfection [22], the prevention of cross-contamination with allergens [16,19]. Cross-contact may be prevented by appropriate production planning: first, products that do not contain allergens or contain allergens that are present in all products are manufactured, and only then products containing specific allergens that are present only in some products are manufactured [17].

Considering the fact that insufficient allergen control may adversely affect the health and quality of consumer's life it is necessary to determine the scope of food allergen control measures and their effectiveness.

[3] ISO 22000:2018 Food safety management systems — Requirements for any organization in the food chain. ISO/TC34/SC17 Management systems for food safety, 2018.

[4] FSSC22000 version 5.1 (Food Safety System Certification 22000). Foundation for Food Safety Certification, 2020.

[5] Global Standard Food Safety (Issue 9). BRCGS, 2022

[6] IFS Food version 7. IFS Management GmbH, 2020.

[7] SQF Code Edition 8.1. Safe Quality Food Institute, 2019.

Previously, currently available methods for the determination of allergens in food products, their advantages and disadvantages were considered [23,24].

The purpose of the study was to analyze the products of the selected enterprise for the presence of allergens, assess the effectiveness of control measures implementation in relation to food allergens at the meat processing enterprise, as well as identify significant noncompliance and take corrective measures developed based on the results of employee survey at the enterprise and direct observation of the production process aimed at improving the control of food allergens.

Materials and methods

A meat processing enterprise located in the Moscow region was chosen as the object of the study. Since 2013, the enterprise has implemented a food safety management system certified for compliance with FSSC 22000 certification scheme and the international standard ISO 22000:2018 "Food safety management systems — Requirements for any organization in the food chain". The enterprise has previously developed and implemented an allergen control program, as required by FSSC 22000 certification scheme and ISO 22000:2018 "Food safety management systems — Requirements for any organization in the food chain".

To assess the relevance of the issue of the allergen control at this enterprise, a study of the manufactured products for the presence of allergens was carried out. A sample in the form of a packed product in the amount of at least 500 g was taken directly at the enterprise in the storage warehouse. Samples were transported in a refrigerated container and stored until testing at a temperature of 2 °C to 4 °C for not more than 24 hours. For the study, 8 types of meat products (15 items) were selected, since they are in the greatest demand among consumers:

1. Small sausages "Molochnye". Grade B meat product;
2. Small sausages "Slivochnye". Grade B meat product;
3. Frankfurters "Doktorskie". Grade B meat product;
4. Cooked sausage "Doktorskaya". Grade A meat product;
5. Cooked sausage "Molochnaya". Grade B meat product;
6. Cooked sausage "Telyach'ya". Grade A meat product;
7. Cooked sausage "Lyubitelskaya". Grade A meat product;
8. Cooked sausage "Russkaya". Grade B meat product;
9. "Ham for breakfast". Grade A boiled pork meat product;
10. Semi-smoked sausage "Krakovskaya". Grade B meat product;
11. Fried sausage "Ukrainian fried". Grade B meat product;
12. Boiled-smoked sausage "Cervelat". Grade A meat product;
13. Boiled-smoked sausage "Bavarskaya". Grade C meat product;
14. Boiled-smoked sausage "Moscovskaya". Grade A meat product;

15. Sausages for frying "Adzharian sausages with herbs". Semi-smoked sausage, Grade C meat product.

Examination of enterprise products for the presence of allergens by high-quality PCR

Products have been tested for the presence of DNA from gluten-containing cereals, soy, mustard and peanuts.

Sample preparation

From each sample of sausages, 100 g were taken. The resulting sample was ground in GRINDOMIX GM 200 homogenizer (Retsch, Haan, Germany) to a homogeneous state. Weight measurement was carried out on HR-150AZ balance (AND, Korea), 150 g weighing limit, accuracy class I.

Extraction of DNA

100 mg samples were taken from the objects of study for DNA extraction. The process itself was carried out using Sorb-GMO-B commercial kits (Sintol CJSC, Russia) according to the instructions. The principle of the method is based on the sorption of free DNA on silica particles.

Real-time PCR conditions

The 30 µl reaction mixture contained 2.5 µl 10x PCR buffer, 2.5 µl MgCl2 at a concentration of 2.5 mM, 2.0 µl dNTP, nucleotides at a concentration of 25 mM, SynTaq polymerases 2.5 EA, and 2 µl of isolated DNA. Primers species-specific to the mitochondrial region of COX1 gene were added to the mixture at a concentration of 300 nM. Reagents were produced by Sintol CJSC, Russia.

Amplification mode: preliminary denaturation at a temperature of 95 °C for 420 s; annealing-elongation at a temperature of 60 °C for 40 s, denaturation at a temperature of 95 °C for 15 s; the duration of the amplification program is 45 cycles. Limit of detection (LOD) of the method is ≤ 0.001%. The sample was amplified in triplicate. Real-time PCR was performed on ANK-32 amplifier (Sintol CJSC, Russia).

Development and verification of the checklist

The study was conducted by interviewing employees of the enterprise and direct on-site observation.

The checklist is based on available literature on the control of food allergens and on the requirements set out in food safety management system standards such as ISO 22000 (ISO/TS 22002–1), FSSC 22000, BRC, IFS.

11 persons were interviewed. These are members of the food safety group, management, and staff of the main workshops.

Results and discussion

The results of the study for the presence of allergens in the products by PCR are presented in Table 2. It was found that two samples contained soy; three samples contained gluten, which may be due to the presence of wheat flour impurities in such ingredients used in the recipe of these sausages as dry milk and dry egg powder; six samples contained mustard; one sample contained peanuts in a small concentration.

The results obtained indicate the presence of allergens in meat products, which, according to the recipe, should not contain them. This, in turn, indicate the need to develop

Table 2. PCR results for the presence of allergens in the products

Sample No.	Sample name	Legumes (soy)	Gluten	Mustard	Peanuts
1.	Small sausages "Molochnye". Grade B meat product.	DETECTED	DETECTED	DETECTED	Not detected
2.	Small sausages "Slivochnye". Grade B meat product.	DETECTED	Not detected	DETECTED	Not detected
3.	Frankfurters "Doktorskie". Grade B meat product.	Not detected	DETECTED	DETECTED	DETECTED
4.	Cooked sausage "Doktorskaya". Grade A meat product.	Not detected	Not detected	Not detected	Not detected
5.	Cooked sausage "Molochnaya". Grade B meat product.	Not detected	Not detected	Not detected	Not detected
6.	Cooked sausage "Telyach'ya". Grade A meat product.	Not detected	Not detected (at the limit of detection)	Not detected	Not detected
7.	Cooked sausage "Lyubitelskaya". Grade A meat product.	Not detected	Not detected	Not detected	Not detected
8.	Cooked sausage "Russkaya". Grade B meat product.	Not detected	Not detected	Not detected	Not detected
9.	«Ham for breakfast». Grade A boiled pork meat product.	Not detected	Not detected	Not detected	Not detected
10.	Semi-smoked sausage "Krakovskaya". Grade B meat product.	Not detected	Detected	Detected	Not detected
11.	Fried sausage «Ukrainian fried». Grade B meat product.	Not detected	Not detected	Detected	Not detected
12.	Boiled-smoked sausage «Cervelat». Grade A meat product.	Not detected	Not detected	Detected	Not detected
13.	Boiled-smoked sausage "Bavarskaya". Grade C meat product.	Not detected	Not detected	Not detected	Not detected
14.	Boiled-smoked sausage "Moscovskaya". Grade A meat product.	Not detected	Not detected	Not detected	Not detected
15.	Sausages for frying "Adzharian sausages with herbs". Semi-smoked sausage, Grade C meat product.	Not detected	Not detected	DETECTED (is labeled)	Not detected

and implement measures aimed at minimizing the risk of allergen transfer during production and the risk of allergen cross-contamination at the selected enterprise.

The effectiveness of any control system implementation is at least 60% dependent on the human factor; on how much the staff will be aware of the need for this process. To determine the degree of enterprise's personnel awareness of the allergen control importance, a questionnaire was developed consisting of 6 questions covering the amount of employees' knowledge about food allergens and their danger to the health of consumers in case of contact with the products of the enterprise.

To assess compliance with allergen control requirements at the enterprise, a checklist was developed (Table 3). At the moment, the checklist includes 7 evaluation criteria and 41 questions that cover all aspects of the production of meat products at the enterprise. According to the criteria "hazard awareness", "identification of food allergens", "washing, packaging and labeling", 5 questions were addressed; according to the criteria "transportation and storage", "cross-contamination", 7 questions were addressed; according to the criterion "management", 6 questions were addressed. It is planned to update this checklist annually based on the results of performance evaluation by changes in the number of criteria and questions, if necessary.

Justification of the criteria and questions included in the checklist

Hazard awareness (1)

When establishing a food safety management system, awareness of food allergens and knowledge of appropriate allergen control measures contained in legislative documents and management system standards are the basis for allergen control. Thus, a topic on allergen control with a section highlighting the risk of unintentionally introduced allergens should be included in the staff training schedule for the year.

Regarding the current situation with personnel awareness of food allergens, data presented in publications related to food service enterprises suggest that staff knowledge is focused on general knowledge about food allergens and first aid rules for anaphylactic reactions [12, 25]. Based on this, questions 1.1 to 1.4 were included in the checklist (Table 3).

As practice shows, visitors to the enterprise may be sources of unintentional introduction of allergens into products and often not all visitors have information about the risks of food allergens and measures to control them. In this regard, question 1.5 was included in the checklist (Table 3).

During the survey at the enterprise under study, it was determined that not all employees were trained in the control of food allergens, but only members. In addition, the Instruction for visitors to the enterprise did not include information about the risks of food allergens and measures to control their transfer into the products of the enterprise.

Identification of food allergens (2)

The presence of allergens in foods may be due to their composition, i. e. intended introduction of allergens, but they may also enter the products as a result of cross-contamination being hidden (unintentionally added) allergens. Complete allergen control requires that both types of allergens are identified [26].

The study by Dzwolak shows that the specifications for purchased raw materials and auxiliary materials were generally absent in enterprises that did not implement food safety management systems in accordance with ISO 22000, BRC or IFS. The implementation of HACCP principles does not lead to the development of specifications for all raw materials and auxiliary materials, since this is not required within the HACCP system [27]. In this regard, questions 2.1, 2.4, 2.5 were included in the checklist (Table 3).

For more than half of the enterprises, the absence of allergens list used in the enterprise is a sign of a significant gap in the control of food allergens, so question 2.2 was included in the checklist (Table 3).

The absence of such a list, which is required by the BRC and IFS standards, contributes to an increased risk of allergen cross-contamination [19,26]. This observation is directly related to the insufficient level of allergen detection when accepting raw materials and auxiliary materials. Therefore, question 2.3 was included in the checklist (Table 3).

Undoubtedly, corrective actions are needed at the sites under study, since full knowledge of allergens upon receipt of raw materials allows to correctly assign a storage location [26]. To avoid cross-contamination, it is also important to implement a policy regarding food brought in by staff, food used in the canteen of the enterprise, and food in vending machines installed at the enterprise. According to a study by Dzwolak, the lack of such a policy in most of the enterprises studied contributed to an increased risk of uncontrolled contact with allergens [27].

Transportation and storage (3)

The transport of raw materials and auxiliary materials is one of those links in the food chain where there is a risk of cross-contamination, but which is often overlooked in a systematic approach to allergen control [28]. If allergenic and non-allergenic ingredients are not separated during transport and storage, this may minimize the effectiveness of Good Manufacturing Practices in subsequent product manufacturing steps. According to [12], the lack of separation of raw materials and auxiliary materials during transportation is a serious gap in preventing cross-contamination with allergens. In this regard, question 3.1 was included in the checklist (Table 3).

A similar problem has been observed with separate storage of raw materials and ancillary materials, where good allergen practice was applied only in some enterprises with current BRC and IFS standards [27]. Therefore, questions 3.3 to 3.6 were included in the checklist (Table 3).

Table 3. Checklist for assessing the control of food allergens at meat processing enterprises

	Questions	Completion mark	Notes % completed
1.	Hazard awareness		
1.1	Have production personnel been trained in the control of food allergens?		
1.2	Have production personnel been informed about which food allergens should not be brought into the enterprise with food?		
1.3	Have production personnel been trained in the procedure/program for food allergen handling?		
1.4	Have production personnel been trained in the control of food allergens?		
1.5	Have visitors been instructed on the principles of the control of food allergens?		
2.	Identification of food allergens		
2.1	Have information on hidden allergens been obtained from all suppliers (raw materials, food additives)?		
2.2	Is there a list of allergenic materials used at the enterprise?		
2.3	Are allergenic materials identified upon arrival of raw materials at the enterprise?		
2.4	Do all product specifications/descriptions at the enterprise contain allergen information?		
2.5	Do all specifications of purchased raw materials and food additives contain information about allergens?		
3.	Transportation and storage		
3.1	Are allergenic and non-allergenic materials separated during transportation?		
3.2	Are allergenic materials labeled (e. g. color code, written label) during storage?		
3.3	If raw materials, additives, semi-finished products are stored in the same storage facility, are allergenic materials separated from non-allergenic ones?		
3.4	If raw materials, additives, semi-finished products are stored in the same storage facility, are there separate and properly designated storage areas for allergenic materials?		
3.5	Are there separate storage facilities for certain allergenic materials?		
3.6	If raw materials, additives, semi-finished products are stored in the same storage facility, are food products containing allergenic materials stored at the lowest level?		
3.7	Are opened packages with raw materials or food additives tightly closed (for example, wrapped in foil or placed in an airtight container)?		
4.	Cross-contamination		
4.1	Have potential cross-contamination sites been identified at the site?		
4.2	Is there a risk of allergen contamination during reprocessing?		
4.3	Does the production plan involve production or packaging in a sequence that reduces cross-contamination (i. e. non-allergenic products prior to allergenic ones)?		
4.4	Is there special production equipment (ladles, sieves, containers, etc.) for allergenic materials?		
4.5	Is special production equipment (ladles, sieves, containers, etc.) for allergenic materials permanently labeled (e. g. marking, color)?		
4.6	Are there special facilities/production lines for foods containing allergenic ingredients?		
4.7	Are allergenic production areas separated by physical barriers from non-allergenic production areas?		
5.	Cleaning		
5.1	If there is no special production equipment (ladles, sieves, containers, etc.) for allergenic materials, are items washed before use with non-allergenic materials?		
5.2	Are the production/packaging lines cleaned when the product changes?		
5.3	Are approved cleaning procedures in place to remove/reduce food allergen residues?		
5.4	Has cleaning been proven to be effective in removing allergen residues (e. g. with ELISA test strips)?		
5.5	Does the hygiene instruction for staff contain a recommendation to wash hands after contact with allergenic materials (i. e. raw materials, semi-finished products, products, personal food)?		
6.	Packaging and labeling		
6.1	Is information about intentionally added allergens printed on packages?		
6.2	Is information about unintentionally added allergens printed on packages?		
6.3	Is food allergen labeling checked for correctness?		
6.4	Is the food allergen information printed on packages checked for correctness when they are accepted?		
6.5	Is food allergen labeling checked when a recipe is changed (new food allergen)?		
6.6	Is compliance with food labeling requirements for allergen information checked?		
7.	Management		
7.1	Are there documented procedures/programs for the control of food allergens?		
7.2	Does the HACCP plan address food allergen hazards?		
7.3	Is the control of food allergens included in the programs of mandatory preliminary activities (PRPs)?		
7.4	Are food allergens included in the traceability system?		
7.5	If claims are related to products (e. g. no peanuts), is there a procedure for verifying such claims?		
7.6	Is the control of food allergens included in the internal audit program?		

The lack of such separation results in the absence of color coding or markings at many sites to identify equipment used when working with allergenic materials. This is addressed by question 3.2 in the checklist (Table 3).

Cross-contamination (4)

Cross-contamination is a complex area of allergen control that combines control measures applied during storage and transportation, cleaning, packaging, allergen identification and personnel activities [26]. Thus, questions 4.1 to 4.7 were included in the checklist (Table 3).

In a study [27], the highest level of compliance observed at 8 sites in terms of identifying cross-contamination areas was observed at all BRC and IFS certified sites and at one ISO 22000 certified site. However, the implementation of a food safety management system that complies with ISO 22000, or is based solely on the principles of HACCP, does not require an accurate analysis of processes in terms of allergen cross-contamination, which may also be due to training gaps in the control of food allergens.

Cleaning (5)

Washing and disinfection are considered effective methods to reduce or even eliminate residual allergens from the surface of equipment and vessels [17,22]. The results of the study [27] showed that more than half of the sites studied had some problems related to washing validation or verification. Therefore, questions 5.1 to 5.5 were included in the checklist (Table 3).

Regarding the washing, it is necessary to introduce reliable methods for verifying the reduction/elimination of allergens based on ELISA, PCR or other available methods and pay more attention to the correct planning of food production (i. e. separation, for example, the production of products with allergens after allergen-free products).

Packaging and labeling (6)

The presence of undeclared food allergens in ingredients and products is a critical food safety issue at all levels of the food supply chain requiring strict and robust food safety management strategies [29].

The high level of compliance with claimed allergen information for consumers (checklist question 6.1) (Table 3) is a result of the mandatory nature of this requirement, as it is prescribed in CU TR 022/2011 on providing food information to consumers, and also due to the ease of identifying allergens in the components of the finished product (declared allergens). In the case of unintentionally introduced allergens, this is more ambiguous, since their presence is the result of cross-contamination [18]. In addition, undeclared allergens in the Russian Federation are subjected to hazard analysis mainly at enterprises that implement a food safety management system in accordance with BRC and IFS standards. At enterprises that have implemented only the principles of HACCP, hidden allergens are usually not considered as a serious risk to food safety [12,28].

A study [30] found that in 2016–2019, among 435 product recalls related to food allergens, incorrect labeling (including "not stated on the label", "wrong advice about the allergen", "wrong label", "unintentional presence", "labelling error" and "unintentionally introduced or undeclared sulfites") was the cause in 54% of the total recalls; improper packaging was the cause in 19% of the total recalls; food allergen contamination was the cause in 14% of the total recalls; lack of labeling in English (allergen(s) not mentioned on the label in English) was the cause in 8% of the total recalls; incorrectly added ingredient was the cause in 2% of the total recalls; and unknown reasons were the cause in 3% of the total recalls.

For this reason, questions 6.1 to 6.6 were included in the checklist (Table 3).

Management (7)

Despite the relatively wide availability of literature describing the requirements for allergen control in food production, the results of the study [27] showed that gaps in allergen control were identified in almost half of the enterprises studied. For this reason, issues related to food allergens have only partially been included in the various elements of the food safety management system. Not including allergen control in elements of the food safety management system such as the HACCP plan, PRP, traceability, and internal audits at many sites studied is a sign of limited allergen control, and in some cases, no control at all. In this regard, questions 7.1 to 7.6 were included in the checklist (Table 3).

As a result of employee surveys at the enterprise and direct on-site observation using the developed checklist, noncompliance was identified in the work of the food safety management system implemented at the enterprise in the field of allergen control. It was determined that not all employees of the enterprise were trained in the control of food allergens. The instruction for visitors did not contain information about the risk of allergens and measures for their control. There is a risk of cross-contamination for allergen-free products when produced on the same line as allergen-containing products. There is no confirmation of the washing effectiveness in terms of the allergen residues presence. These results largely duplicate the results obtained [27], according to which only at 4 enterprises the staff received written information that it is forbidden to bring products containing allergens to the enterprise. Due to lack of funds or lack of space, half of the surveyed sites did not implement practices to prevent cross-contamination. More than half of the enterprises studied had problems with validation or verification of the washing effectiveness. Thus, it can be concluded that most enterprises have common problems in the development of procedures for allergen control. They may be avoided if there are resources and specific requirements for the structure of allergen control programs and the activities that this program includes.

Procedure development for allergen control in the production of meat products

As a part of the activities to reduce and eliminate the risk of allergens presence in the finished products at the selected enterprise, it was decided to expand the existing food safety management system.

All 11 interviewed employees of the enterprise are aware of the risk to consumer health when using products with allergens. However, only members of the food safety team (7 people) were trained in the control of food allergens.

The likelihood of cross-contamination with allergens was then assessed at each stage of the food production process, from the input control of raw materials to the sale of the finished product. In this case, the physical form of the allergens used must be assessed, for example, liquid and powder have a different risk of cross-contamination. So, milk powder during weighing may get into products through the ventilation system or from personnel clothing, while when adding liquid milk, this risk is lower if certain measures are observed (isolation by physical barriers, distance between products).

In cases where risk of contamination was identified (during the production on the same line of products that do not include allergenic components and allergen-containing products), measures were taken to reduce the unintentional transfer of allergens into the product. For this purpose, the principles of Good Manufacturing Practice (GMP) have been successfully applied within the organization of the production process. To ensure food safety, GMP requires all personnel to maintain strict discipline. Key aspects of allergen control in the production of meat products are shown in Figure 1.

Since the manufacturer is obliged to know about the presence of allergens in all raw materials used, which is achieved during work with the supplier and due to the input control of the transport documents for raw materials, all suppliers were requested to provide information on the content of food allergens in raw materials in the form of:

a) main ingredients indicated in the composition (for example, soy vegetable protein in the composition of a complex food additive);
b) auxiliary ingredients (e. g. food additive derived from an allergenic source, for example wheat amylase);
c) undeclared ingredients introduced due to industrial cross-contamination with allergens.

Suppliers of raw materials have been properly trained and aware of the risks that may result from contamination of products with allergens and provide relevant information. All ingredients are fully described on the label and in the specifications for raw materials, since the use of generalized names, such as, for example, "vegetable oils and fats", is unacceptable.

After the input control, when placed in the manufacturer's warehouses, raw materials containing allergens were identified, and separate storage of such ingredients was also provided.

The only approach to completely avoid allergen cross-contamination during the manufacturing process is to use separate manufacturing sites. However, this was not possible at the selected enterprise. In this regard, measures were taken to separate products that contain allergens from those that do not contain allergens:

— separation of production into different areas; establishing physical barriers between production lines;
— provision of dedicated equipment, inventory and containers;

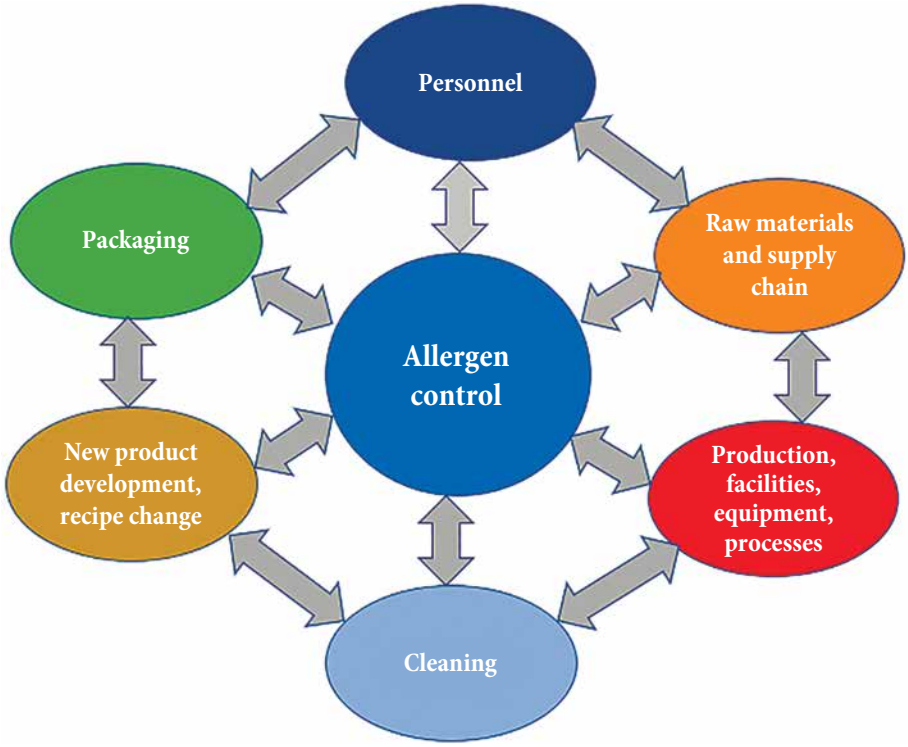

Figure 1. Key areas to consider when establishing the allergen control system (adapted from [31])

— minimization of unnecessary movement of materials; appropriate planning of production cycles, including cleaning of equipment between production cycles;
— organization of a separate air supply, where it is possible.

At the stage of input control of the main raw materials and auxiliary materials, their compliance with regulatory and technical documentation, including information on the presence of allergens, was verified. Responsible employees were trained in allergen awareness and control in accordance with their responsibilities. Transport documents were controlled; the incoming raw materials were identified for compliance with the information and visually assessed. Next, clear labeling was carried out, which indicated whether it is a potential allergen (factories may use color coding or other means to identify allergenic ingredients), and there was a separation of incoming raw materials batches. At the stage of production planning, storage and production areas of the main raw materials and auxiliary materials with allergens and free from them were separated. Areas for the storage of allergens were prepared and allocated. Special shipping containers identified by color coding were purchased and used. Allergenic raw materials are placed in a dedicated and marked area of the warehouse, separate from raw materials that do not contain allergens; physical barriers are used. Instructions on the prevention of cross-contamination have been developed and distributed in appropriate sites. When transporting allergenic raw materials from the warehouse to the spice preparation site, special marked closed containers are used. Allergen, finished product and waste routes are separated over time (space) to prevent cross-contamination. After transportation, the premises are cleaned along the route and the transport equipment is sanitized. When storing and using allergens, racks, scales for weighing, inventory (ladles, tanks, bags), storage areas for cleaning equipment and the cleaning equipment itself are marked. Special clothing is used for the personnel and control over the timely shift is carried out. The operation of the exhaust system is controlled. In the production of meat products, it is planned to sequence the production of allergenic products after those free from allergens. After the end of the production process, a thorough washing of equipment and inventory is carried out. It is necessary to draw up sanitization schedules and instructions, control the quality of equipment washing, separate instruments, develop rules for cleaning up spilled substances and unmounting equipment during washing. It is also necessary to carry out identical measures and controls when packaging products with allergens and free from them. All allergenic ingredients are declared on the label; product labeling is carried out in accordance with the requirements of CU TR 022/2011 regarding the indication of allergen contents. Control over the recycling of products and the disposal of food waste is carried out.

The developed Procedure for Allergen Control in the production of meat products complements and expands the previous Allergen Control Program developed and implemented at the enterprise by including additional control points, in particular, confirmation of the cleaning program effectiveness by commercial ELISA test kits, and control over the product recycling and disposal of food waste.

Meat processing enterprises are heavily responsible both for compliance with the requirements of the law and for the health of consumers. Therefore, in order to minimize the unintending transfer of allergens into finished products, it is necessary to develop, implement and maintain an allergen control program, analyze the causes of allergenic products sales and organize resource management.

Conclusion

During this work, the products of the selected enterprise were examined for the presence of gluten, soy, mustard and peanuts. Of the 15 samples studied, 2 samples contained soy, 3 samples contained gluten, 6 samples contained mustard, 1 sample contained peanuts, and 4 samples contained 2 to 3 allergenic ingredients at the same time. These results confirmed the need to develop and implement an allergen control procedure at the selected enterprise. Since the enterprise is certified in accordance with the requirements of the ISO 22000:2018 "Food safety management systems — Requirements for any organization in the food chain" and the FSSC 22000 certification scheme, an allergen control program has been developed and implemented as a part of the implemented food safety management system. However, its effectiveness is rather low, which was shown by employee survey at the enterprise, as well as by allergens found in finished products. Actions to improve the allergen control programs at the surveyed enterprise include activities such as increasing the proportion of staff involved in training on the control of food allergens, creating guidelines to define good practice for allergens, especially with regard to avoiding cross-contamination. These actions also include improving cleaning procedures that use proven cleaning methods and test the effectiveness of reducing/eliminating food allergens. It is planned that washing programs will be supported and validated by precise methods, such as commercial ELISA test kits, instead of non-specific testing methods that are based on total protein determination and visual inspection. The procedure for allergen control in the production of meat products developed based on the results of the research will be tested at the selected enterprise in 3 months. Previously tested products will be sampled and assessed for allergens. Based on the results of the repeated study, a conclusion will be made about the effectiveness/ineffectiveness of the proposed measures.

REFERENCES

1. Spotz, K. (2018). Allergens: An enhanced focus. *Journal of AOAC International*, 101(1), 56–59. https://doi.org/10.5740/jaoacint.17–0435
2. Martin, H. (2021). Review of celiac disease: Clinical manifestations, diagnosis, and management. *Physician Assistant Clinics*, 6(4), 569–580. https://doi.org/10.1016/j.cpha.2021.06.001
3. Solymosi, D., Sárdy, M., Pónyai, G. (2020). Interdisciplinary significance of food-related adverse reactions in adulthood. *Nutrients*, 12(12), Article 3725. https://doi.org/10.3390/nu12123725
4. Du Toit, G., Katz, Y., Sasieni, P., Mesher, D., Maleki, S.J., Fisher, H.R. et al. (2008). Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *Journal of Allergy and Clinical Immunology*, 122(5), 984–991. https://doi.org/10.1016/j.jaci.2008.08.039
5. Sicherer, S.H., Sampson, H.A. (2010). Food allergy. *Journal of Allergy and Clinical Immunology*, 125(2, Suppl. 2), S116-S125. https://doi.org/10.1016/j.jaci.2009.08.028
6. Maleki, S.J., Chung, S.-Y., Champagne, E., T., Raufman, J.-P. (2000). The effects of roasting on the allergenic properties of peanut proteins. *Journal of Allergy and Clinical Immunology*, 106(4), 763–768. https://doi.org/10.1067/mai.2000.109620
7. Remington, B. C., Westerhout, J., Meima, M. Y., Blom, W. M., Kruizinga, A. G., Wheeler, M. W. et al. (2020). Updated population minimal eliciting dose distributions for use in risk assessment of 14 priority food allergens. *Food and Chemical Toxicology*, 139, Article 111259. https://doi.org/10.1016/j.fct.2020.111259
8. Holzhauser, T., Johnson, P., Hindley, J. P., O'Connor, G., Chan, C.-H., Costa, J. et al. (2020). Are current analytical methods suitable to verify VITAL® 2.0/3.0 allergen reference doses for EU allergens in foods? *Food and Chemical Toxicology*, 145, Article 111709. https://doi.org/10.1016/j.fct.2020.111709
9. Blom, W. M., van Os-Medendorp, H., Bijlsma, S., van Dijk, A., Kruizinga, A. G., Rubingh, C. et al. (2020). Allergen risk assessment: Food intake levels of the general population represent those of food allergic patients. *Food and Chemical Toxicology*, 146, Article 111781. https://doi.org/10.1016/j.fct.2020.111781
10. Houben, G. F., Baumert, J. L., Blom, W. M., Kruizinga, A. G., Meima, M. Y., Remington, B. C. et al (2020). Full range of population Eliciting Dose values for 14 priority allergenic foods and recommendations for use in risk characterization. *Food and Chemical Toxicology*, 146, Article 111831. https://doi.org/10.1016/j.fct.2020.111831
11. VITAL (2022). Further information for VITAL Online Users on the update from version 2.3.30 (previous version) to version 3.0.0 (updated version). Retrieved from https://vital.allergenbureau.net/wp-content/uploads/2021/04/further_information_for_vital_online_users_on_the_update_from_version_2330_previous_version_to_version_300_updated_version.pdf Accessed August 15, 2022
12. Gendel, S.M., Khan, N., Yajnik, M. (2016). A survey of food allergen control practices in the U.S. food industry. *Journal of Food Protection,* 76(2), 302–306. https://doi.org/10.4315/0362–028X.JFP-12–373
13. Dupuis, R., Meisel, Z., Grande, D., Strupp, E., Kounaves, S., Graves, A. et al. (2016). Food allergy management among restaurant workers in a large U.S. city. *Food Control,* 63, 147–157. https://doi.org/10.1016/j.foodcont.2015.11.026
14. Mortimore, S., Wallace, C. (2013). HACCP. A Practical Approach. Third Edition. Springer: New York, Heidelberg, Dordrecht, London, 2013. https://doi.org/10.1007/978–1–4614–5028–3
15. Cianferoni, A., Spergel, J. M. (2009). Food allergy: review, classification and diagnosis. *Allergology International*, 58(4), 457–466. https://doi.org/10.2332/allergolint.09-RAI-0138
16. Crevel, R.W.R., Taylor S. L., Pfaff, S., Alldrick, A. (2013). Managing Food Allergens: Case Histories and How They Were Handled. Chapter in a book: Risk Management for Food Allergy. Academic Press, Oxford, Waltham. https://doi.org/10.1016/B978–0–12–381988–8.00009–9
17. Pachołek, B., Sady, S., Kupińska-Adamczyk, E. (2018). Management of food allergens in the food industry. *Journal of Agribusiness and Rural Development*, 47(1), 73–80. https://doi.org/10.17306/J.JARD.2018.00388
18. Röder, M., Weber, W. (2016). Allergen management in the food industry. *Gesundheitsschutz,* 59(7), 900–907. https://doi.org/10.1007/s00103–016–2367-y (In German)
19. Stein, K. (2015). Effective allergen management practices to reduce allergens in food. Chapter in a book: Handbook of Food Allergen Detection and Control. Woodhead Publishing, UK. https://doi.org/10.1533/9781782420217.1.103
20. Taylor, S.L., Hefle, S.L. (2005). Allergen management. *Food Technology*, 59(2), 40–43.
21. Muñoz-Furlong, A., Sampson, H.A. (2008). The Management of Food Allergy. Chapter in a book: Food Allergy: Adverse Reactions to Foods and Food Additives. Blackwell Publishing Ltd, Massachusetts. https://doi.org/10.1002/9781444300062.ch36
22. Jackson, L.S., Al-Taher, F.M., Moorman M., DeVries J.W., Tippett R., Swanson K. M.J. et al. (2008). Cleaning and other control and validation strategies to prevent allergen cross-contact in food-processing operation. *Journal of Food Protection*, 71(2), 445–458. https://doi.org/10.4315/0362–028X–71.2.445
23. Kryuchenko, E.V., Zamula, V.S., Kuzlyakina, Yu.A., Chernukha I.M. (2020). Overview of modern methods for detecting allergens in food. *Vsyo o Myase*, 5S, 169–172. https://doi.org/10.21323/2071–2499–2020–5S–169–172 (In Russian)
24. Chernukha, I.M., Kryuchenko, E.V., Kuzlyakina, Yu.A., Zamula, V.S. (2022). *Qualimetric assessment of methods for the determination of allergens in meat products*. IOP Conference Series: Earth and Environmental Science, 1052(1), Article 012125. https://doi.org/10.1088/1755–1315/1052/1/012125
25. Dupuis, R., Meisel, Z., Grande, D., Strupp, E., Kounaves, S., Graves, A. et al. (2016). Food allergy management among restaurant workers in a large U.S. city. *Food Control*, 63, 147–157. https://doi.org/10.1016/j.foodcont.2015.11.026
26. Crevel, R., Cochrane, S. (2014). Allergens. In: Food Safety Management. A Practical Guide 529 for the Food Industry [Ed. by Motarjemi, Y. and Lelieveld, H.], Academic Press, London, 2014.
27. Dzwolak, W. (2017). Assessment of food allergen management in small food facilities. *Food Control*, 73, 323–331. https://doi.org/10.1016/j.foodcont.2016.08.019
28. Dzwolak, W. (2022). Allergen cross-contact control plan supporting the implementation of food allergen management (FAM) in small food businesses. *Food Control*, 135, Article 108777. https://doi.org/10.1016/j.foodcont.2021.108777
29. Koeberl, M., Clarke, D., Allen, K. J., Fleming, F., Katzer, L., Alice, L. N. et al. (2018). Food allergen management in Australia. *Journal of AOAC International*, 101(1), 60–69. https://doi.org/10.5740/jaoacint.17-0386
30. Jia, L., Evans, S. (2021). Improving food allergen management in food manufacturing: An incentive-based approach. *Food Control*, 129, Article 108246. https://doi.org/10.1016/j.foodcont.2021.108246
31. Yurchak, Z.A., Kuznetsova, O.A., Starchikov, A.D. (2015). Prevention and minimization of production food allergens. *Vsyo o Myase*, 5, 19-21. (In Russian)

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EFFECT OF PEPPER AND SALT BLENDS ON MICROBIAL QUALITY OF *QUANTA*: ETHIOPIAN DRIED RED MEAT

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Keywords: *Quanta, spice blends, pre-drying treatment, microbial quality*

Abstract
This study was conducted to assess the effect of spice blends varying in salt and pepper concentrations on the microbial quality of Quanta: Ethiopian dried red meat. The experiment had seven treatments: 25% spices, 25% salt, and 50% pepper (T1); 25% spices, 20% salt, and 55% pepper (T2); 25% spices, 15% salt, and 60% pepper (T3); 25% spices, 10% salt, and 65% pepper (T4); 25% spices, 5% salt, and 70% pepper (T5); 100% spices (without salt and pepper), a positive control (T6); a negative control without any added ingredient (T7). Microbiological analyses were performed initially on the raw sliced meat and spice blends, and after application of the treatments on the 10th and 20th days of drying. High initial loads of total bacteria (APC) and Enterobacteriaceae (EC) were observed in the raw meat samples and spice blends and increased over the drying periods (10 and 20 days) in all treatments. No significant difference ($p>0.05$) was observed among the treatments (T1-T7) for APC and EC at a given drying period and between the drying periods. Salmonella spp. was not detected in any of the seven treatments either on the 10th and 20th days of drying. However, Escherichia coli was detected in six (T1-T6) of the dry meat samples except in T7 both on the 10th and 20th days of drying suggesting that the spice blends served as a source of contamination of the dried meat samples with E. coli. However, the spice blends used in combination with drying were effective in inhibiting the growth of Salmonella species in the dry meat samples. Spices as well as the raw meat used for Quanta preparation should be produced and handled under hygienic conditions to minimize the microorganisms that they harbor.

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Introduction

The world population is expected to reach 9.7 billion in 2050 [1]. The increased population growth will lead to increased demand for food including meat and meat products [1,2]. Red meat is rich in many nutrients such as minerals, vitamins, and essential fatty acids and it is an excellent protein source, as the bioavailability of nutrients in meat is high compared to plant-based protein sources [2]. Meat and meat products provide primarily vitamin B12, highly digestible protein and bioavailable iron [3].

In recent years, there appears to be a shift in the consumption pattern of meat (mainly red meat) among the world population with consumption increasing in developing countries and decreasing in developed countries [2]. It is predicted that the consumption of animal protein from red meat such as beef, sheep and goat will increase over the next 2–3 decades among the middle-class population of developing countries especially in Africa and Asia [1,2].

There has been a significant increase in consumption of animal-based foods in the last 50 years in the world owing to economic growth [4]. According to FAO statistics, global meat and fish consumption has increased from 23 kg per capita in 1961 to 42 kg per capita in recent years [4]. Moreover, Henchion et al. [1] reported that animal-source protein supply (g/capita/day) is projected to increase in all regions from 2012 to 2050.

It has been reported that most of the future growth in meat and fish consumption is likely to occur in low-income countries, including Sub-Saharan Africa (SSA), where the current consumption levels are still very low [4]. Strong population growth and urbanization in SSA will reinforce growth in total demand for animal-based foods [4]. Meat production in SSA is projected to increase by 2.7% per annum till 2030, which is high compared to the expected increase in global meat production of 1.4% [4]. In SSA, consumption of animal-based foods is expected to increase by 54–69% if GDP of the region doubles [4].

Ethiopia has the largest livestock population in Africa [5]. However, consumption of animal source food has always been low in the country and declining as a result of

the low livestock productivity and continuously growing human population [6,7]. According to the 2020 FAO statistics, the total meat production in Ethiopia was estimated at 918,564 tonnes [8]. The majority of meat production in Ethiopia comes from cattle (beef) which accounts for 47.14% (433,025 tonnes) of the total meat production in the country [8]. According to Shawel and Kawashima [9], the consumption of meat declined from 20 kg/person/year in 1961 to 8 kg/person/year in 2004 in Ethiopia. The average per capita consumption (kg/year/capita) of meat in Ethiopia based on the FAO food balance sheet data was reported to be 7.99 in 2020 [4].

Fresh meat is a highly perishable product due to its biological composition [3,10]. The diverse nutrient composition of meat makes it an ideal environment for the growth and propagation of meat spoilage micro-organisms and common food-borne pathogens [3]. It is therefore essential that adequate preservation technologies are applied to maintain its safety and quality [10].

Food handling, preparation, and preservation practices in Ethiopia are based on indigenous knowledge that is handed over to the present generation, which is an invaluable and intangible asset as they are the outcome of repeated research and practical experiment by many generations [11]. Moreover, research on meat and meat products in Ethiopia has been given the lowest attention [12]. The traditional dried meat product of Ethiopia and East African countries [13], called "*Quanta*" (in Amharic), is similar to the dried meat Biltong. People in Ethiopia prepare *Quanta* with the application of salt (salting) and different spices [14] on the surface of red meat. Blends of spices used in the pre-treatment of the raw material used for the preparation of *Quanta*, that is, the sliced fresh meat include *Mitmita (Capisicum frutescene)* and *Berbere (Capisicum anmum)* and they are prepared as cooking aid or condiment [15]. The composition of the ingredients for the above-mentioned pre-treatment of raw meat is reported to be 50% pepper, 25% salt, and 25% spices [13].

However, the ingredients used in the preparation of the pre-treatment materials vary in their type and amount due to different factors and their inhibitory effect against pathogens and spoilage microorganisms has not been studied to date. Besides, the proportion of the ingredients (pepper, salt, and spices) in the blend which results in better inhibitory effects without affecting the sensory quality of the meat has also not been studied so far. Improvement and proper use of traditional meat preservation techniques like preparation of *Quanta* can play a significant role to ensure sustainable food supply through reduction of post-harvest (post-slaughter) losses of meat. Moreover, improving the quality of *Quanta* through effective pre-drying treatments may alleviate the problem of seasonal availability of meat in the country, may help to develop a uniform type of dried meat, and create an opportunity for exporting meat in a dried form. Therefore, this experiment was conducted to evaluate the treatment effect of blends varying in concentrations of salt and pepper on the microbial quality of dried red meat (beef), *Quanta.*

Methodology and methods

Preparation of blends used for pre-drying treatment

Mareqo type red pepper *(Capisicum anmum)* which is African chilies indigenous to Ethiopia [16], and table salt (NaCl) were purchased from Assela market on the relative quality basis (cleanness, color, and size) with the help of experienced women. The items purchased for the blend preparation were taken to a blend processing place (a private compound in Assela town which was rented for this experimental season) with independent plastic bags and they were subjected to wet cleaning (washing) and/or dry cleaning (picking, trimming, etc.) based on the requirement of each ingredient. Each type of spices, pepper and salt were sun-dried for 10 days in independent plastic trays. The sole spice blend was made to consist of a combination of 4% Basil (*Ocimum basilicum*), 4% Rue (*Ruta graveolens*), 4% Rosemary (*Rosmarinus officialis*), 7% Fenugreek (*Trigonella foenum-graecum*), 7% Bishop's weed (*Carum copticum* L.), 7% Black cumin (*Nigella sativa* L.), 15% Garlic (*Allium sativum*), 15% Shallot (*Allium cepa*), 15% Ginger (*Zingiber officinale*) and 22% Ethiopian Cardamom (*Aframomum corrorima*). The spices were mixed by pounding them with a mortar and pestle. The red pepper was also pounded separately with a mortar and pestle to a size of about 5 mm with the traditional size reduction process called "*shikesheka*" in Amharic.

The preparation of blends, which were used to treat meat slices, was based on FAO [13] ingredients estimation for traditional meat drying of *Quanta* viz., 50% pepper, 25% salt, and 25% spices. Thus, this blend level was prepared and used as one pre-drying treatment in the experiment. Besides, the other four blends varying in the proportion of pepper and salt but having the same amount of the other blend of spices were prepared. The blending of the mixtures was done by pounding with a mortar and pestle. This blending step is called '*Deleza*' in Amharic. A blend formulated with spices only was prepared to be used as a positive control. The blends were placed in the sun again for 3 days for drying. All six lots of blends were subjected to a careful traditional art of low heat treatment, further drying, on a metal sheet one after the other independently. This traditional art of heat drying is called '*Emesa*' in Amharic. Finally, milling of the above-stated six blends was done independently, one after the other, in one of the commercial mills in the town of Assela.

Therefore, the compositions of spice (S) blends prepared for the pre-drying treatments and their respective treatments were as follows: S1 with 25% spices, 25% salt, and 50% pepper (T1); S2 with 25% spices, 20% salt, and 55% pepper (T2); S3 with 25% spices, 15% salt, and 60% pepper (T3); S4 with 25% spices, 10% salt, and 65% pepper (T4); S5 with 25% spices, 5% salt, and 70% pepper (T5);

a positive control, S6 with 100% spices (without salt and pepper) (T6); and, a negative control, without any added ingredient (T7). Therefore, the experiment had a total of seven treatments.

Preparation of meat slices

Meat from two pairs of hind legs of two male beef cattle (Arsi breed) was purchased from private butchers in Assela town. The two hind legs of both oxen were cut into whole cut meat (deboned meat) and sliced into strips 1 cm thick and 40 cm long according to FAO [13]. Slicing was performed by the researchers with the assistance of two experienced women.

Pre-drying treatment of meat slices

The amount of blend used for the pre-drying treatment of sliced meats was determined according to Jay et al. [17]. The minimum inhibitory concentration (MIC) of most spices required to inhibit growth of sensitive organisms ranges from 1 to 5%. Thus, by taking the average concentration level, which is about 3%, dilutions were prepared by mixing the pre-drying treatment blends with distilled water. About 4 L of pre-drying treatments were prepared and used for every six treatments including a blend of sole spices. Two kilos of sliced meat lots were used for each treatment and uniformly treated by dipping in their respective dilutions for 10 minutes by turning them up and down. Similarly, the negative control, without any spice treatment, was treated with distilled water to control the deviation that could occur because of the water used in the dilution of other treatment blends. Seven clean plastic pans (bowls) were used to treat the seven experimental treatments individually in the first block (sliced meat lots of the first ox) and the bowls were reused for the second block (sliced meat lots of the second ox) of similar treatments after thorough cleaning and disinfection with 70% alcohol.

Drying of meat samples

Drying of the meat slices was done in a room with a 4 m × 4 m area having windows and a door for adequate ventilation, and the room openings were covered with mesh wire to prevent the entrance of flies according to FAO [13]. The ambient temperature of the experimental site, Assela town, was between 9.17 °C and 22.63 °C during experimentation. A drying bed (string) 2 m high, 2 m wide and 3 m long was constructed in the drying room. The drying bed had two blocks with a 50 cm gap between them. Both of the drying blocks were made to have 14 rows (7 pairs) of stretched ropes (5 mm diameter) with a 20 cm gap between the rows. Drying was done by suspending sliced meat lots on ropes (Figure 1). Each treatment had two independent hanging rows across the blocks. A uniform arrangement of meat slices was made with no surface contact between the neighboring meat slices. Drying was done for twenty days and identification cards were suspended together with drying meat slices.

Figure 1. Treated meat samples hanging on ropes during drying

Experimental design

The design used to conduct this experiment was Randomized Complete Block Design (RCBD). Two sources of meat, meat samples obtained from two different oxen, were used as a block after having been sliced in order to avoid the variation in meat quality obtained from the two animals. About 14 kg of slices were made from the first ox meat and divided into seven lots each containing two kilos. The seven treatments were randomly allocated to the seven meat slices in the first block. The same was done to the slices made from the second ox meat in the second block. In this experiment, two varying factors, namely salt and pepper, were used. Five different blends were prepared from the spices, salt, and pepper by varying only the ratio of pepper to salt and keeping the level of spices constant in all five blends. In addition, two types of controls were used in the experiment; a positive control that was a blend made from spices only (without pepper and salt), and the other was a negative control without any pre-drying treatment with spices or salt. A total of seven treatments, six spice blends, and the negative control (without any pre-drying spice treatment), were applied to each of the two blocks.

Microbiological analysis

In the current study, microbiological analysis was done at four different times to assess: the aerobic plate count (APC), *Enterobacteriaceae* count, and presence of the pathogens, *Escherichia coli* and *Salmonella* spp. The first microbiological analysis was done on the sliced fresh meat samples of both meat sources (from two types of sliced meats sourced from different oxen) before they were treated and dried, and on the six different types of spice blends used (Table 1) to assess their initial microbial load. The second, third and fourth microbiological analyses were conducted after the application of pre-drying treatments and on the 10^{th} and 20^{th} days of the drying experiment, respectively to determine the change in microbial population over the drying period.

Sampling

About 200 g of samples were taken from each sliced fresh meats, spice blends, and sliced and dried meat samples in the respective microbiological analysis seasons. Sampling was done randomly and samples were transported to the Microbiology Laboratory of Quality and Standards Authority of Ethiopia by putting them in an icebox after they were packed into polyethylene bags and labeled properly. Sampling of spice blends and meat lots for microbiological analysis was done by aseptically weighting 25 g from each sample type. Sample dilution (1:10) was performed with 225 ml of buffered peptone water [18] and homogenized for two minutes using a stomacher (Seward Medical, London). Serial (10-fold) dilutions (10^{-1} to 10^{-7}) were prepared by transferring 1 ml of the previous dilution (1:10) into test tubes containing 9 ml of 0.1% peptone water [18]. Separate sterile pipettes were used for transferring samples during serial dilutions and all dilutions were thoroughly mixed before they were plated. The presence of the pathogens *Escherichia coli* and *Salmonella* spp. was detected using samples from the initial dilution level (1:10). However, the aerobic plate count (APC) and *Enterobacteriaceae* count were made using the appropriate dilutions that yielded countable colonies (30–300 colonies/dish). Every analysis was performed in triplicate.

Escherichia coli detection

Detection of *Escherichia coli* was done according to the method described by Roberts and Greenwood [19] following four sequential incubation steps. The first incubation step was done at 37 °C for 48 h [19] by transferring 1 ml representative sample from 1:10 (10^{-1}) dilution into test tubes containing Lauryl Tryptose (LT) broth (Lab M Limited, UK). The second incubation step was done by transferring 1 ml representative sample from the Lauryl Tryptose (LT) broth into test tubes containing Brilliant Green Bile (BGB) broth (Lab M Limited, UK) and incubated at 35 °C for 24 h, and gas production was considered as an indicative test for the presence of *Escherichia coli* according to ISO [19]. The third incubation step was done in the selective media for pathogenic *Escherichia coli*, MacConkey Sorbitol medium (Lab M Limited, UK), for 24 h at 45.5 °C [19] after transferring 1 ml representative sample from Brilliant Green Bile (BGB) broth. Colonies grown on this medium were subjected to the fourth and the final confirmatory test. The fourth and the confirmatory test was done by transferring about 10% of typical colonies grown on MacConkey Sorbitol medium into test tubes containing peptone water and incubated at 44 °C for 24 h. Then, the peptone water in the test tubes was tested with Kovac's reagent (5 ml) for the presence of indole. The production of bluish color was considered as an indicator for the presence of indole in the samples, that is, a positive test for the existence of *Escherichia coli* [19].

Detection of Salmonella spp.

Detection of *Salmonella* spp. was done according to ISO [20] following five consecutive incubation steps. The first incubation step, pre-enrichment, was performed at 37 °C for 48 h by taking about 150 ml of representative samples from the 1:10 dilution levels. The second incubation step was done by transferring an aliquot from the completed pre-enrichment step into a selective enrichment medium, Rappaport Vassiliadis Soya (RVS) broth (Lab M Limited, UK), and incubating at 41 °C for 24 h. The third incubation step was performed by plating the sample enriched in the second incubation step into solidified Hektoen Enteric (HE) selective medium (Lab M Limited, UK) and incubating at 37 °C for 24 h. Then, the fourth incubation step was done by transferring about 10% of typical colonies selectively grown on Hektoen Enteric (HE) medium into solidified Nutrient agar medium (Lab M Limited, UK) and incubating at 37 °C for 24 h. The fifth and final incubation step was done by transferring about 10% of typical colonies grown on Nutrient agar, plating into Urea agar medium (Lab M Limited, UK) and incubating at 37 °C for 24 h. Finally, smooth colonies (colorless, translucent, or pale colonies) that were 2–4 mm in diameter were considered as a positive test for the presence of *Salmonella* spp.

Aerobic plate count
and Enterobacteriaceae count

Aerobic plate count and *Enterobacteriaceae* count were made using samples from appropriate dilution levels. One milliliter of a sample was pipetted into appropriately marked Petri dishes. Enumeration of the APC was performed after incubating samples using plate count agar (Lab M Limited, UK) at 30 °C for 72 h according to ISO [18] method 4833. Incubation of *Enterobacteriaceae* was done using Violet Red Bile (VRB) agar (Park Scientific Limited, UK) at 30 °C for 48 h and all red-pink colonies with a diameter of greater than 1 mm were counted [21]. The colonies were counted using a colony counter (Wissenschaftlich Technische, Werkstatten, Germany) and the estimated average numbers per gram of sample were calculated according to Maurin and James [21] for APC and *Enterobacteriaceae* count, and then data were presented in $\log_{10}$ cfu/g.

Statistical analysis

Analysis of variance (ANOVA) for Randomized Complete Block Design (RCBD) was carried out using PROC General Linear Model (GLM) of the Statistical Analysis System [22] Version 9.1. Microbial counts were first transformed to logarithmic values ($\log_{10}$) before statistical analysis. Differences between treatment means were determined using the least significant difference (LSD) technique. All comparisons were made at a 5% level of significance.

Results and discussion

Red meat is a highly perishable product and soon becomes unfit to eat and possibly dangerous to health through microbial growth, chemical change, and breakdown by endogenous enzymes [23]. Drying is amongst the effective and simple methods for the preservation of red meat [24]. Although commercialization and production of *Quanta* can help in alleviating the variation in meat availability and price in the country, to date there is no documented information about its processing steps, and no research has been conducted to enhance its quality attributes and storage stability. The current study investigated the effect of spice blends varying in concentrations of salt and pepper on microbial quality of the Ethiopian dried red meat (beef), *Quanta.*

Detection of pathogens in the raw material

The results for the detection of the pathogens *Escherichia coli* and *Salmonella* spp. in the raw materials, the sliced raw meat and the spice blends used in this experiment are presented in Table 1. *Escherichia coli* is the most frequently identified pathogen associated with beef products [25]. The incidence of *E. coli* is not very variable in domestic or export beef meat [26]. Since its discovery by Theodor Escherich in 1885, it has been receiving much greater importance due to the pathogenicity of certain strains to both humans and animals [27]. In the current study, *Escherichia coli* was not detected in the two sliced raw meat samples. The finding disagrees with that of Gwida et al. [28] who reported a high percentage of *E. coli* isolated from raw meat and unprocessed ready-to-eat products. However, *E. coli* was detected in all the six spice blends analyzed. Possible sources of contamination of spices by pathogenic microorganisms were reported to include storage equipment, handling, unhygienic surroundings, vehicular transmission, atmospheric particles and air [29]. On the other hand, *Salmonella* spp. was not detected in any of the spice blends; however, its presence was exhibited in the sliced raw meat of both animal sources. In the process of converting live animals into meat, microbial contamination of carcass surfaces is unavoidable [25]. While most of the microfloras transferred to the carcasses during the slaughtering process are nonpathogenic, there is a possibility that pathogens like *Salmonella* spp. may be present and it represents one of the most critical safety challenges for the meat industry [25]. *Salmonella* spp. is most commonly associated with animal products and is only present in vegetables through cross-contamination [30,31]. This may explain the absence of detection of the pathogen *Salmonella* spp. in the spice blends analyzed.

Table 1. Occurrence of the pathogens *Escherichia coli* and *Salmonella* spp. in raw sliced meat samples and different spice blends used in the experiment

Type of sample	Occurrence of the pathogens	
	Escherichia coli	*Salmonella* spp.
Sliced meat (B1)	-ve	+ve
Sliced meat (B2)	-ve	+ve
Spice blend (S1)	+ve	-ve
Spice blend (S2)	+ve	-ve
Spice blend (S3)	+ve	-ve
Spice blend (S4)	+ve	-ve
Spice blend (S5)	+ve	-ve
Spice blend (S6)	+ve	-ve

n = 3, number of samples; B1 and B2 are sliced meat samples from two different sources used in block 1 and block 2, respectively; S1 up to S6 are spice blends used to treat the meat samples in this study; -ve shows the absence of the pathogen and +ve shows the presence of the pathogen.

Enumeration of microorganisms in the raw materials

Evaluation of the microbiological quality and safety of food products is commonly carried out by determination of total viable counts and the indicator organisms *Enterobacteriaceae* and *E. coli* [32,33]. Aerobic plate count is used to estimate the bacterial population in a food sample. It is not an evaluation of the entire bacterial population nor does it indicate differences among bacterial types in a food product. It provides an estimate of the number of microorganisms that can grow aerobically at ambient temperatures. The APC may be used to judge sanitary quality, sensory acceptability, and conformance with good manufacturing practices (GMPs) [34]. The results for the aerobic plate count and *Enterobacteriaceae* count ($\log_{10}$ cfu/g) of the raw sliced meat samples and different spice blends used for the treatment of the meat samples are presented in Table 2. A very low APC of <1 $\log_{10}$ cfu/g was found in the meat sample obtained from the first block (B1), while 5.91 $\log_{10}$ cfu/g was found in the raw meat samples obtained from the second block (B2). The variation in the APC between the two meat sources could be from the hygienic practice followed during slaughtering and post slaughtering of the animals. The high count exhibited in one of the beef sources (B2) was also in the range (4.0 to 7.05 $\log_{10}$ cfu/g) of earlier research reports for the microbiological status of fresh beef cuts at different countries' retail markets [35,36,37]. A major problem in food hygiene is the fecal contamination of beef and chicken meat with the family *Enterobacteriaceae* [28]. *Enterobacteriaceae* are a large family of facultatively anaerobic, gram-negative bacilli that inhabit the intestines of many animal species. This family includes pathogenic *Escherichia*, *Salmonella* serovars, and *Klebsiella* species [28,38]. The high prevalence of *Enterobacteriaceae* could be attributed to inadequate sanitary conditions and poor general hygiene. In the present study, a very low *Enterobacteriaceae* count (<1 $\log_{10}$ cfu/g) were found in the raw sliced meat samples of the two beef sources used in the preparation of *Quanta*. Crowley et al. [39] reported *Enterobacteriaceae* levels ranging from 6.54 to 6.98 $\log_{10}$ cfu/g in fresh, unpackaged, and minced beef. Abdelrahman et al. [40] reported $6.3 \times 10^4 \pm 2.8 \times 10^4$ cfu/g counts for fresh ground beef. Zulfakar et al. [37] identified 5.05 ± 0.87 $\log_{10}$ cfu/g in a bacterial contamination study on beef sold at selected wet markets in Selangor and Kuala

Lumpur. As compared to these earlier studies, a substantially lower APC and *Enterobacteriaceae* count reflects the hygienic status of the sliced meats from two different sources used in the current study.

Table 2. Aerobic plate count and *Enterobacteriaceae* count (log_{10} cfu/g) in raw sliced meat samples and different spice blends used for treatment of the meat samples

Type of sample	Bacterial count (log_{10} cfu/g)	
	APC	Enterobacteriaceae
Sliced meats (B1)	<1	<1
Sliced meats (B2)	5.91 ± 0.11	<1
Spice blend (S1)	5.89 ± 0.04	5.28 ± 0.01
Spice blend (S2)	6.15 ± 0.04	5.28 ± 0.15
Spice blend (S3)	6.24 ± 0.01	<1
Spice blend (S4)	5.89 ± 0.16	5.79 ± 0.13
Spice blend (S5)	5.83 ± 0.08	5.79 ± 0.04
Spice blend (S6)	6.44 ± 0.03	6.07 ± 0.01

n = 3, number of observations; B1 and B2, and S1 up to S6 are as indicated in Table 1; APC is aerobic plate count; Values in the table are means ± SD of three observations.

On the other hand, the APC and *Enterobacteriaceae* count of most of the spice blends analyzed were above 5.0 log_{10} cfu/g except for the spice blend S3 where a low detectable count of *Enterobacteriaceae* was found (Table 2). Bakobie et al. [29] reported that spices and herbs can serve as sources of microbial contamination of foods, in which they are used as condiments or cooking aids. In the present study, high initial loads of both total bacteria and *Enterobacteriaceae* were observed in the spice blends that ranged from 5.89 to 6.44 log_{10} cfu/g and from <1 log_{10} cfu/g to 6.07 log_{10} cfu/g for the aerobic plate count and *Enterobacteriaceae* count, respectively. The high bacterial load in the spices is an indication of unhygienic practices during their preparation. In the microbiological quality study of the spice used in the production of Kilishi which is a product similar to that in our study, *quanta*, a comparable high aerobic plate count of 8 log_{10} cfu/g was reported by Shamsuddeen [41]. According to Shamsuddeen [41], spices like other food substances may carry some bacteria, yeasts, molds spores, and even some insects. The predominant flora is generally composed of aerobic spore-forming bacteria; non-spore-forming bacteria, indicator organisms, and some pathogens can also be found according to the International Commission on Microbiological Specifications for Foods [42].

Detection of pathogens in the treated meat samples

With an increase in global trade and consumer awareness of the hygienic quality of meat in recent years, international attention is being focused on ways to improve the microbial quality and safety of foods [25]. Rapid, accurate, and reliable detection and identification of bacterial foodborne pathogens are critical for food safety. The occurrence of *Escherichia coli* in the treated meat samples over a drying period of 10 and 20 days and that of *Salmonella* spp. in the raw meat sample is indicated in Table 3. *Escherichia coli* is a member of the family *Enterobacteriaceae*. *E. coli* is known to microbiologists as «enteric bacteria», because it lives in the intestinal tract of humans and animals. *E. coli* colonizes the gastrointestinal tracts of a wide range of wild and domestic animals, especially animals raised for human consumption [43]. *Escherichia coli* was not detected in the raw sliced meat samples as indicated in Table 3; however, it was detected in six (T1-T6) of the dry meat samples treated with spice blends on the 10th and 20th days of the drying experiments. *Escherichia coli* was not detected in T7 (the negative control, which did not contain the spice blend) throughout the experimental period. As *E. coli* was not detected in the raw meat samples, the detection of *E. coli* on the 10th and 20th days of the drying experiments was attributed to the presence of *E. coli* in the spice blends used in the experiment (Table 1). Thus, it seems that the spice blends used served as a source of contamination of the meat by *E. coli*. This calls for careful and scrupulous hygienic measures during handling and preparation of spices used for treatment of the dried meat *Quanta*. Similarly, occurrence of microorganisms that are potentially pathogenic in spices used in Suya (dried smoked meat) and Kilishi (sun dried spiced and grilled meat snack) preparation was reported as a major cause of gastrointestinal disturbances resulting from the consumption of these meat products in Nigeria [41]. Contaminated spices were reported to be

Table 3. Occurrence of *Escherichia coli* and *Salmonella* spp. in raw sliced meat (day 1) and meat samples treated with different spice blends (day 10 and 20 of drying)

Treatments	Occurrence of pathogens					
	Escherichia coli			*Salmonella* spp.		
	Day 1	Day 10	Day 20	Day 1	Day 10	Day 20
T1	-ve	+ve	+ve	+ve	-ve	-ve
T2	-ve	+ve	+ve	+ve	-ve	-ve
T3	-ve	+ve	+ve	+ve	-ve	-ve
T4	-ve	+ve	+ve	+ve	-ve	-ve
T5	-ve	+ve	+ve	+ve	-ve	-ve
T6 (positive control)	-ve	+ve	+ve	+ve	-ve	-ve
T7 (negative control)	-ve	-ve	-ve	+ve	-ve	-ve

n= 3, number of observations; T1 up to T7 are experimental treatments (sliced meat samples treated with different spice blends as indicated in Table 1; spice blend number corresponds to the treatment number except for T7, which did not receive pre-drying spice treatment); +ve refers to presence and –ve refers to absence.

causes of food-borne illness and spoilage of food and were associated with food-borne pathogenic microorganisms [44,45]. According to Toldra [46], unless spices are treated to reduce their microbial content, they may add high numbers and undesirable kinds of organisms to food, in which they are used. In the current study, the spice blends used for the pre-drying treatment of the sliced meats were treated with low heat treatment during the preparation step. However, the heat treatment applied was mild as it was intended only for drying and did not help in reducing the bacterial contamination of the spices. Therefore, spices should be subjected to treatment that would reduce their microbial load to avoid the introduction of undesirable kinds of spoilage and pathogenic organisms.

Salmonella spp. was detected on day one in all the raw meat samples (treatments) (Tables 1 and 3); however, it was not detected in the treated meat samples on the 10th and 20th days of drying (Table 3). The absence of *Salmonella* spp. in the meat samples on the 10th and 20th days of the drying period suggests that the different concentrations of the spice blends used in combination with drying are effective in inhibiting the growth of *Salmonella* species in the meat samples. Drying inhibits microbial growth in foods by reducing its water activity. According to Murano [47], the removal of biologically active water through drying helps stop the growth of microbes. In general, bacteria other than halophiles will not grow at 0.83 a_w or below, and most are inhibited markedly at 0.90 a_w or less [48]. Ghaly et al. [49] documented that the growth of pathogens is prevented by a_w at 0.85 and USDA [50] reported that the minimum water activity for growth of *Salmonellae* associated with dried meat products is 0.94. In the present study the disappearance of *Salmonella* spp. can be related to the combined inhibitory effect of the spice blends and drying that led to a reduction of water activity of all treatments.

Enumeration of microorganisms in the treated meat samples

The APC and *Enterobacteriaceae* count (log_{10} cfu/g) of meat samples on the 10th and 20th days of drying after application of the treatments are indicated in Table 4. As compared to the total plate count in the raw materials (meat samples and spice blends) reported in Table 2, an increase in the APC was observed in the treated meat samples over the drying period (10th and 20th days). All the meat samples had APC > 7.0 log_{10} cfu/g (Table 4). No significant difference ($p > 0.05$) in the APC was observed among the treatments (T_1-T_7) at a given drying period (10th and 20th days) and also between the two drying times for a given treatment (Table 4). Some researchers stated that the *Enterobacteriaceae* as a whole, and not just *E. coli*, should be taken into account when considering the sanitary standards and hygiene of dry and low-moisture foods [51,52]. High *Enterobacteriaceae* count in food samples is an indication of possible contamination from enteric sources [53].

Similar to the aerobic plate count, all the meat samples (T1-T7) had the *Enterobacteriaceae* count of > 7.0 log_{10} cfu/g (Table 4) during the drying experiment. No significant difference ($p > 0.05$) in the *Enterobacteriaceae* count was observed among the different treatments (T_1-T_7) at a given drying time and also between the 10th and 20th days of the drying period for a given treatment (Table 4). The high microbial counts observed in the spice blends used for pre-treatment of the meat samples (Table 2) in the present study may be responsible for the very high (>7.0 log_{10} cfu/g) APC and *Enterobacteriaceae* count observed in the dried meat samples after 10 and 20 days of treatment. Thus, this calls for scrupulous hygienic measures during the handling and preparation of spice blends used for the treatment of meat samples. According to Frazier and Westhoff [54], spices do not have a marked bacteriostatic effect in the concentrations used in meat products and they may even serve as a source of contamination of the processed product. According to Jay et al. [17], components used as seasoning and other formulation ingredients/additives such as spices can be sources of additional microorganisms. This may explain the increases in the *Enterobacteriaceae* count and total bacteria count over the drying periods (10 and 20 days) of the present study.

Table 4. Aerobic plate count (APC) and *Enterobacteriaceae* count (log_{10} cfu/g) of meat samples on the 10th and 20th days of drying after application of the treatments

Treatments	Bacterial counts (log_{10} cfu/g)			
	Aerobic plate count (APC)		*Enterobacteriaceae* (EC)	
	10th day	20th day	10th day	20th day
T1	7.69 ± 0.18	7.78 ± 0.06	7.70 ± 0.16	7.70 ± 0.20
T2	7.83 ± 0.01	7.73 ± 0.04	7.60 ± 0.16	7.49 ± 0.01
T3	7.75 ± 0.03	7.82 ± 0.15	7.44 ± 0.25	7.55 ± 0.33
T4	7.72 ± 0.01	7.83 ± 0.06	7.56 ± 0.31	7.55 ± 0.29
T5	7.71 ± 0.04	7.74 ± 0.13	7.70 ± 0.23	7.44 ± 0.30
T6	7.42 ± 0.04	7.86 ± 0.04	7.41 ± 0.14	7.65 ± 0.16
T7	7.89 ± 0.11	7.87 ± 0.03	7.72 ± 0.28	7.75 ± 0.05

n = 3, number of observations; T1 up to T7 are as indicated in the Table 3; Values in the table are means ± SD of three observations; No significant difference ($p > 0.05$) in the APC and EC was observed among the treatments (T_1-T_7) at a given drying period (10th and 20th days) and also between the two drying days for a given treatment.

Conclusion

The results of the present study showed that treatment of meat samples with the spice blends served as a source of contamination of the dried meat samples with *E. coli*. However, the spice blends used in combination with drying were effective in inhibiting the growth of *Salmonella* species and resulted in absence of *Salmonella* spp. in the dry meat samples on the 10th and 20th days of the drying period. Based on the findings of this study, the following recommendations are made to improve the quality of dried meat, *Quanta*:

- Spices and herbs applied on meat used for preparation of *Quanta* should be produced and handled under hygienic conditions and should be subjected to treatments that would reduce their microbial load during the blend preparation.
- Extraction of essential oils and active agents of spices may increase the antimicrobial and preservative effects of spices on dried meat. Thus, this needs further investigation.

REFERENCES

1. Henchion, M., Moloney, A.P., Hyland, J., Zimmermann, J., McCarthy, S. (2021). Review: Trends for meat, milk and egg consumption for the next decades and the role played by livestock systems in the global production of proteins. *Animal*, 15, Article 100287. https://doi.org/10.1016/j.animal.2021.100287
2. Ponnampalam, E.N., Bekhit, A.E.D., Bruce, H., Scollan, N.D., Muchenje, V., Silva, P., Jacobs, J.L. (2019). Production strategies and processing systems of meat: current status and future outlook for innovation — a global perspective. Chapter in a book: Sustainable Meat Production and Processing. London: Academic Press. 17–44.
3. Rudy, M., Kucharyk, S., Duma-Kocan, P., Stanisławczyk, R., Gil, M. (2020). Unconventional methods of preserving meat products and their impact on health and the environment. *Sustainability (Switzerland)*, 12(15), Article 5948. https://doi.org/10.3390/su12155948
4. Desierea, S., Hungb, Y., Verbekeb, W., D'Haese, M. (2018). Assessing current and future meat and fish consumption in Sub-Sahara Africa: Learnings from FAO Food Balance Sheets and LSMS household survey data. *Global Food Security*, 16, 116–126. https://doi.org/10.1016/j.gfs.2017.12.004
5. Solomon, A., Workalemahu, A., Jabbar, M.A., Ahmed, M.M., Hurissa, B. (2003). Livestock marketing in Ethiopia: A review of structure, performance and development initiatives. Socio Economic and Policy Research. Working Paper 52. Nairobi, Kenya: International Livestock Research Institute.
6. FAO. (2005). Supply utilization accounts and food balances. FAOSTAT on-line database. Retrieved from http://faostat.fao.org/faostat. Accessed December 14, 2019.
7. UN. (2005). World population prospect. Retrieved from http://esa.un.org/unpp/. Accessed October 24, 2019.
8. FAOSTAT. (2020). Production Statistics. Live Animals. FAO Statistics Division. Rome, Italy: Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/faostat/en/#data/QA. Accessed June 05, 2020.
9. Betru, S., Kawashima, H. (2009). Pattern and determinants of meat consumption in urban and rural Ethiopia. *Livestock Research for Rural Development*, 21(9), Article 143.
10. Zhou, G.H., Xu, X.L., Liu, Y. (2010). Preservation technologies for fresh meat — A review. *Meat Science*, 86(1), 119–128. https://doi.org/10.1016/j.meatsci.2010.04.033
11. Kuyu, C.G., Bereka, T.Y. (2020). Review on contribution of indigenous food preparation and preservation techniques to attainment of food security in Ethiopian. *Food Science and Nutrition*, 8(1), 3–15. https://doi.org/10.1002/fsn3.1274
12. Dagne, T., Ameha, N. (2017). Review on beef eating quality attributes (tenderness, juiciness and flavor) and quality standards in Ethiopia. *Food Science and Quality Management*, 62, 23–30.
13. FAO. (1990). Manual of Simple Methods of Meat Preservation. FAO Animal Production and Health Paper No. 79. Rome, Italy: Food and Agriculture Organization of the United Nations. Retrieved from https://www.fao.org/3/x6932e/x6932e00.htm. Accessed April 25, 2019
14. AACCSA (2015). Value chain study on meat processing industry in Ethiopia. A project on strengthening the private sector in Ethiopia. Addis Ababa, Ethiopia: Addis Ababa Chamber of Commerce and Sectoral Associations (AACCSA). Retrieved from http://mau.addischamber.com/pdf/Business-Opportunity-for-meat-processing-VC.pdf. Accessed February 09, 2018)
15. Abebe, P., Asfaw, Z., Bekele, T., Agize, M. (2018). Diversity, use and conservation of spices and condiments in the home gardens (Derkuwa) of Konta Special District (Woreda), southern Ethiopia. *International Journal of Current Research and Academic Review*, 6(3), 27–42. https://doi.org/https://doi.org/10.20546/ijcrar.2018.603.006
16. Quality and Standards Authority of Ethiopia. (2002). Spices and condiments. *Oleoresin capsicum* specification. Ethiopian Standard: 680. Addis Ababa, Ethiopia: Ethiopian Quality and Standard Authority.
17. Jay, J.M., Loessner, M.J., Golden, D.A. (2005). Modern Food Microbiology, 7th ed. New York, USA: Springer Science + Business Media Inc.
18. ISO. (2003). Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of microorganisms — colony count at 30 °C by the pour plate technique. ISO Method 4833:2003. Geneva, Switzerland: International Organization for Standardization.
19. Roberts, D., Greenwood, M., 2003. Practical Food Microbiology, 3rd ed. Massachusetts, USA: Blackwell Publishing Ltd.
20. ISO. (2002). Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella* spp. ISO. Method 6579. Geneva, Switzerland: International Organization for Standardization.
21. Maurin, L., James, T.P., 2001. Bacteriological Analytical Manual, 8th ed. USA: US Food and Drug Administration (FDA).
22. SAS. (2004). Statistical Analysis Systems, *SAS* Version 9.1. Cary, NC, USA: *SAS* Institute Inc. Retrieved from https://support.sas.com/documentation/onlinedoc/91pdf/sasdoc_91/stat_ug_7313.pdf. Accessed May 11, 2017)
23. Henchion, M., McCarthy, M., Resconi, V., Troy, D. (2014). Meat consumption, trends and quality matters. *Meat Science,* 98(3), 561–568. https://doi.org/10.1016/j.meatsci.2014.06.007
24. Yuma, Y.S., Beta, A.M., Basore, B.A. (2020). Red meat processing and preservation technologies: a review. *World Journal of Dairy and Food Sciences*, 15(2), 78–87. https://doi.org/10.5829/idosi.wjdfs.2020.78.87
25. Biswas, A.K., Kondaiah, N., Anjaneyulu, A.S.R., Mandal, P.K. (2011). Causes, concerns, consequences and control of microbial contaminants in meat — a review. *International Journal of Meat Science*, 1(1), 27–35. https://doi.org/10.3923/ijmeat.2011.27.35
26. Hazarika, R.A., Singh, D.K., Kapoor, K.N., Agarwal, R.K., Pandey, A.B., Rajkumar, D.N. (2005). Detection and characterization of verotoxin producing *E. coli* (VTEC) isolated from buffalo meat. *Journal of Food Safety*, 24(4), 281–290. https://doi.org/10.1111/j.1745–4565.2004.00536.x
27. Biswas, A.K., Mandal, P.K. (2017). Meat-borne pathogens and use of natural antimicrobials for Food Safety. Chapter in a book: Foodborne pathogens and antibiotic resistance New Jersey: John Wiley & Sons, Inc. 225–245.
28. Gwida, M., Hotzel, H., Geue, L., Tomaso, H. (2014). Occurrence of *Enterobacteriaceae* in raw meat and in human samples from Egyptian retail sellers. *International Scholarly Research Notices*, 2014, Article 565671. https://doi.org/10.1155/2014/565671
29. Bakobie, N., Addae, A.S., Duwiejuah, A.B., Cobbina, S.J., Miniyila, S. (2017). Microbial profile of common spices and spice blends used in Tamale, Ghana. *International Journal of Food Contamination*, 4(1), Article 10. https://doi.org/10.1186/s40550–017–0055–9
30. Heredia, N., García, S. (2018). Animals as sources of foodborne pathogens: A review. *Animal Nutrition*, 4(1), 250–255. https://doi.org/10.1016/j.aninu.2018.04.006
31. Ehuwa, O., Jaiswal, A.K., Jaiswal, S. (2021). Salmonella, food safety and food handling practices. *Foods*, 10(5), Article 907. https://doi.org/10.3390/foods10050907

32. Moore, G., Griffith, C. (2002). A comparison of surface sampling methods for detecting coliforms on food contact surfaces. *Food Microbiology*, 19(1), 65–73. https://doi.org/10.1006/fmic.2001.0464
33. EFSA. (2010). The assessment of the comparison of the Australian monitoring programme for carcasses to requirements in Regulation (EC) No. 2073/2005 on microbiological criteria on foodstuffs. *EFSA Journal*, 8(3), Article 1452. https://doi.org/10.2903/j.efsa.2010.1452
34. Mendonca, A., Thomas-Popo, E., Gordon, A. (2020). Microbiological considerations in food safety and quality systems implementation. Chapter in a book: Food Safety and Quality Systems in Developing Countries. Volume III: Technical and Market Considerations. Jamaica: Technological Solutions Limited, Kingston. 185–260.
35. Kim, H.-J., Kim, D., Kim, H.-J., Song, S.-O., Song, Y.-H., Jang, A. (2018). Evaluation of the microbiological status of raw beef in Korea: Considering the suitability of aerobic plate count guidelines. *Korean Journal for Food Science of Animal Resources*, 38(1), 43–51. https://doi.org/10.5851/kosfa.2018.38.1.043
36. Stopforth, J.D., Lopes, M., Shultz, J.E., Miksch, R.R., Samadpour, M. (2006). Microbiological status of fresh beef cuts. *Journal of Food Protection*, 69(6), 1456–1459. https://doi.org/10.4315/0362–028x-69.6.1456
37. Zulfakar, S.S., Baharudin, N., Bakar, N.F.A. (2017). Bacterial contamination on beef sold at selected wet markets in Selangor and Kuala Lumpur. *Journal of Agricultural Science*, 9(13), 89–95. https://doi.org/10.5539/jas.v9n13p89
38. Ruby, J.R., Ingham, S.C. (2009). Use of *Enterobacteriaceae* analysis results for predicting absence of *Salmonella* serovars on beef carcasses. *Journal of Food Protection*, 72(2), 260–266. https://doi.org/10.4315/0362–028x-72.2.260
39. Crowley, H., Cagney, C., Sheridan, J., Anderson, W., McDowell, D., Blair, I.S. et al. (2005). *Enterobacteriaceae* in beef products from retail outlets in the Republic of Ireland and comparison of the presence and counts of *E. coli* O157: H7 in these products. *Food Microbiology*, 22(5), 409–414. https://doi.org/10.1016/j.fm.2004.09.013
40. Abdelrahman, H., Ahmed, A.M., Shaheen, H. (2014). Quantitative and qualitative studies on *Enterobacteriaceae* in ground beef. *Suez Canal Veterinary Medicine Journal*, 19(2), 77–88. https://doi.org/10.21608/scvmj.2014.65324
41. Shamsuddeen, U. (2009). Microbiological quality of spice used in the production of kilishi a traditionally dried and grilled meat product. *Bayero Journal of Pure and Applied Sciences*, 2(2), 66–69. https://doi.org/10.4314/bajopas.v2i2.63767
42. ICMSF. (1986). Microorganisms in foods 2. Sampling for microbiological analysis: principles and specific applications, 2nd ed. International Commission on Microbiological Specifications for Foods (ICMSF). Oxford: Blackwell Scientific Publications. 213–216.
43. Ramos, S., Silva, V., de Lurdes Enes Dapkevicius, M., Caniça, M., Tejedor-Junco, M.T., Igrejas, G. et al. (2020). *Escherichia coli* as commensal and pathogenic bacteria among food-producing animals: Health implications of extended spectrum β-lactamase (ESBL) production. *Animals*, 10, Article 2239. https://doi.org/10.3390/ani10122239
44. Lanciotti, R., Gianotti, A., Patrignani, F., Belletti, N., Guerzoni, M.E., Gardini, F. (2004). Use of natural aroma compounds to improve shelf life and safety of minimally processed fruits. *Trends in Food Science and Technology*, 15(3–4), 201–208. https://doi.org/10.1016/j.tifs.2003.10.004
45. Li, H., Tajkarimi, M., Osburn, B.I. (2008). Impact of vacuum cooling on *Escherichia coli* O157: H7 infiltration into lettuce tissue. *Applied and Environmental Microbiology*, 74(10), 3138–3142. https://doi.org/10.1128/AEM.02811–07
46. Toldra, F. (2002). Dry-Cured Meat Products. Connecticut, USA: Food & Nutrition Press, Inc.
47. Murano, S.P. (2003). Understanding Food Science and Technology. Belmont, US: Thomson Learning Academy Resource Center.
48. Huang, T.C., Nip, W.K. (2001). Intermediate-moisture meat and dehydrated meat. Chapter in a book: Meat Science and Applications. New York: Marcel Dekker, 403–442.
49. Ghaly, A.E., Dave, D., Budge, S., Brooks, M.S. (2010). Fish spoilage mechanisms and preservation techniques: Review. *American Journal of Applied Sciences*, 7(7), 846–864.
50. USDA. (2005). Principles of preservation of shelf-stable dried meat products. United State Department of Agriculture. Food Safety and Inspection Service. Retrieved from: http://www.fsis.usda.gov/PDF/FSRE_SS_7Principl es.pdf. Accessed June 10, 2022
51. Buchanan, B.L., Oni, R. (2012). Use of microbiological indicators for assessing hygiene controls for the manufacture of powdered infant formula. *Journal of Food Protection*, 75(5), 989–997. https://doi.org/10.4315/0362–028X.JFP-11–532
52. Codex Alimentarius Commission. (2018). Code of hygienic practice for low-moisture foods. Codex Standard CXC75–2015. Rome, Italy: Food and Agriculture Organization of the United Nations. Retrieved from https://higieneambiental.com/sites/default/files/images/halimentaria/codex-alimentarius-bajaaw.pdf. Accessed July 13, 2020
53. Ratsimba, A., Rakoto, D., Jeannoda, V., Andriamampianina, H., Talon, R., Leroy, S. et al. (2019). Physicochemical and microbiological characteristics of kitoza, a traditional salted/dried/smoked meat product of Madagascar. *Food Science and Nutrition*, 7(8), 2666–2673. https://doi.org/10.1002/fsn3.1122
54. Frazier, M., Westhoff, W.C. (2006). Food Microbiology, 3rd ed. New York: McGraw Hill Publishing Company Limited, 163–165.

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PREVALENCE OF LISTERIA MONOCYTOGENES IN MEAT PRODUCTS DURING 2017–2019 DEPENDING ON TECHNOLOGICAL FACTORS AND SEASONS

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Keywords: *Listeria monocytogenes, semi-finished meat products, monitoring, technological factors, sample preparation, seasonality*

Abstract

Microbiological examination of contamination of imported and domestic meat products with pathogenic bacteria Listeria monocytogenes depending on a meat type, technology and season was carried out during 2017–2019. In total, 2777 product samples were analyzed; the presence of this pathogen was revealed in 8.8% of products (244 positive samples). It was found that the prevalence of L. monocytogenes in meat products increased over three years of observation (2017–2019). The highest occurrence of this pathogen was found in poultry meat (on average 18.7%) followed by products from beef (13.2%). Meat products from mixed raw materials (beef and pork) accounted for 5.3% of tested samples, while in pork semi-finished products L. monocytogenes was found only in 3.2% of cases. It was noted that the technology of semi-finished products significantly affected the level of contamination of meat products with L. monocytogenes. Various technological approaches are used in the production process increasing the risk of contamination of the finished product since there is no timely data on Listeria contamination of raw materials used for production of a particular product. It has been established that a significant role in microbiological studies is played by various approaches to sample preparation of analyzed samples of meat cuts, semi-finished products in large and small pieces, as well as minced semi-finished products. Not knowing the real level of surface contamination with L. monocytogenes of carcasses, half-carcasses, semi-finished products in large pieces, manufacturers use such raw materials for the subsequent production of other types of semi-finished meat products, increasing the risk of manufacturing unsafe products with following contamination of equipment, work surfaces and other objects of the production environment. The highest occurrence of L. monocytogenes in meat products during three years of observation was found in the summer period (14.2%). The proportions of positive samples in the winter, spring and autumn months varied on average within 6.7–7.1%.

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Introduction

Listeria monocytogenes (L. monocytogenes) is a facultative Gram-positive intracellular pathogen, which causes an infectious illness called listeriosis. In terms of the lethality and severity of clinical course, listeriosis exceeds salmonellosis and campylobacteriosis turning into one of the most significant foodborne infections in the world [1]. This pathogen is widely distributed in the environment, where it is frequently found in foods, and poses a serious problem in the food chain, especially for ready-to-eat (RTE) food products [2,3].

Over the last decades, the majority of large epidemic outbreaks of listeriosis with the high percent of fatal cases have been associated with food consumption, first of all, cheese (especially soft), milk and other dairy products, as well as meat semi-finished products and salads [1]. With that, a leading role is played by ready-to-eat (RTE) foods supporting the growth of *L. monocytogenes* that are stored

in the refrigerated conditions for a long time and subjected to cross-contamination during storage. At low positive temperatures, the pathogen can slowly multiply in foods, including meat products [4]. According to the data of the European Food Safety Authority (EFSA), 2,480 cases of listeriosis were reported in the EU countries in 2017 [5]. The number of reported confirmed human cases of listeriosis in the EU countries was 2,545 in 2018 and 2,621 in 2019 [6]. These data show the stable high number of recorded cases of listeriosis among the EU population.

At the beginning of the 21st century, in the Russian Federation, the corresponding changes were made in SanPiN2.3.2.1078–01[1] by introducing the norm for controlling *L. monocytogenes* in foods and SanPiN3.1.7.2817–10[2] "Prevention of listeriosis in humans" by introducing the periodicity of the control of *Listeria* in food industry enterprises. These documents allowed organizing the effective control of pathogenic *Listeria*. If measures on the control of this microorganism in the food production environment are ineffective, it can persist, which leads to cross-contamination of foods [7]. McCarthy Z. et al. [8] assessed a risk of changes in the level of contamination with pathogens at different technological links in the places of poultry slaughter and meat processing, and found that the complexity and continuity of a technological process can easily lead to cross-contamination. Different *L. monocytogenes* strains can survive and proliferate in food processing enterprises due to the corresponding phenotypic properties such as the attachment to surfaces, biofilm-forming ability and increased resistance to environmental stress [9,10]. Bacteria organized in a biofilm develop resistance to harsh environmental conditions: desiccation, nutrient deprivation or sanitary treatment [11,12,13,14]. In the study carried out by Bonsaglia et al. [15], almost all *L. monocytogenes* strains isolated from the food production environment were able to form biofilm on stainless steel and glass surfaces.

Since 2011, the safety of food products regarding pathogenic *Listeria* has been ensured by the Technical Regulation of the Customs Union (TR TU 021/2011[3]). Methods for controlling foods for the presence of pathogenic *Listeria* according to GOST 32031–2012[4] "Food products. Methods for detection of *Listeria monocytogenes*" were developed and introduced into practice in Russia. The modern methodological base allows fast and effective detection of pathogenic *Listeria* in foods along with other pathogens and opportunistic pathogens.

With that, the number of recorded listeriosis cases in the Russian Federation is not high, although its registration as a distinct nosological form of human illness was introduced in the RF in 1992. In Russia, only sporadic listeriosis cases were detected. In 2005–2017, 644 listeriosis cases were recorded in Russia with the highest number (75) of cases in 2006–2007. During this period, 229 listeriosis cases were recorded in Moscow accounting for 35.6% of all cases reported in Russia [16]. In 2019, the clinical diagnosis of listeriosis was laboratory confirmed in the RF in 86 cases (19 fatal cases) [17]. It can be stated that there is a certain imbalance between the confirmed level of *Listeria* contamination of foods and the revealed level of listeriosis incidence.

In our view, Russia has significant reserves to increase the effectiveness of the epidemiological surveillance of the *Listeria* infection based on the improvement of the laboratory diagnostics of the main clinical forms of listeriosis (meningitis, meningoencephalitis, sepsis; abortion and stillbirth in pregnant women), introduction of the obligatory epidemiological investigation of listeriosis cases with an emphasis on the foodborne transmission, and analysis of the level of *Listeria* contamination of foods in the conditions of the technological chain of modern food production. Only few studies on detection of *Listeria* in the conditions of modern meat processing plants were carried out in Russia [18,19]. In this study, quite extensive investigations of *L. monocytogenes* contamination of imported and domestic meat products depending on a meat type, technology and seasons were carried out.

The aim of the study was to analyze the prevalence of *L. monocytogenes* in meat products and semi-finished products depending on a meat type, production technology and season during a period from 2017 to 2019.

Objects and methods

The following samples were investigated: raw poultry semi-finished products (natural, minced and with spices), pork and beef semi-finished products (in large pieces, in small pieces and minced), semi-finished products (minced and in dough) made from mixed meat types (beef and pork), as well as ready-to-eat (RTE) meat products.

Sample preparation for microbiological analysis included thawing (when necessary), opening packages under aseptic conditions (when analyzing packed meat products), flaming of the sample surface or sampling without flaming of the surface, and comminution of samples.

Sampling from pork and beef semi-finished products in large pieces as well as from poultry carcasses was carried out according to GOST R ISO 6887–2–2013[5] and

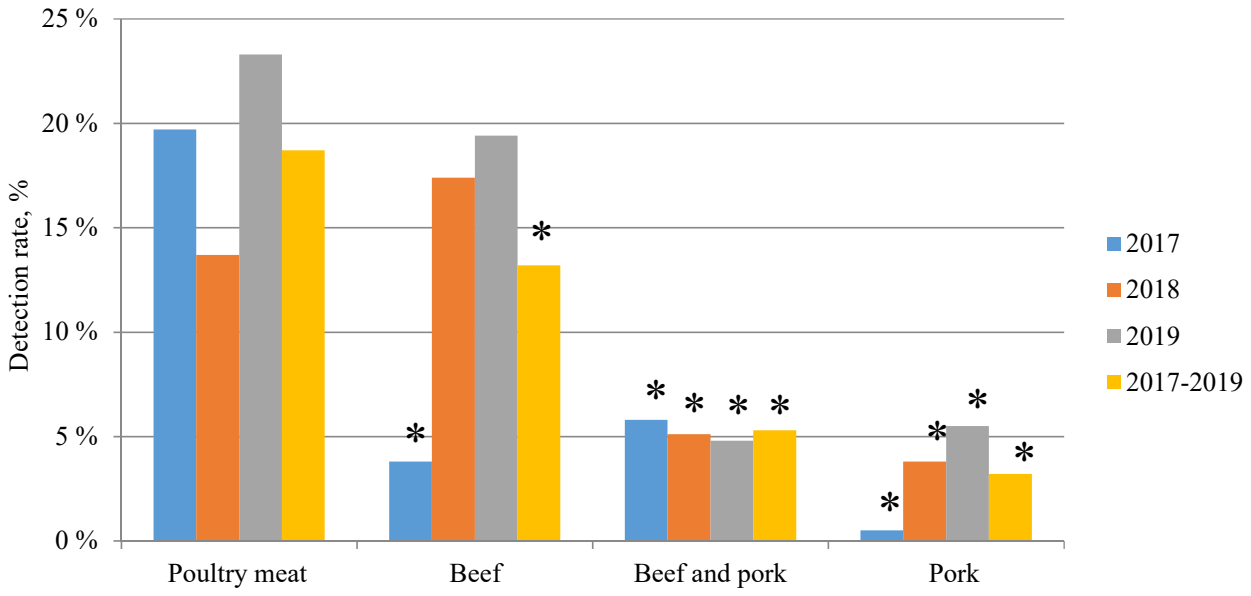
[1] Additions and changes No 22 to SanPiN 2.3.2.1078–01. Sanitary and epidemiological rules and regulations SanERR 2.3.2. 2804–10 "Hygienic requirements for the safety and nutritional value of food products". Retrieved from https://base.garant.ru/12183206/53f89421bbdaf741eb2d1ecc4ddb4c33/ Accessed August 25, 2022. (In Russian)

[2] SanPiN 3.1.7.2817–10. Sanitary and epidemiological rules and regulations "Prevention of listeriosis in humans". Retrieved from https://36.rospotrebnadzor.ru/documents/san_nor/6082 Accessed August 25, 2022. (In Russian)

[3] Technical regulation of the Customs Union TR CU 021/2011"On food safety". (Adopted by The decision of the Council of the Eurasian economic Commission of December 9, 2011 No. 880). Moscow, 2011. Retrieved from https://docs.cntd.ru/document/902320560. Accessed August 24, 2022. (In Russian)

[4] GOST 32031–2012 "Food products. Methods for detection of *Listeria monocytogenes*" Retrieved from https://docs.cntd.ru/document/1200105310 Accessed August 24, 2022. (In Russian)

[5] GOST R ISO 6887–2–2013 «Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 2. Specific rules for the preparation of meat and meat products» Retrieved from https://docs.cntd.ru/document/1200104686 Accessed August 24, 2022 (In Russian)



* p < 0.05 when compared to poultry meat during the same period.

Figure 1. Detection rate of contaminated meat product samples by meat types in 2017–2019

GOST R 54354–2011[6] as follows. A package was removed with the adherence to the aseptic rules and use of sterile instruments. After that, a layer 2 mm thick was cut out from a product surface area of 50 × 50 mm. The surface of this site was flamed up to carbonization, and then the carbonized layer with an area of 40 × 40 mm and thickness of 10 mm was removed with other sterile instruments. Analytical units of 25 g each were taken with sterile forceps and scalpel, and placed into sterile bags for homogenization.

Analytical units of 25 g each were taken from semi-finished products in small pieces, minced semi-finished products and semi-finished products in dough without treating sample surfaces.

All analytical units were tested on the presence of *Listeria monocytogenes* according to GOST 32031–2012.

Statistical analysis was performed using software MS Excel 2019 (Microsoft, USA) and Statistica 12.0 (Statsoft, USA). To assess statistical significance of differences in data, Pearson's chi-squared test and Fisher's exact test were used. Differences were considered significant at $p < 0.05$.

Results and discussion

During the period from January 2017 to December 2019 (inclusively), 2777 samples of meat semi-finished products were analyzed. Among them, 244 samples (8.8%) were positive for *L. monocytogenes* (Table 1).

Analysis of the obtained data allows us to note that the frequency of detection of *L. monocytogenes* rose steadily from 2017 to 2019. The percent of samples positive for *L. monocytogenes* grew year after year and increased practically twofold during the studied period despite the fact that the smallest number of samples was analyzed in 2019.

[6] GOST 54354–2011 «Meat and meat products. General requirements and methods of microbiological testing» Retrieved from https://docs.cntd.ru/document/1200087716 Accessed August 24, 2022 (In Russian)

Table 1. Results of the investigation of different meat types and the number of samples positive for *L. monocytogenes* in 2017–2019

Meat type	2017 r. (analyzed/ positive)	2018 r. (analyzed/ positive)	2019 r. (analyzed/ positive)
Poultry meat	122/24 (19.7%)	226/31 (13.7%)	223/52 (23.3%)#
Beef	156/6 (3.8%)#	132/23 (17.4%)*	144/28 (19.4%)*
Beef and pork	411/24 (5.8%)	394/20 (5.1%)	336/16 (4.8%)
Pork	213/1 (0.5%)#	239/9 (3.8%)*	181/10 (5.5)*
Total	902/55 (6.1%)	991/83 (8.4%)	884/106 (12.0%)#

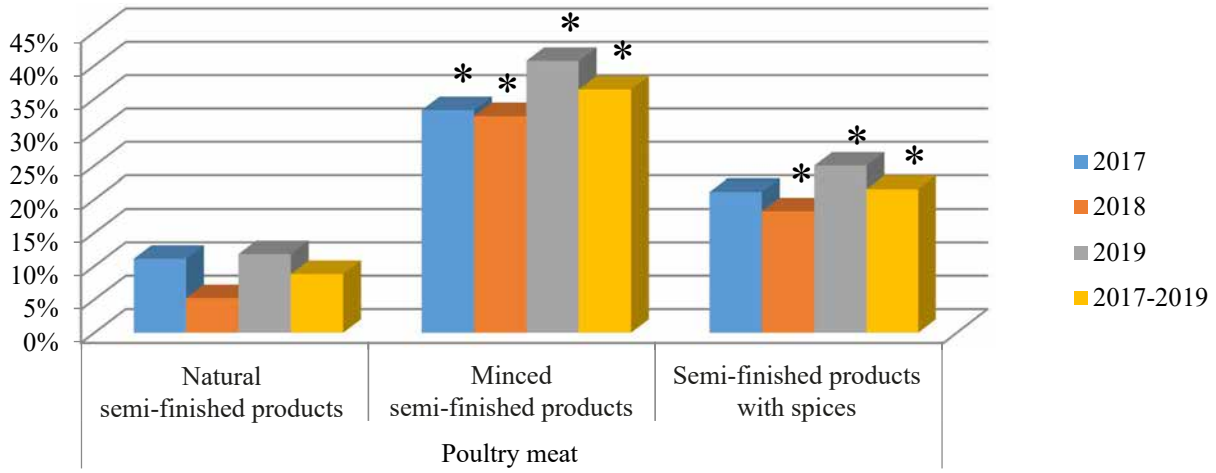
* p < 0.05 when compared with 2017,
# p < 0.05 when compared with 2018.

Data obtained for 2019 agree with the results of the researchers from Brazil [20], who studied the prevalence of *L. monocytogenes* in different meat types in Brazil using a systematic review and meta-analysis of scientific studies published during the period from 2009 to 2019. The total prevalence of *L. monocytogenes* in meat products in Brazil was 13% [20].

It was interesting to assess an impact of different conditions and factors on detection of *L. monocytogenes* during 2017–2019. The results of the investigation were ranked by meat types, technologies of product manufacture, years and seasons.

When analyzing the raw material composition of the tested meat products (Figure 1), it is possible to state with reasonable confidence that the most vulnerable in terms of *L. monocytogenes* contamination were poultry meat and beef. The data on the frequency of detection of *L. monocytogenes* in different meat types during the period of 2017–2019 are presented in the histogram below.

In 2017, the detection rate of *L. monocytogenes* in poultry meat (19.7%) significantly differed from that in other meat types, showing the maximum number of positive samples among all meat types. The proportions of positive



* p < 0.05 when compared with natural semi-finished products from poultry meat.

Figure 2. Detection rate of *L. monocytogenes* in different types of poultry semi-finished products in 2017–2019

samples from mixed raw materials (beef and pork) and from beef were at a level of 5.8 and 3.8%, respectively, while pork was the least contaminated (only 0.5%).

In 2018, products from beef accounted for the maximum proportion of positive samples (17.4%), followed by poultry meat (13.7%), products from mixed raw materials (beef and pork) and pork (5.1 and 3.8%, respectively).

In 2019, it was established with certainty that during the studied period the highest proportion of all positive samples was in poultry meat (23.3%), the second place in terms of the contamination degree was occupied by beef products (19.4%), which also showed the maximum number of positive samples over the period of 2017–2019. The proportion of meat products from mixed raw materials (beef and pork) reduced to 4.8% of tested products showing the insignificant trend towards a decrease in the prevalence over the studied period. At the same time, the prevalence of *L. monocytogenes* in pork increased to 5.5%.

Our data indicate with certainty that *L. monocytogenes* most often occur in poultry meat and beef, which is in complete agreement with the results of other researchers on the RF territory [21]. However, studies carried out abroad, on the contrary, indicate the maximum contamination of pork [20].

The obtained statistical data show an insignificant reduction (from 5.8 to 4.8%) in the detection rate in meat products from mixed raw materials (beef and pork) and a clear increase in the detection rate in pork (from 0.5 to 5.5%).

It was interesting to analyze detection of the pathogen in products depending on the methods for sample preparation in microbiological examination, technological processes applied to meat raw materials during production and different seasons of the investigations.

Products from poultry meat were divided into three types of semi-finished products depending on the technology of their production according to GOST 31936–2012[7]: a) natural semi-finished products, which included carcasses and parts of carcasses, semi-finished products in pieces (boneless and bone-in), b) minced semi-finished products, and c) semi-finished products with the use of spices.

Figure 2 presents the results of the investigation as a diagram, which clearly demonstrates that among the tested poultry semi-finished products, natural semi-finished products were the least contaminated with the pathogen under study. We established by statistical data processing that natural semi-finished products differed significantly from other types in all cases (23 positives out of 262 tested samples) except semi-finished products with spices in 2017.

Minced semi-finished products were the most contaminated (43 positives out of 118 tested samples), which is probably linked with the technology of their production (the maximum product area comes into contact with production objects during mincing). Over the studied period, semi-finished products with spices occupied the intermediate position (41 out of 191), which can be linked with the inhibitory action of preserving agents being constituents of the final composition of these products.

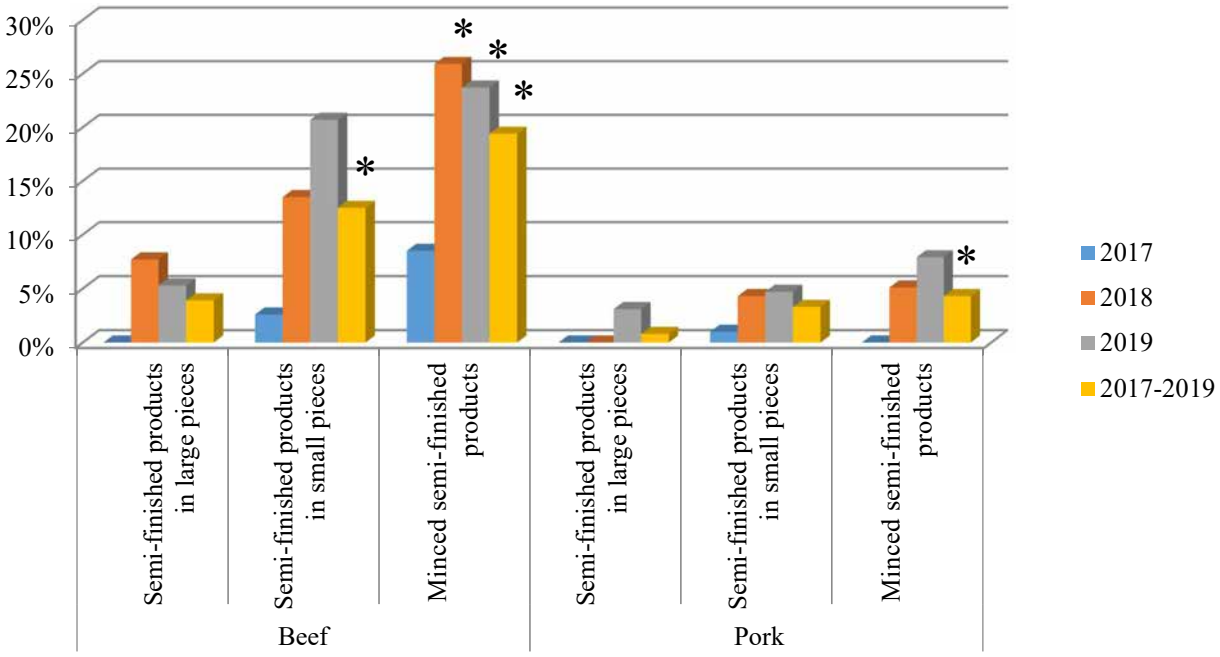
Fifty two poultry carcasses were tested during the indicated period; with that, contaminated samples were not found.

Results of investigations depend to a greater degree on sampling methods. A choice of these methods is directly linked with the aim of the research. According to the documents on sampling (GOST 7702.2.0–2016[8] and GOST R ISO 6887–2–2013[9]), two sampling methods are used for products from poultry meat to assess microbiological safety: the destructive method (tissue dissection) with surface treatment used for taking samples from deep layers of the pectoral muscle of poultry carcasses and the

[7] GOST 31936–2012 «Semi-prepared poultry meat and poultry offal. General specifications». Retrieved from https://docs.cntd.ru/document/1200103353 Accessed August 24, 2022 (In Russian)

[8] GOST 7702.2.0–2016 «Poultry slaughtering products, poultry meat ready-to-cook products and the objects of production environment. Sampling methods and the preparation to microbiological analyses» Retrieved from https://docs.cntd.ru/document/1200139190 Accessed August 24, 2022 (In Russian)

[9] GOST R ISO 6887–2–2013 «Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 2. Specific rules for the preparation of meat and meat products» Retrieved from https://docs.cntd.ru/document/1200104686 Accessed August 24, 2022 (In Russian)



* $p < 0.05$ when compared with semi-finished products in large pieces from beef and pork.

Figure 3. Detection rate of L. monocytogenes in different types of semi-finished products from beef and pork in 2017–2019

destructive method without treatment of the semi-finished product surface used in examination of all other poultry semi-finished products. The overall microbiological status of a product with consideration for its contamination during production is assessed by simultaneous examination of the surface and deep layers of semi-finished products [22]. At the same time, examination of poultry carcasses that are raw materials in the subsequent semi-finished product manufacture by the first method of sample preparation allows making a conclusion about the presence of *L. monocytogenes* only in the deep layers not reflecting possible contamination of the surface layers during primary processing. This statement was confirmed by our data obtained in 2017–2019 in examination of whole poultry carcasses using the destructive method of sampling from deep layers with surface treatment.

An important role in meat product contamination is played by the fact that various technological manipulations are used in the production process (for example, when mincing a semi-finished product) increasing a risk of microbial contamination of the finished product.

Moreover, *Listeria* occurs in the poultry intestine according to data of several scientists (the prevalence in poultry fecal samples was 33% for *Listeria* spp. and 33% for *L. monocytogenes*) [23]. Upon improper primary processing practice, they can contaminate poultry superficial skin layers as well as objects of production environment including floor drains that are considered to be the main point of *Listeria* location in poultry processing plants. This, in turn, leads to extremely high level of product contamination linked exactly with floor drains during the production process. A percent of *Listeria* detection in floor drains in poultry processing plants is almost three times higher than in meat processing plants [24]. Poor construction of the building can lead to accumulation of water in the drainage creating ideal conditions for *L. monocytogenes* survival and biofilm formation [25,26]. Finally, poor sanitary conditions, such as using high pressure hoses for washing floors, can generate aerosols potentially spreading *Listeria* from non-food contact surfaces (NFCS) to food contact surfaces (FCS), or new niches of NFCS [26].

According to GOST 32951–2014[10] and GOST 33102–2014[11], all tested meat semi-finished products were divided depending on the production technology and analyzed on the presence of *L. monocytogenes*.

As can be seen from Figure 3, it was calculated with confidence that among semi-finished products made both from beef and from pork, the lowest *L. monocytogenes* contamination was observed in semi-finished products in large pieces.

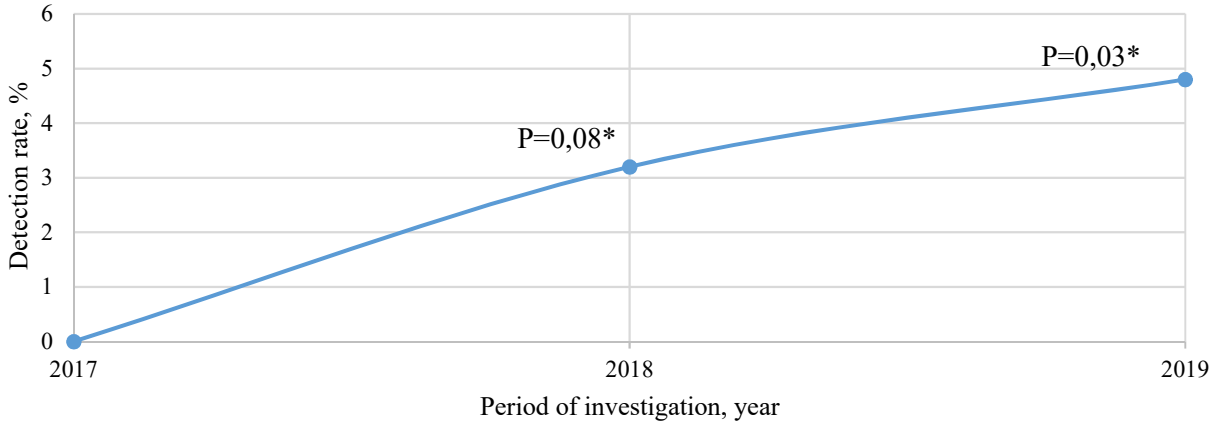
One of the reasons of obtaining such results is the fact that today different approaches to sample preparation for microbiological examination are used for meat cuts, semi-finished products in large pieces, semi-finished products in small pieces and minced semi-finished products.

Moreover, an important role is played by the technology of minced semi-finished product manufacture, where there is an increased risk of additional contamination of raw materials in meat grinders, mincemeat mixers, hamburger patty molders, from the surfaces of objects of the production environment in meat processing plants and so on.

Large scale investigations performed in Italy showed that the most frequently contaminated with *L. monocyto-*

[10] GOST 32951–2014 «Semi-prepared meat and meat-contained product. General specifications» Retrieved from https://docs.cntd.ru/document/1200113849 Accessed August 24, 2022 (In Russian)

[11] GOST 33102–2014 «Products of meat industry. Classification» Retrieved from https://docs.cntd.ru/document/1200114757 Accessed August 24, 2022 (In Russian)



* significance levels are given in comparison with 2017.

Figure 4. Detection rate of contaminated RTE meat product samples in 2017–2019

genes were the equipment (9%) and machinery (32.3%), as well as constructions, such as floor, walls, drains, (10%) and cleaning tools (26.7%) [27].

It is evident that the prevalence of this pathogen in beef semi-finished products was higher than in pork semi-finished products. The obtained data make it possible to conclude that pork is less susceptible to *L. monocytogenes* contamination, which corresponds to the scientific data of other researchers [21].

Our previous studies [4] allow stating that the surfaces of 20% of cattle carcasses after hide removal are contaminated with *L. monocytogenes* and 20–80% are contaminated with other *Listeria* species. At the same time, deep layers of beef and pork cuts, as a rule, are free from *L. monocytogenes*.

According to the existing normative documentation, sampling from meat cuts as well as from meat in carcasses, half-carcasses, quarters, semi-finished products in large pieces is performed from deep layers; that is, after surface sterilization by its flaming and removal of this area. Microbiological criteria indicated in TR CU 021/2011[12] are given for assessment of deep layers.

Therefore, when testing the above mentioned semi-finished products, only deep layers are assessed, while surface contamination with pathogenic microorganisms is not taken into account.

At the same time, when testing semi-finished products in small pieces and minced semi-finished products, another method for sample preparation is specified, namely, without flaming of the surface. Consequently, the surface and deep layers are assessed in total. This incompatibility in assessment distorts the true situation. Not knowing the real level of surface contamination of carcasses, half-carcasses and semi-finished products in large pieces with *L. monocytogenes*, producers use such raw materials to manufacture other types of semi-finished products increasing a risk of production of unsafe foods and contamination of the equipment, surfaces and other objects of the production environment. For example, scientific research demonstrated the features and routes of cross-contamination of meat products with pathogenic *Listeria*. Swabs (n = 240) from different production zones of a meat processing plant (slaughtering, deboning, cutting and packaging lines, shipping zones, refrigeration chambers) were investigated and *Listeria* was identified in 53 swabs [28]. At the same time, the use of GOST R ISO 17604–2011[13] for detection and enumeration of microorganisms on the carcass surface during processing of slaughter animals and poultry allows detecting a level of safety and establishing the risk-oriented approach to controlling the spread of pathogenic microorganisms including *L. monocytogenes*.

It was also interesting to establish the number of positive samples of ready-to-eat (RTE) meat products. Much attention is given to this particular group of products worldwide and today monitoring of the presence of *L. monocytogenes* is shifted from raw materials to RTE products. With that, quantification of this microorganism (not more than 100 CFU/g) is performed [29].

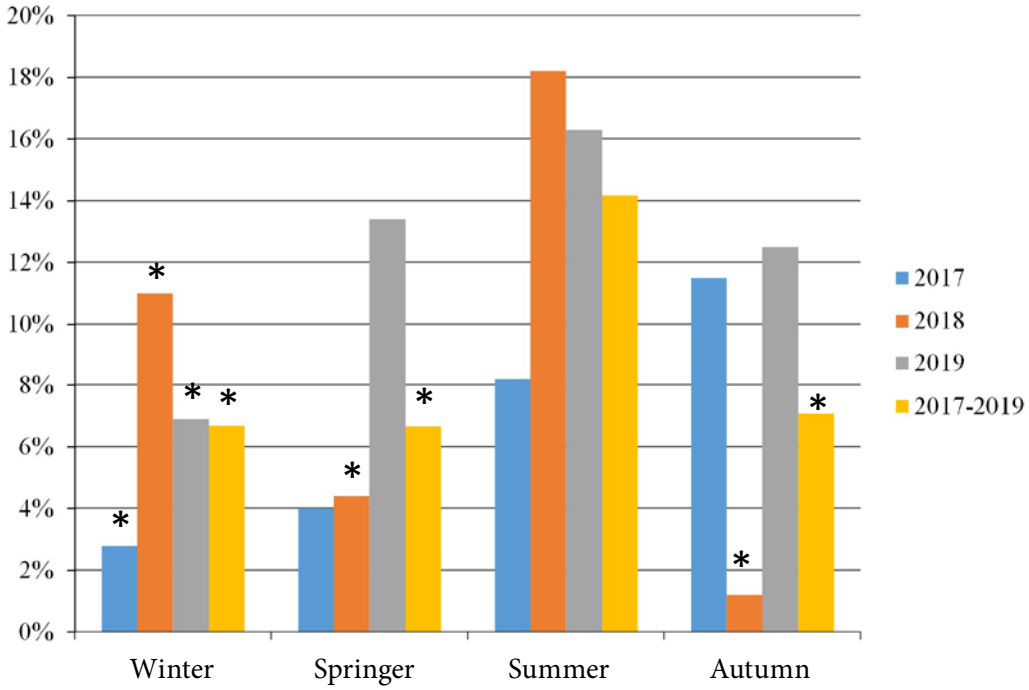
It can be seen from Figure 4 that positive samples were not revealed in all tested RTE meat products (n = 95) in 2017. In 2018, the prevalence of *L. monocytogenes* in the RTE meat products was 3.2% (4 positives out of 125 samples); in 2019, it was as high as 4.8% (7 positives out of 146 tested samples) taking into account the fact that the maximum number of samples was tested that year.

Analysis of the revealed dynamics allows suggesting that the number of RTE products contaminated with *L. monocytogenes* is increasing year after year.

When comparing the average total prevalence of *L. monocytogenes* in RTE meat (11%) found in the studies carried out by the Brasilian researchers [20] with that in other countries, it is possible to note lower values of overall prevalence (0.5%, 2.1% and 3.2%, respectively) for the United States, European Union and China [30,31].

[12] Technical regulation of the Customs Union TR CU 021/2011"On food safety". (Adopted by The decision of the Council of the Eurasian economic Commission of December 9, 2011 No. 880). Moscow, 2011. Retrieved from https://docs.cntd.ru/document/902320560. Accessed August 24, 2022. (In Russian)

[13] GOST 17604–2011 « Microbiology of food and animal feeding stuffs. Carcass sampling for microbiological analysis» Retrieved from https://docs.cntd.ru/document/1200089425. Accessed August 24, 2022 (In Russian)



* $p < 0.05$ when compared with summer.

Figure 5. Detection rate of contaminated meat product samples depending on a season in 2017–2019

Prediction and prevention of epidemiologically unfavorable situations should also be based on determination of seasonal peculiarities in circulation of pathogenic bacteria. We studied the frequency of detection of *L. monocytogenes* in meat products depending on a season. The results obtained in the investigation are presented in Figure 5.

Over three years, the highest prevalence of *L. monocytogenes* was observed in summer (on average 14.2%). Several epidemiologists also note an increase in acute intestinal infections associated with pathogenic bacteria precisely in the warm period of the year [32]. Apparently, this peculiarity is linked with more favorable conditions for microbial growth and is determined by an increase in the ambient temperature, for example, as in a cold chain breach in food logistics. Another explanation for the high detection rates of the pathogen in summer can be found in the studies showing that wild birds living nearby agricultural objects can be vectors for *L. monocytogenes* transmission and facilitate the spread of the bacterium through feces in pastures, soil, water, and feed [33,34].

For example, seagulls that are feeding at sewage facilities and rooks (to a smaller degree) were earlier identified as carriers of *L. monocytogenes* in feces. With that, the bacterial load increased in the nesting season and coincided with the peak period for listeriosis in sheep [34,35].

In 2017–2019, the detection rate of *L. monocytogenes* in winter, spring and autumn was in a range of 6.7–7.1% without clear predominance in this indicator depending on a season contrary to summer.

Conclusion

The results of the study show that the prevalence of *L. monocytogenes* in meat products dynamically grew year after year during the period from 2017 to 2019 making up 6.1, 8.4 and 12%, respectively.

The obtained data allow making a conclusion that poultry meat was definitely the most susceptible to *L. monocytogenes* contamination (its proportion was on average 18.7% of all products in 2017–2019), followed by beef (the detection rate was 13.2%).

Among the tested poultry semi-finished products that were sampled without flaming of the surface, natural semi-finished products were the least contaminated with *L. monocytogenes*. When analyzing the whole poultry carcasses (with flaming), this pathogen was not found in the deep layers.

Furthermore, the lowest *L. monocytogenes* contamination was found in semi-finished products in large pieces made from different meat types compared to semi-finished products in small pieces and minced semi-finished products. This can be explained by the fact that today different approaches to sample preparation are used for cuts and semi-finished products in large and small pieces.

Analysis of the obtained data indicates that detection of *L. monocytogenes* depends on the product composition (a type of meat raw materials), production technology and method of sample preparation for microbiological analysis.

A higher prevalence of *L. monocytogenes* was observed in beef semi-finished products compared to semi-finished products made from pork. Pork is less susceptible to *L. monocytogenes* contamination.

The number of ready-to-eat (RTE) products contaminated with *L. monocytogenes* is increasing every year.

The highest prevalence (14.2%) of *L. monocytogenes* in meat products was observed in summer, which was probably conditioned by a stable increase in the ambient tem-

perature, possibly, with a cold chain breach in food logistics and so on.

The obtained results showing quite a high level of *Listeria* contamination at different stages and under different conditions of meat product manufacture can be used for the preparation of modern guidance on the control of *Listeria* in food processing plants as well as methodical recommendations for analysis of this pathogen in raw materials, ingredients and objects of the production environment.

REFERENCES

1. Tartakovskij, I. S. (2000). Listeria: role in human infectious pathology and laboratory diagnostics. *Clinical Microbiology and Antimicrobial Chemotherapy*, 2(2), 20–30. (In Russian)
2. Bertsch, D., Muelli, M., Weller, M., Uruty, A., Lacroix, C., Meile, L. (2014). Antimicrobial susceptibility and antibiotic resistance gene transfer analysis of foodborne, clinical, and environmental Listeria spp. isolates including Listeria monocytogenes. *MicrobiologyOpen*, 3(1), 118–127. https://doi.org/10.1002/mbo3.155
3. Chaitiemwong, N., Hazeleger, W. C., Beumer, R. R., Zwietering, M. H. (2014). Quantification of transfer of Listeria monocytogenes between cooked ham and slicing machine surfaces. *Food Control*, 44, 177–184. https://doi.org/10.1016/j.foodcont.2014.03.056
4. Bataeva, D. S., Zaiko, E.V., Yushina, Yu. K. (2019). Assessment of the microbiological stability of semi-finished meat products during storage. Vsyo o Myase, 5, 24–27. https://doi.org/10.21323/2071–2499–2019–5–24–27 (In Russian)
5. EFSA. (2018). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA Journal*, 16(12), Article e05500. https://doi.org/10.2903/j.efsa.2018.5500
6. EFSA. (2019). The European Union one health 2018 zoonoses report. *EFSA Journal*, 17(12), Article e05926. https://doi.org/10.2903/j.efsa.2019.5926
7. Jordan, K., McAuliffe, O. (2018). Listeria monocytogenes in foods. *Advances in Food and Nutrition Research*, 86, 181–213. https://doi.org/10.1016/bs.afnr.2018.02.006
8. McCarthy, Z., Smith, B., Fazil, A., Ryan, S. D., Wu, J., Munther, D. (2019). An individual-carcass model for quantifying bacterial cross-contamination in an industrial three-stage poultry scalding tank. *Journal of Food Engineering*, 262, 142–153. https://doi.org/10.1016/j.jfoodeng.2019.05.013
9. Koreňová, J., Oravcová, K., Véghová, A., Karpiskova, R., Kuchta, T. (2016). Biofilm formation in various conditions is not a key factor of persistence potential of Listeria monocytogenes in food-processing environment. *Journal of Food and Nutrition Research*, 55(2), 189–193.
10. Di Ciccio, P., Vergara, A., Festino, A. R., Paludi, D., Zanardi, E., Ghidini, S. et al. (2015). Biofilm formation by Staphylococcus aureus on food contact surfaces: Relationship with temperature and cell surface hydrophobicity. *Food Control*, 50, 930–936. https://doi.org/10.1016/j.foodcont.2014.10.048
11. Alavi, H. E. D., Hansen, L. T. (2013). Kinetics of biofilm formation and desiccation survival of Listeria monocytogenes in single and dual species biofilms with Pseudomonas fluorescens, Serratia proteamaculans or Shewanella baltica on food-grade stainless steel surfaces. *Biofouling*, 29(10), 1253–1268. https://doi.org/10.1080/08927014.2013.835805
12. Esbelin, J., Santos, T., Hébraud, M. (2018). Desiccation: An environmental and food industry stress that bacteria commonly face. *Food Microbiology*, 69, 82–88. https://doi.org/10.1016/j.fm.2017.07.017
13. Ferreira, V., Wiedmann, M., Teixeira, P., Stasiewicz, M. J. (2014). Listeria monocytogenes persistence in food-associated environments: epidemiology, strain characteristics, and implications for public health. *Journal of Food Protection*, 77(1), 150–170. https://doi.org/10.4315/0362–028X.JFP-13–150
14. Zoz, F., Grandvalet, C., Lang, E., Iaconelli, C., Gervais, P., Firmesse, O. et al. (2017). Listeria monocytogenes ability to survive desiccation: Influence of serotype, origin, virulence, and genotype. *International Journal of Food Microbiology*, 248, 82–89. https://doi.org/10.1016/j.ijfoodmicro.2017.02.010
15. Bonsaglia, E. C. R., Silva, N. C. C., Júnior, A. F., Júnior, J. A., Tsunemi, M. H., Rall, V. L. M. (2014). Production of biofilm by Listeria monocytogenes in different materials and temperatures. *Food Control*, 35(1), 386–391. https://doi.org/10.1016/j.foodcont.2013.07.023
16. Kovaljov, V.A., Filatov, N.N., Aljoshina, E.N., Simonova, E.G. (2019). Sickness of listeriosis in Russian Federation. *Science of the Young (Eruditio Juvenium)*, 7(4), 509–517. https://doi.org/10.23888/HMJ201974509–517 (In Russian)
17. State report "On the state of sanitary and epidemiological welfare of the population in the Russian Federation in 2019". Retrieved from https://www.rospotrebnadzor.ru/documents/details.php? ELEMENT_ID=14933 Accessed September 14, 2022. (In Russian)
18. Nityaga, I. M. (2016). Listeria contamination of meat products and express their identity using methods based on PCR *Russian Journal Problems of Veterinary Sanitation, Hygiene and Ecology,* 1(17), 17–22. (In Russian)
19. Nityaga I. M. (2005). Improving the efficiency of detection of L, monocytogenes and enterohemorrhagic E. coli in meat and meat products. Author's abstract of the dissertation for the scientific degree of Candidate of Biological Sciences Moscow: Moscow State University of Applied Biotechnology, 2005. (In Russian)
20. Cavalcanti, A. A. C., Limeira, C. H., de Siqueira, I. N., de Lima, A. C., de Medeiros, F. J. P., de Souza, J. G. et al. (2022). The prevalence of Listeria monocytogenes in meat products in Brazil: A systematic literature review and meta-analysis. *Research in Veterinary Science,* 145, 169–176. https://doi.org/10.1016/j.rvsc.2022.02.015
21. Sinelnikova, M. A., Buzoleva, L.S., Bespechuk, N. Yu., Koltun, G.G. (2017). Indication of listeria monocytogenes in meat and meat products in the territory of agricultural province. *Hygiene and Sanitation, Russian Journal,* 96(6), 590–593. https://doi.org/10.18821/0016–9900–2017–96–6–590–593 (In Russian)
22. Bataeva, D.S., Makhova, A.A. (2017). Analysis the dependence of the results of microbiological studies to methods for sampling meat and meat products. *Vsyo o Myase*, 2, 43–45. (In Russian)
23. Skovgaard, N., Morgen, C. A. (1988). Detection of Listeria spp. in faeces from animals, in feeds, and in raw foods of animal origin. *International Journal of Food Microbiology*, 6(3), 229–242. https://doi.org/10.1016/0168–1605(88)90015–3
24. Loganin, P. (2017). Listeria at a poultry processing plant. *Sfera, PTICEPROM*, 2 (36), 56–59. (In Russian)
25. Djordjevic, D., Wiedmann, M., McLandsborough, L. A. (2002). Microtiter plate assay for assessment of Listeria monocytogenes biofilm formation. *Applied and Environmental Microbiology*, 68(6), 2950–2958. https://doi.org/10.1128/AEM.68.6.2950–2958.2002
26. Hammons, S. R., Etter, A. J., Wang, J., Wu, T., Ford, T., Howard, M. T. et al. (2017). Evaluation of third-party deep cleaning as a Listeria monocytogenes control strategy in retail delis. *Journal of Food Protection*, 80(11), 1913–1923. https://doi.org/10.4315/0362–028X.JFP-17–113
27. Antoci, S., Iannetti, L., Centorotola, G., Acciari, V. A., Pomilio, F., Daminelli, P. et al. (2021). Monitoring Italian establishments exporting food of animal origin to third countries: SSOP compliance and *Listeria monocytogenes* and *Salmonella* spp. contamination. *Food Control,* 121, Article 107584. https://doi.org/10.1016/j.foodcont.2020.107584
28. Tutelyan, A.V., Jushina, Yu.K., Sokolova, O.V., Bataeva, D.S., Fesyun, A.D., Datiy, A.V. (2019). Formation of biological films by microorganisms in food productions. *Problems of Nutrition,* 88(3), 32–43. https://doi.org/10.24411/0042–8833–2019–10027 (In Russian)
29. Control of Listeria monocytogenes in Ready-To-Eat Foods: Guidance for Industry Draft Guidance. Retrieved from https://www.fda.gov/files/food/published/Draft-Guidance-for-Industry — Control-of-Listeria-monocytogenes-in-Ready-To-Eat-Foods-%28PDF%29.pdf Accessed September 15, 2022.
30. Liu, Y., Sun, W., Sun, T., Gorris, L. G. M., Wang, X., Liu, B. et al. (2020). The prevalence of Listeria monocytogenes in meat products in China: A systematic literature review and novel meta-analysis approach. *International Journal of Food Microbiology*, 312, Article 108358. https://doi.org/10.1016/j.ijfoodmicro.2019.108358

31. FSIS risk assessment for Listeria monocytogenes in deli meats. (2003). Retrieved from https://www.fsis.usda.gov/node/2013 Accessed September 10, 2022.
32. Podkolzin, A.T., Fenske, E.B., Abramycheva, N. Yu., Shipulin, G.A., Doroshina, E.A., Kozina, E.A. et al. (November 28–30, 2007*). Seasonality and age structure of the incidence of acute intestinal infections in the Russian Federation*. VI All-Russian Scientific and Practical Conference "Genodiagnostics of Infectious Diseases — 2007 (Molecular Diagnostics — 2007)" Moscow: University Book, 2007. (In Russian)
33. Schoder, D., Melzner, D., Schmalwieser, A., Zangana, A., Winter, P., Wagner, M. (2011). Important vectors for *Listeria monocytogenes* transmission at farm dairies manufacturing fresh sheep and goat cheese from raw milk. *Journal of Food Protection,* 74(6), 919–924. https://doi.org/10.4315/0362–028X.JFP-10–534
34. Rodriguez, C., Taminiau, B., Garcia-Fuentes, E., Daube, G., Korsak, N. (2021). *Listeria monocytogenes* dissemination in farming and primary production: Sources, shedding and control measures. *Food Control,* 120, Article 107540. https://doi.org/10.1016/j.foodcont.2020.107540
35. Fenlon, D. R. (1986). Growth of naturally occurring *Listeria* spp. in silage: A comparative study of laboratory and farm ensiled grass. *Grass and Forage Science*, 41(4), 375–378. https://doi.org/10.1111/j.1365–2494.1986.tb01828.x

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INFLUENCE OF DRYING AND PRETREATMENT METHODS ON CERTAIN PARAMETERS OF YELLOW MEALWORM LARVAE (*TENEBRIO MOLITOR*)

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Abstract

Nowadays alternative protein sources like edible insects are becoming widely used as human food. One of the most popular insect is yellow mealworm (Tenebrio molitor) due to its high nutrition value. However, pretreatment and drying are necessary to increase the food shelf life and the efficiency of its use. Due to this, the purpose of the present work was the determination of influence of pretreatment methods (freezing of larvae for 1 month, freezing for 2 hours, freezing for 1 month followed by defrosting for 2 hours at room temperature, blanching) and drying methods (convection drying at 40 °C and 60 °C, microwave drying) of yellow mealworm on its color (determination of L, a*, b*, ΔE_{lab}, Ch, H, BI), moisture content (gravimetric method), fatty acid composition (determined by gas chromatography with mass spectroscopy) and time of drying (time required to reach constant weight). It was found that all used pretreatment and drying methods had no effect on the fatty acid composition of the larvae. In terms of drying rate and color retention, microwave drying showed better results than convection drying at 40 °C and 60 °C. Meanwhile, convection drying at 40 °C leads to the higher final moisture content of the samples. Among the pretreatment methods, only blanching the larvae samples before drying retained their color better and also accelerated the rate of the convection drying.*

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Introduction

By 2050 the world's population may reach 9–11 billion people. It will lead to an increase in food production by 70% due to necessity of providing an adequate supply of food for the population [1]. Meanwhile, food industry itself has a significant impact on the environment [2–5]. In particular, animal husbandry contributes to greenhouse gas emissions, soil acidification, nitrification and soil erosion [6]. Moreover, water expenses in the agricultural sector are about 70% of total consumption [7], and 1/3 of this volume is used for raising animals [8].

Edible insects, which variety accounts for more than 2,000 species, can potentially be used for partial replacement of meat in the human diet, as well as a feed additive for farm animals [9]. Currently in the world, insects are regularly consumed by about two billion people [10]. The cultivation of insects has a lower environmental impact, in comparison with the cultivation of cattle, pigs and poultry, since this type of cultivation requires less feed, soil and water [11]. Moreover, the high nutritional value of insects and easiness of their breeding led to the intensive development of the insects-producing industry and insect-based food products manufacture even in European countries [12–14]. In [9,15] the edible insects are characterized as one of the food sources that are able to cover the growing need for food and can prevent world famine. In the countries where human entomophagy is a tradition, the insects are considered to be culinary delight and a valuable source of protein [14].

The yellow mealworm (*Tenebrio molitor*) is one of the most common edible insect species. Currently, yellow mealworm is already approved as a novel food in the European Union [16]. Experts from the European Food Safety Authority have acknowledged it to be safe as an ingredient in biscuits, snacks, snack bars and pasta [17]. According to scientific literature data, the taste of the yellow mealworm resembles the taste of nuts, umami and cereals [18,19]. In the other research, its taste is described as savory, similar to the taste of dried shrimp [17]. Yellow mealworm contains approximately 46 wt.%. protein and 33 wt.%. fat, the main fatty acids

are linoleic, oleic and palmitic ones [20]. *Tenebrio molitor* contains essential amino acids, polyunsaturated fatty acids, minerals and vitamins [11, 21–23]. In addition, the edible fraction of the yellow mealworm accounts for almost 100%, which significantly exceeds the edible fraction in chickens and pigs (55%) and cattle (40%) [17].

Killing and drying are among the main stages of insect processing that affect the quality of the final product and its shelf life [24]. Killing method influences significantly on the physical and chemical parameters and microbiota of insects [25]. Among the methods tested, it was shown that longer time of killing can cause stress, which contributes to acceleration of oxidative processes in insects, including the breakdown of triglycerides into fatty acids and acylglycerol [26]. In this regard the most humane methods of killing insects are freezing and blanching [27,28].

Meanwhile, the larvae contain a lot of water (59–68 wt.%) [29,30], which makes them vulnerable to lipid oxidation, enzymatic, non-enzymatic reactions and microbiological deterioration. In fact, when the optimum level of moisture content is reached, the rates of oxidation reactions and microbiological contamination are reduced to minimum, thus the shelf life of the product increases [31]. Based on this, reducing the moisture content of biomass is an important condition for maintaining the proper quality of the food product. Moreover, drying significantly decreases the mass of insects, which helps to reduce the cost of transportation and storage.

Dehydration can be achieved using various methods such as freeze drying, convection, infrared, vacuum and microwave drying [32]. In addition to the type of drying, the quality of the final product is influenced by the drying parameters, which can vary over a wide range. Thus, manufacturers encounter the challenge of finding the appropriate drying technologies to preserve the quality characteristics of insects for a long time [30]. One of the most efficient methods of drying is freeze drying [24]. In this case, the frozen product is dehydrated as a result of the sublimation of moisture under vacuum. The freeze-dried products almost completely retain their original characteristics due to the low temperature and lack of oxygen during the drying process. However, freeze drying takes a long time, which implies high capital expenses and energy costs. Based on this, it is relevant to study more affordable and faster drying methods, for example — convection drying and microwave drying.

The research [32] represents the results of the comparison of the samples colors obtained in case of applying various drying methods. The samples dried by convection drying have dark color, which can be described as brownish, contrasting with the golden color. At the same time, the degree of the color change did not depend on the drying temperature from 60 °C to 80 °C. [33]. Browning produces consumers' negative perception of the food appearance, although the brown pigments, formed during drying, do not affect the sensory characteristics of the final product. The authors reported the efficiency of blanching before drying the larvae of *Tenebrio molitor* to preserve their color, due to the fact that during the temperature treatment, the activity of enzymes leading to the browning of the biomass is reduced [34]. In [29], yellow mealworm larvae, previously killed by freezing, were dried by various methods like freeze drying, fluid bed drying, vacuum drying, convection and microwave drying. The results of the study showed that vacuum and microwave drying can be an alternative to freeze-drying of mealworms. However, currently there are no data on the influence of pretreatment methods (freezing, blanching) on the quality characteristics of mealworm dried by convection drying or microwave drying. Based on this, the aim of the work was to determine the effect of the pretreatment methods (freezing for 2 months, freezing for 2 hours, freezing for 2 months, followed by defrosting at room temperature for 2 hours, blanching) and drying (convection drying at 40 °C and 60 °C, microwave drying) on moisture content, drying rate, fatty acid composition, appearance and color of the mealworm larvae.

Objects and methods

The larvae of the yellow mealworm at the age of two months were used for the study. The insects were taken from the breeding stock of the yellow mealworm (*Tenebrio molitor*) of the Laboratory for the Structural Processing of Bioresources of the All-Russian Research Institute for Food Additives.

Determination of humidity

The water content of the larvae samples (moisture) was determined in accordance with the procedure described in AOAC950.46 [35]: the larvae sample with known weight was dried in the convection oven at 105 °C to constant weight, then the dry residue was weighed on an analytical balance GR-200 (AND, Japan, measurement range 0.01–210 g, resolution 0.1 mg), and the moisture was calculated using the following equation (1):

$$W = \frac{m_0 - m_{dry}}{m_0} \times 100\% \quad (1)$$

where:

W — moisture of the sample, %;

m_0 — sample weight before drying, g;

m_{dry} — sample weight after drying at 105 °C for 8 hours, g.

The moisture of each sample was measured three times, then the average value was calculated and the confidence interval was estimated at a significance level of 0.05. Calculations were carried out using the software Microsoft Excel 2016 (Microsoft Office, US).

Processing and drying methods

As a pretreatment stage, yellow mealworm larvae were killed by freezing (2 hours or 30 days, –20 °C) or blanching (20 seconds, 100 °C). Moreover, some of the frozen insects

were defrosted at room temperature for 2 hours before drying. Then, each of the samples was dried to a constant weight using three following ways:

- CD 40 °C — convection drying in the drying oven UF110plus (Memmert, Germany) at 40 °C;
- CD 60 °C — convection drying in the drying oven at 60 °C;
- MW — microwave drying in the UOMO-T150 microwave system (Omitex, Russia) at the frequency of 2450 MHz.

The following terms are introduced in the research:

1) Blanched — larvae processed in boiling water for 20 seconds;
2) Frozen (2 hours) — larvae frozen at –20 °C for 2 hours;
3) Frozen (1 month) — larvae frozen at –20 °C with a shelf life of 1 month;
4) Defrosted — Frozen (1 month) larval samples kept at room temperature for two hours before analysis.

During the drying insect samples were periodically weighed to assess the rate of moisture removal. The moisture of the larvae at different times was determined based on the data on moisture of the larvae at the end of the experiment and the change in the sample weight during drying.

The drying rate of insect samples was calculated using equation (2) given in [31]:

$$DR = \frac{M_t - M_{t+\Delta t}}{\Delta t} \quad (2)$$

where:

DR — drying rate, 1/h;
M_t — moisture content at the time t;
$M_{t+\Delta t}$ — moisture content at the time $t + \Delta t$;
Δt — considered time interval, h.

Moisture content M_t was defined by the following equation (3) [36]:

$$M_t = \frac{m_t - m_{dry}}{m_{dry}} \times 100\% \quad (3)$$

where:

m_t — sample weight at the time t, g;
m_{dry} — sample weight after drying for 8 hours at 105 °C, g.

Analysis of the fatty acid composition of insects

The fatty acid composition was determined by gas chromatography with mass spectrometric detection with the gas chromatographic analyzer Varian 450-GC (Varian, USA) with the mass spectrometric detector Varian 240-MS. The following materials and equipment were used: capillary column Varian WCOT fused silica 50M X 0.25MM ID Coating CP-WAX 58 (FFAP)-CB DF=0.2 (Varian, USA); thermostat Termit (DNA-Technology, Russia); helium (grade 6.0), sulfuric acid (≥ 95,6%, JSC «Shchekinoazot», Russia); methanol (HPLC gradient grade CHIMMED, Russia); chloroform (≥99,8%, EKOS-1, Russia); deionized water; fatty acid methyl ester standards (18919 1AMP Supelco F. A.M.E. Mix, C4-C24, USA): butyric acid (C4:0); caproic acid (C6:0); caprylic acid (C8:0); capric acid (C10:0); undecanoic acid (C11:0); lauric acid (C12:0); tridecanoic acid (C13:0); myristic acid (C14:0); myristoleic acid (C14:1); pentadecanoic acid (C15:0); cis-10-pentadecenoic acid (C15:1); palmitic acid (C16:0); palmitoleic acid (C16:1); heptadecanoic acid (C17:0); cis-10-heptadecenoic acid (C17:1); stearic acid (C18:0); elaidic acid (C18:1ω9t); oleic acid (C18:1ω9c); linoleic acid (C18:2ω6t); linoleic acid (C18:2ω6c); arachidic acid (C20:0); gamolenic acid (C18:3ω6); cis-gondoic acid (C20:1); linolenic acid (C18:3ω3); heneicosylic acid (C21:0); cis-11,14-eicosadienoic acid (C20:2); behenic acid (C22:0); dihomo-γ-linolenic acid (C20:3ω6); erucic acid (C22:1ω9); cis-11,14,17-eicosatrienoic acid (C20:3ω3); tricosanoic acid (C23:0); methyl cis-5,8,11,14-eicosatetraenoic acid (C20:4ω6); cis-13,16-docosadienoic acid (C22:2); lignoceric acid (C24:0); cis-5,8,11,14,17-eicosapentaenoic acid (C20:5ω3); nervonic acid (C24:1); cis-4,7,10,13,16,19-docosahexaenoic acid (C22:6ω3).

Analysis conditions were as follows: the flow rate of carrier gas was 1 ml/min, injector temperature 250 °C, split 1:15, start of chromatogram registration: from the 9th minute. The temperature program is presented in the Table 1.

Table 1. Temperature program of the analysis

Temperature, °C	Heating rate, °C/min	Time at a given temperature, min	Total time, min
50	—	4	4
190	6	15	42,33
250	4	10	67,33

Sample preparation: 600 µl of the 15% solution of sulfuric acid in methanol and 600 µl of chloroform were added to microtube (Eppendorf, Germany) with the sample. The microtube was carefully sealed with parafilm and placed in the thermostat for 1 hour at 65 °C. Then the sample was cooled, 200 µl of deionized water was added and thoroughly stirred. After that the organic layer was taken and 1 µl of it was directly injected into the chromatograph using the CPAL autosampler and the 10 µl Hamilton chromatographic syringe. The measurement error was 15%.

Analysis of color characteristics

The color of the yellow mealworm larvae was determined in accordance with the method described in [37]. L^* (whiteness or lightness/darkness), a^* (redness/greenness), b^* (yellowness/blueness) values were determined using Adobe Photoshop CS6 software. Samples photos were taken using the iPhone 11 smartphone in daylight (6500 K).

The parameter ΔE_{lab} (total color difference) was determined according to the following equation (4) [33]:

$$\Delta E_{lab} = \sqrt{(L^* - L_1^*)^2 + (a^* - a_1^*)^2 + (b^* - b_1^*)^2} \quad (4)$$

where:

L^* — lightness of untreated live larvae;
L_1^* — lightness of treated larvae;
a^* — redness/greenness of untreated live larvae;
a_1^* — redness/greenness of treated larvae;
b^* — yellowness/blueness of untreated live larvae;
b_1^* — yellowness/blueness of treated larvae.

Chroma (*Ch*) was determined according to the equation (5) [38]:

$$Ch = (a^{*2} + b^{*2})^{\frac{1}{2}} \quad (5)$$

Hue angle (*H*) was determined according to the equation (6):

$$H = \text{tg}^{-1}\left(\frac{b^*}{a^*}\right) \quad (6)$$

Browning index (*BI*) was determined according to the equations (7) and (8):

$$BI = \frac{100 \cdot (x - 0.31)}{0.17} \quad (7)$$

$$x = \frac{(a^* + 1.75L^*)}{(5.654L^* + a^* - 3.012b^*)} \quad (8)$$

The L^*, a^*, b^* values were determined at 6 different points, selected at random on each sample image. The image of untreated larvae was used as a control reference. The final values L^*, a^*, b^* were the average of 6 values. Next, the standard deviation and confidence interval were calculated with a confidence level of 0.95. All calculations were carried out using the software Microsoft Excel 2016 (Microsoft Office, US).

Results and discussion

Samples drying

The water content of the larval samples before and after drying is presented in Table 2. The final moisture values obtained were similar for all samples, slightly higher values were observed in case of insects dried in the convection oven at 40 °C.

Table 2. Moisture of the *Tenebrio molitor* larvae samples

Sample	Drying method	*W* before drying, %	*W* after drying, %
Blanched	MW	63.2 ± 4.0	4.0 ± 0.6
	CD 40 °C		4.8 ± 0.7
	CD 60 °C		4.0 ± 0.6
Frozen (2 hours)	MW	58.8 ± 12.5	3.5 ± 0.5
	CD 40 °C		8.8 ± 1.2
	CD 60 °C		3.7 ± 0.5
Frozen (1 month)	MW	60.3 ± 10.0	3.3 ± 0.5
	CD 40 °C		6.5 ± 0.9
	CD 60 °C		4.4 ± 0.6
Defrosted	MW	60.8 ± 8.0	4.6 ± 0.6
	CD 40 °C		7.7 ± 1.1
	CD 60 °C		5.6 ± 0.8

Data on the change in larvae samples moisture during microwave drying are presented in Figure 1. The dependences obtained for insects pretreated in various ways were similar. The difference was observed for Frozen (1 month) larvae, however, this was most likely caused by different drying mode: drying of Frozen (1 month) larvae was carried out in short-term (1–2 min) periods between which the sample was weighed and cooled, while in the other cases there was an initial long drying period (10–12 min), after which the moisture of the sample was already below 30 wt.%.

According to the literature data [39], during the microwave drying after the evaporation of the main amount of water, the moisture of dried samples becomes approximately constant and does not change even during prolonged drying. This fact proves the inexpediency of prolonged microwave drying.

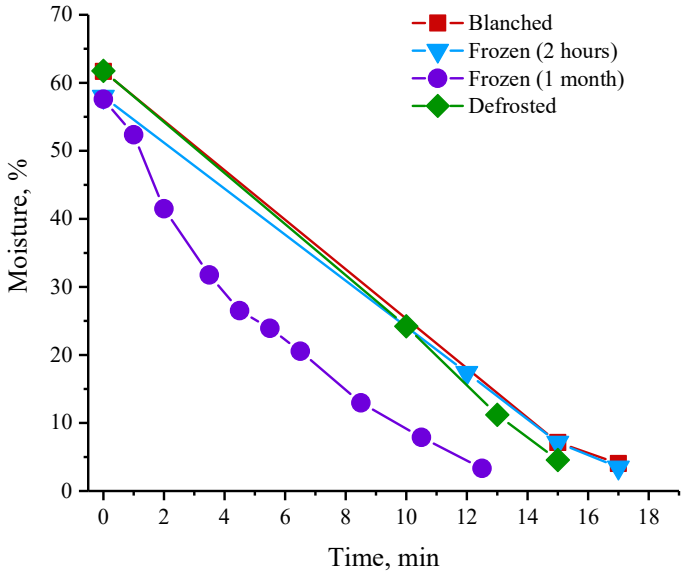

Figure 1. The change of the *Tenebrio molitor* larvae samples moisture during microwave drying

Figures 2 and 3 show the change in moisture of samples during convective drying at 40 °C and 60 °C, respectively. In case of the microwave drying of the *Tenebrio molitor* larvae, the moisture of about 7 wt.% was achieved in 14 minutes on average (Figure 1), while in case of the convective drying at 60 °C the same values were achieved in 19 hours (Figure 3), and with the convective drying at 40 °C — in 72 hours (Figure 2). Thus, larval samples can be dried by the microwave drying approximately 81 times faster than in the convection oven at 60 °C and 309 times faster than by the convection drying at 40 °C.

For more objective comparison of the drying modes kinetics the drying rate (*DR*) at different times was calculated for several samples. Figure 4 shows the rate of drying of Blanched and Frozen (2 hours) samples by convection drying at 40 °C and 60 °C as a function of moisture content. As a rule, food drying curves feature an initial period of increasing of drying rate associated with heating of the product, followed by a period of the rate decreasing [40,41]. In this study, during the initial period of drying, the samples weight was not measured; therefore, the period of rate increase was not recorded. During the convection drying at 40 °C, a plateau on the dependence of the drying rate (*DR*) on the moisture content was observed. The presence of the constant *DR* period is not typical for most food products [31,40,42,43] and, in this case, may be associated with frequent cooling of the samples during the weighing process and subsequent heating when placing them back in the drying oven. During convection drying

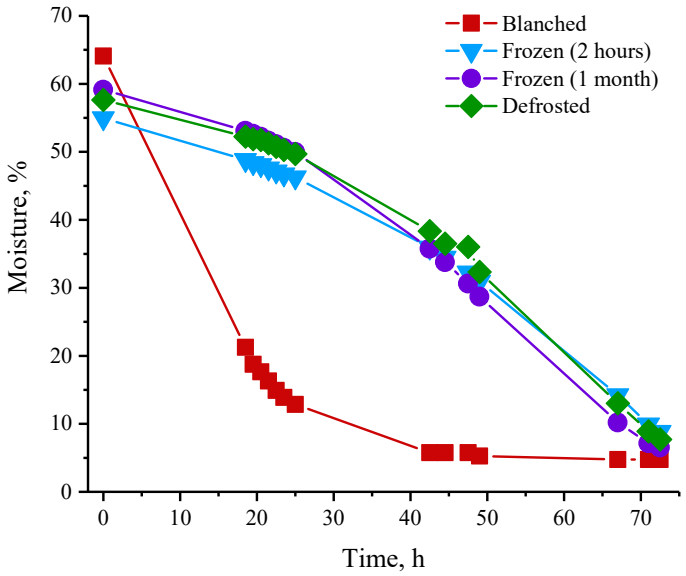

Figure 2. The change of the *Tenebrio molitor* larvae samples moisture during convective drying at temperature 40 °C

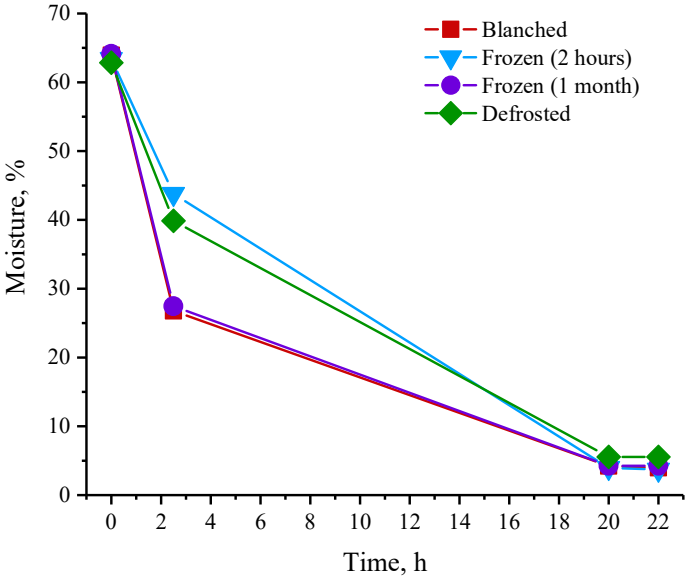

Figure 3. The change of the *Tenebrio molitor* larvae samples moisture during convective drying at temperature 60 °C

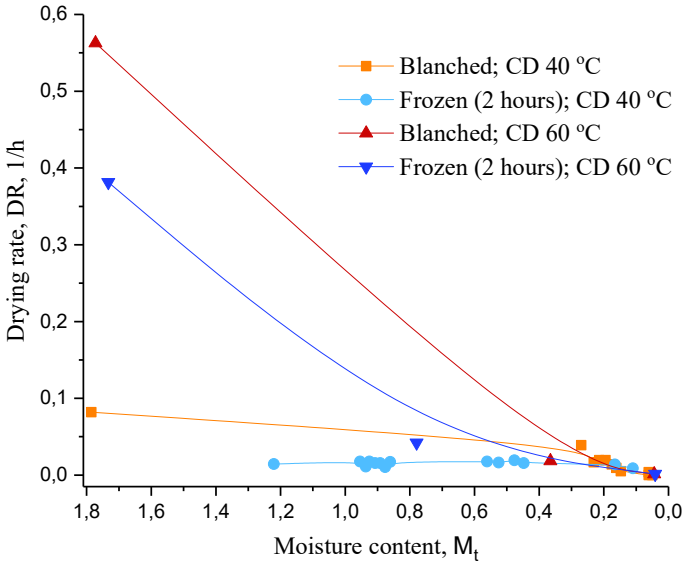

Figure 4. The dependence of the drying rate on moisture content of the *Tenebrio molitor* larvae samples

at 60 °C, the drying rate monotonically decreased during all the experiments (Figure 4).

DR was significantly affected by the drying temperature — the rate increased with increasing temperature (Figure 4). This coincides with the literature data [41]. Similar to the data in [42], the influence of temperature on the drying rate increases with the increase in the moisture content of the samples. It is often assumed that during the period corresponding to the decrease in the drying rate, the mechanism that determines the kinetics of the process is moisture diffusion to the surface [43], and the diffusion coefficient, in its turn, increases due to the temperature increase [44].

The obtained data also showed that the drying rate of blanched samples was higher than that of killed by freezing at –20 °C (Figure 4). During the blanching process, proteins can undergo structural changes such as denaturation, crosslinking, and interaction with lipids. This leads to a decrease in the number of hydrophilic water binding sites (polar side chains, carbonyl and amino groups), as well as to the destruction of the cell membranes. These structural changes can lead to an increase in the drying rate [36] and a decrease in the hygroscopicity of samples of the *Tenebrio molitor* larvae [31].

Fatty acid content

Among the identified fatty acids, myristic, palmitic, palmitoleic, oleic, linoleic and linolenic acids had the highest content. Analysis of the results (Figure 5) showed that the considered methods of drying and pretreatment of insects had no significant effect on their fatty acid composition.

The same results were observed in [24], which reported minor differences in the composition of mealworm larvae dried by various methods. According to [45], *Tenebrio molitor* larvae contain a large amount of unsaturated fatty acids with a high content of oleic and linoleic acids, as well as saturated fatty acids, which characterizes the larvae as potentially beneficial to human health.

Analysis of the color characteristics of the samples

Appearance is one of the main factors that the consumer uses to evaluate the quality of a food. For example, the inclusion of insects in food products can lead to their browning, which gives rise to the negative reaction in neophobic consumers and, therefore, refusal to purchase [46]. Figure 6 and Table 3 show photographs of larvae before and after treatment/drying.

Table 4 shows the color parameters of larvae after various combinations of their pretreatment and drying.

All treated larvae had an uneven color, due to which the L^*, a^*, b^* parameters have a significant degree of deviation.

The ΔE_{lab} reflects the degree of the color difference between untreated and treated larvae [33]. According to the literature, differences in perceived color can be classified

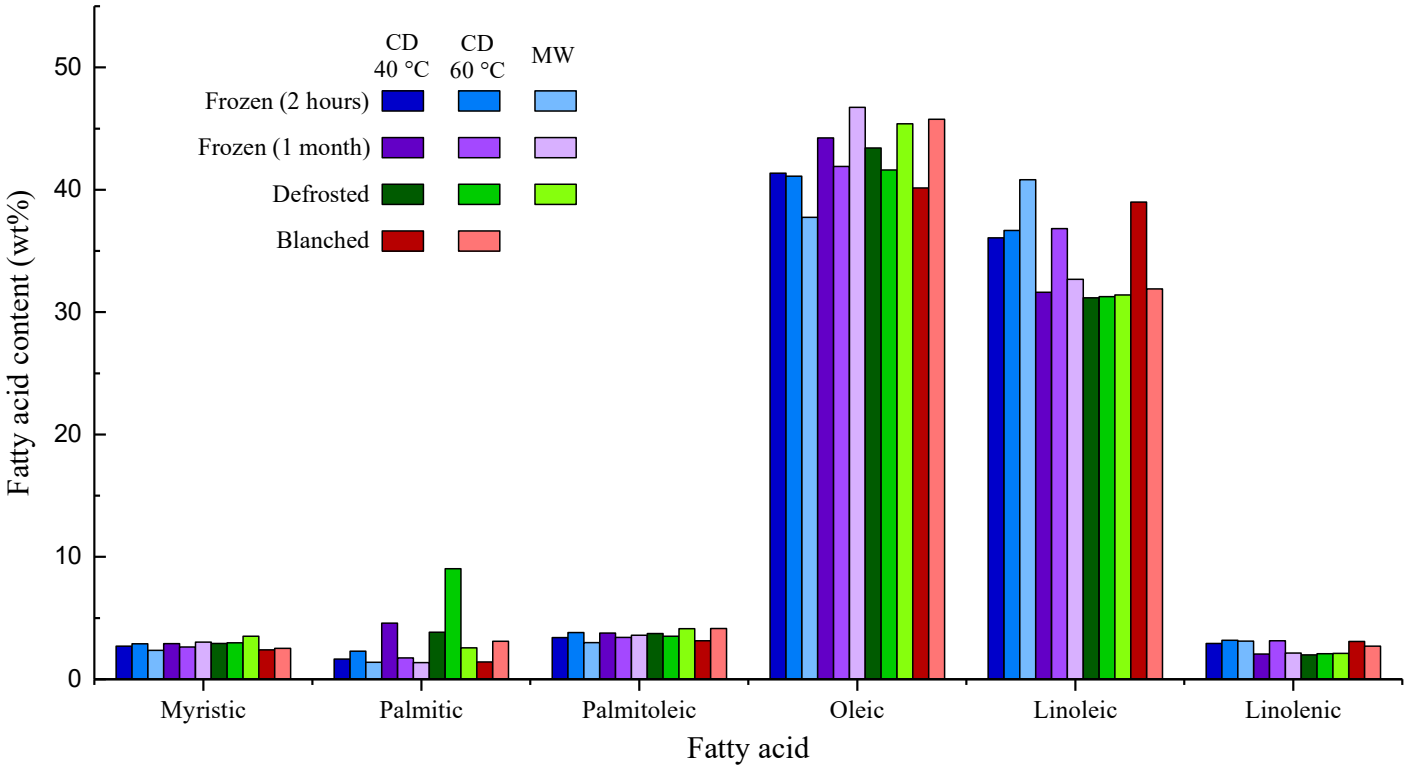

Figure 5. Fatty acid content in the samples of *Tenebrio molitor* larvae

as "significant" ($\Delta E > 3$), "visible" ($1.5 < 3$) and "minor" ($\Delta E < 1.5$). Thus, all methods of processing larvae cause significant changes in their color compared to untreated live larvae.

Blanching before drying comparing to the other treatment methods led to the browning reduction. It can occur as a result of inactivation of browning enzymes, such as polyphenol oxidase. In crustaceans, for example, polyphenol oxidase loses its activity after 2 min at 60 °C [33].

Browning caused by drying at low temperatures between 40 °C and 60 °C may result from the combination of enzymatic and non-enzymatic processes. Non-enzymatic browning occurs due to the degradation of carbohydrates as a result of Maillard reaction or caramelization [47]. Thus, exposure to high temperature for a long time contributes to non-enzymatic browning processes, which leads to the formation of colored Maillard products, and as a result the dried larvae feature darker color [30]. Enzymatic browning is associated with the formation of dark brown pigments through melanosis [31]. After initiating an enzymatic reaction in the presence of oxygen, melanosis continues as a chemical condensation reaction, which rate is not reduced by high temperature.

Figure 6. Live larvae before processing/drying

As a result of prolonged convection drying at 40 °C, all larvae, except for blanched ones, showed low values of lightness, chroma, and browning index. As can be seen from Table 3 and 4, these larvae had a dark gray color, which is likely to be negatively perceived by consumers. Increasing the drying temperature up to 60 °C led to an increase in chroma and browning index of larvae. The reason may be the predominance of the non-enzymatic browning reactions at this temperature as a result of the inactivation of polyphenol oxidase.

Microwave-dried larvae had higher lightness and browning index values compared to convection-dried larvae. This may be explained by the fact that during microwave drying the larvae were exposed to high temperature for a short period of time (up to 17 min). Thus, the high treatment temperature inactivated the enzymatic reaction, and the low duration of the process reduced the duration of non-enzymatic reactions.

Conclusion

In this study the influence of the pretreatment and drying methods on the drying rate, fatty acid composition and color of the yellow mealworm (*Tenebrio molitor*) larvae were investigated. All considered methods had no significant effect on the larvae fatty acid composition. On the other hand, the pretreatment and drying of the samples

Table 3. Appearance of larvae after processing/drying

Before drying	MW	CD 40 °C	CD 60 °C
Blanched			
			
Frozen (2 hours)			
			
Frozen (1 month)			
			
Defrosted			
			

Table 4. Parameters L^*, a^*, b^*, ΔE_{lab}, *Ch, h, BI* of the *Tenebrio molitor* larvae treated with various methods

Sample	L^*	a^*	b^*	ΔE_{lab}	*Ch*	*H*	*BI*	Color
Initial (not treated)	42.50 ± 6.40	20.33 ± 4.23	39.50 ± 1.41	—	44.43	1.10	212.00	
Blanched	53.83 ± 6.93	15.83 ± 2.60	42.00 ± 7.97	15.69	44.89	1.21	152.66	
Frozen (2 hours)	38.50 ± 13.24	12.50 ± 2.47	31.67 ± 8.22	11.38	34.04	1.20	167.10	
Frozen (1 month)	39.67 ± 8.79	15.84 ± 5.73	33.67 ± 12.99	12.39	37.20	1.13	180.12	
Defrosted	37.00 ± 10.49	6.33 ± 0.97	21.50 ± 9.52	18.85	22.41	1.28	95.59	
Blanched; CD 40 °C	39.83 ± 16.27	11.67 ± 2.24	28.83 ± 8.82	12.72	31.10	1.18	137.45	
Frozen (2 hours); CD 40 °C	34.17 ± 17.64	4.83 ± 3.66	7.33 ± 6.19	33.23	8.78	0.99	34.11	
Frozen (1 month); CD 40 °C	22.50 ± 21.25	4.17 ± 2.93	5.00 ± 5.10	39.89	6.51	0.88	38.22	
Defrosted; CD 40 °C	23.00 ± 13.59	3.17 ± 2.23	4.50 ± 4.52	40.12	5.50	0.96	31.46	
Blanched; CD 60 °C	29.00 ± 9.58	11.17 ± 3.52	26.50 ± 3.91	19.65	28.76	1.17	200.82	
Frozen (2 hours); CD 60 °C	27.33 ± 13.88	5.67 ± 2.46	18.83 ± 9.07	25.64	19.67	1.28	122.48	
Frozen (1 month); CD 60 °C	22.17 ± 14.70	8.33 ± 6.61	21.00 ± 15.33	27.66	22.59	1.19	212.46	
Defrosted; CD 60 °C	18.17 ± 8.23	9.83 ± 5.74	14.83 ± 6.27	34.95	17.80	0.98	179.29	
Blanched; MW	39.83 ± 8.06	5.33 ± 1.09	8.67 ± 5.00	30.95	10.18	1.02	33.94	
Frozen (2 hours); MW	35.50 ± 10.45	10.67 ± 5.96	14.00 ± 6.50	26.99	17.60	0.92	71.17	
Frozen (1 month); MW	36.17 ± 15.16	9.33 ± 2.85	24.50 ± 12.74	16.78	26.22	1.21	123.45	
Defrosted; MW	31.17 ± 11.31	11.33 ± 2.36	29.83 ± 10.31	16.08	31.91	1.21	215.45	

affected the drying rate and the color parameters of the resulting samples. Microwave drying was the fastest and most efficient drying method. It also provided a product with good color characteristics regardless of the insect pretreatment. The convective drying at 60 °C produced the samples of a similar final moisture content compared to the microwave drying, but convection drying was 81 times longer, resulting in browning of the insects. The convective drying at 40 °C was even longer than the drying at 60 °C, and the obtained samples were considerably brown. However, it has been shown that pre-blanching the larvae increases the speed of drying by the convective method and also allows keeping the color of the product close to the untreated one. Thus, convection drying of *Tenebrio molitor* larvae, in combination with pre-blanching, also allows obtaining a product with acceptable color characteristics and low water content. No significant influence of the defrosting of larvae or their prolonged storage at –20 °C on their drying rate or color characteristics was found.

REFERENCES

1. The State of World Fisheries and Aquaculture. (2000). Food and Agriculture Organization of the United Nations. Fisheries Department, 2000.
2. Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G. et al. (2013). Edible insects: future prospects for food and feed security (No. 171). Food and agriculture organization of the United Nations, Rome, 2013.
3. Mitsuhashi, J. (2010) The future use of insects as human food. Chapter in a book: Forest Insects as Food: Humans Bite Back. FAO of the United Nations Regional Office for Asia and the Pacific, Bangkok, 2010.
4. Zonderland-Thomassen, M. A., Lieffering, M., Ledgard, S. F. (2014). Water footprint of beef cattle and sheep produced in New Zealand: water scarcity and eutrophication impacts. *Journal of Cleaner Production*, 73, 253–262. https://doi.org/10.1016/j.jclepro.2013.12.025
5. Tilman, D., Clark, M. (2014). Global diets link environmental sustainability and human health. *Nature*, 515, 518–522. https://doi.org/10.1038/nature13959
6. Fluet, M. J., Vescovi, L., Bokoye, A. I. (2009). Water and Climate Change (Citizen mobilization, a source of solutions). Retrieved from: https://books.google.ru/books?hl=ru&lr=&id=tRzGWUWFEkQC&oi=fnd&pg=PA1&dq=The+United+Nations+World+Water+Development+Report%E2%80%93N+32009%E2%80%93Water+and+Climate+Change+(Citizen+mobilization,+a+source+of+solutions).+UNESCO&ots=4pE9yKabLu&sig=UNv2FdiATgjZbpk_AN82ujiPdk&redir_esc=y#v=onepage&q=The%20United%20Nations%20World%20Water%20Development%20Report%E2%80%93N%2032009%E2%80%93Water%20and%20Climate%20Change%20(Citizen%20mobilization%2C%20a%20source%20of%20solutions).%20UNESCO&f=false Accessed November 18, 2022
7. Ridoutt, B. G., Sanguansri, P., Freer, M., Harper, G. S. (2012). Water footprint of livestock: comparison of six geographically defined beef production systems. *The International Journal of Life Cycle Assessment*, 17(2), 165–175. https://doi.org/10.1007/s11367–011–0346-y
8. Mekonnen, M. M., Hoekstra, A. Y. (2012). A global assessment of the water footprint of farm animal products. *Ecosystems*, 15(3), 401–415. https://doi.org/10.1007/s10021–011–9517–8
9. Dobermann, D., Swift, J. A., Field, L. M. (2017). Opportunities and hurdles of edible insects for food and feed. *Nutrition Bulletin*, 42(4), 293–308. https://doi.org/10.1111/nbu.12291
10. Ramos-Elorduy, J. (2009). Anthropo-entomophagy: Cultures, evolution and sustainability. *Entomological Research*, 39(5), 271–288. https://doi.org/10.1111/j.1748–5967.2009.00238.x
11. Miglietta, P. P., De Leo, F., Ruberti, M., Massari, S. (2015). Mealworms for food: A water footprint perspective. *Water*, 7(11), 6190–6203. https://doi.org/10.3390/w7116190
12. Ghaly, A. E., Alkoaik, F. N. (2009). The yellow mealworm as a novel source of protein. *American Journal of Agricultural and Biological Sciences*, 4(4), 319–331. https://doi.org/10.3844/ajabssp.2009.319.331
13. Siemianowska, E., Kosewska, A., Aljewicz, M., Skibniewska, K. A., Polak-Juszczak, L., Jarocki, A. et al. (2013). Larvae of mealworm (*Tenebrio molitor* L.) as European novel food. *Agricultural Sciences*, 4(6), 287–291. https://doi.org/10.4236/as.2013.46041
14. Rumpold, B. A., Schlüter, O. K. (2013). Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science and Emerging Technologies*, 17, 1–11. https://doi.org/10.1016/j.ifset.2012.11.005
15. Rumpold, B. A., Schlüter, O. K. (2013). Nutritional composition and safety aspects of edible insects. *Molecular Nutrition and Food Research*, 57(5), 802–823. https://doi.org/10.1002/mnfr.201200735
16. Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Kearney, J., Maciuk, A. et al. (2021). Safety of dried yellow mealworm (*Tenebrio molitor* larva) as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, 19(1), e06343. https://doi.org/10.2903/j.efsa.2021.6343
17. Errico, S., Spagnoletta, A., Verardi, A., Moliterni, S., Dimatteo, S., Sangiorgio, P. (2022). *Tenebrio molitor* as a source of interesting natural compounds, their recovery processes, biological effects, and safety aspects. *Comprehensive Reviews in Food Science and Food Safety*, 21(1), 148–197. https://doi.org/10.1111/1541–4337.12863
18. Elhassan, M., Wendin, K., Olsson, V., Langton, M. (2019). Quality aspects of insects as food — nutritional, sensory, and related concepts. *Foods*, 8(3), Artricle 95. https://doi.org/10.3390/foods8030095
19. Evans, J., Flore, R., Frøst, M. B. (2017). On eating insects: essays, stories and recipes. Phaidon Press, 2017.
20. Paul, A., Frederich, M., Megido, R. C., Alabi, T., Malik, P., Uyttenbroeck, R. et al. (2017). Insect fatty acids: A comparison of lipids from three Orthopterans and *Tenebrio molitor* L. larvae. *Journal of Asia-Pacific Entomology*, 20(2), 337–340. https://doi.org/10.1016/j.aspen.2017.02.001
21. Nowak, V., Persijn, D., Rittenschober, D., Charrondiere, U. R. (2016). Review of food composition data for edible insects. *Food Chemistry*, 193, 39–46. https://doi.org/10.1016/j.foodchem.2014.10.114
22. Christensen, D. L., Orech, F. O., Mungai, M. N., Larsen, T., Friis, H., Aagaard-Hansen, J. (2006). Entomophagy among the Luo of Kenya: a potential mineral source? *International Journal of Food Sciences and Nutrition*, 57(3–4), 198–203. https://doi.org/10.1080/09637480600738252
23. Son, Y. -J., Choi, S. Y., Hwang, I. -K., Nho, C. W., Kim, S. H. (2020). Could defatted mealworm (*Tenebrio molitor*) and mealworm oil be used as food ingredients? *Foods*, 9(1), Article 40. https://doi.org/10.3390/foods9010040
24. Lenaerts, S., Van Der Borght, M., Callens, A., Van Campenhout, L. (2018). Suitability of microwave drying for mealworms (*Tenebrio molitor*) as alternative to freeze drying: Impact on nutritional quality and colour. *Food Chemistry*, 254, 129–136. https://doi.org/10.1016/j.foodchem.2018.02.006
25. Larouche, J., Deschamps, M. -H., Saucier, L., Lebeuf, Y., Doyen, A., Landenberg, G. W. (2019). Effects of stunning methods on lipid oxidation, colour and microbial load of black soldier fly (*Hermetia illucens*) larvae. *Animals*, 9(4), Article 182. https://doi.org/10.3390/ani9040182
26. Singh, Y., Cullere, M., Kovitvadhi, A., Chundang, P., Dalle Zotte, A. (2020). Effect of different stunning methods on physicochemical traits, nutritional characteristics, in vitro human digestibility and oxidative stability during storage of the house cricket (*Acheta domesticus* L.). *Innovative Food Science and Emerging Technologies*, 65, Article 102444. https://doi.org/10.1016/j.ifset.2020.102444
27. van Huis, A. (2019). Welfare of farmed insects. *Journal of Insects as Food and Feed*, 5(3), 159–162. https://doi.org/10.3920/JIFF2019.x004
28. El Hajj, R., Mhemdi, H., Besombes, C., Allaf, K., Lefrançois, V., Vorobiev, E. (2022). Edible insects' transformation for feed and food uses: An overview of current insights and future devel-

opments in the field. *Processes*, 10(5), Article 970. https://doi.org/10.3390/pr10050970
29. Kröncke, N., Böschen, V., Woyzichovski, J., Demtröder, S., Benning, R. (2018). Comparison of suitable drying processes for mealworms (*Tenebrio molitor*). *Innovative Food Science and Emerging Technologies*, 50, 20–25. https://doi.org/10.1016/j.ifset.2018.10.009
30. Kröncke, N., Grebenteuch, S., Keil, C., Demtröder, S., Kroh, L., Thünemann, A. F. et al. (2019). Effect of different drying methods on nutrient quality of the yellow mealworm (*Tenebrio molitor* L.). *Insects*, 10(4), Article 84. https://doi.org/10.3390/insects10040084
31. Azzollini, D., Derossi, A., Severini, C. (2016). Understanding the drying kinetic and hygroscopic behaviour of larvae of yellow mealworm (*Tenebrio molitor*) and the effects on their quality. *Journal of Insects as Food and Feed*, 2(4), 233–243. https://doi.org/10.3920/JIFF2016.0001
32. Parniakov, O., Mikhrovska, M., Wiktor, A., Alles, M., Ristic, D., Bogusz, R. et al. (2022). Insect processing for food and feed: A review of drying methods. *Drying Technology*, 40(8), 1500–1513. https://doi.org/10.1080/07373937.2021.1962905
33. Purschke, B., Brüggen, H., Scheibelberger, R., Jäger, H. (2018). Effect of pre-treatment and drying method on physicochemical properties and dry fractionation behaviour of mealworm larvae (*Tenebrio molitor* L.). *European Food Research and Technology*, 244(2), 269–280. https://doi.org/10.1007/s00217–017–2953–8
34. Vandeweyer, D., Lenaerts, S., Callens, A., Van Campenhout, L. (2017). Effect of blanching followed by refrigerated storage or industrial microwave drying on the microbial load of yellow mealworm larvae (*Tenebrio molitor*). *Food Control*, 71, 311–314. https://doi.org/10.1016/j.foodcont.2016.07.011
35. AOAC (2000) Official Methods of Analysis of the Association of Official Analytical Chemists, Official methods of analysis (17th Edition), vol. 2. Gaithersburg, MD, USA, 2000.
36. Figueira, G. M., Park, K. J., Brod, F. P. R., Honorio, S. L. (2004). Evaluation of desorption isotherms, drying rates and inulin concentration of chicory roots (*Cichorium intybus* L.) with and without enzymatic inactivation. *Journal of Food Engineering*, 63(3), 273–280. https://doi.org/10.1016/j.jfoodeng.2003.06.001
37. Lashkari, H., Halabinejad, M., Rafati, A., Namdar, A. (2020). Shelf life extension of veal meat by edible coating incorporated with *Zataria multiflora* essential oil. *Journal of Food Quality*, 2020, Article 8871857. https://doi.org/10.1155/2020/8871857
38. Maskan, M. (2001). Kinetics of colour change of kiwifruits during hot air and microwave drying. *Journal of Food Engineering*, 48(2), 169–175. https://doi.org/10.1016/S0260–8774(00)00154–0
39. Pande, R., Mishra, H. N., Singh, M. N. (2012). Microwave drying for safe storage and improved nutritional quality of green gram seed (*Vigna radiata*). *Journal of Agricultural and Food Chemistry*, 60(14), 3809–3816. https://doi.org/10.1021/jf204540n
40. Hosseinpour, S., Rafiee, S., Mohtasebi, S. S., Aghbashlo, M. (2013). Application of computer vision technique for on-line monitoring of shrimp color changes during drying. *Journal of Food Engineering*, 115(1), 99–114. https://doi.org/10.1016/j.jfoodeng.2012.10.003
41. Monica, P., Sharma, H.K., Sarkar, B.C., Singh, C. (2010). Kinetics of drumstick leaves (*Moringa oleifera*) during convective drying. *African Journal of Plant Science*, 4(10), 391–400.
42. Sharma, G. P., Prasad, S. (2001). Drying of garlic (*Allium sativum*) cloves by microwave–hot air combination. *Journal of Food Engineering*, 50(2), 99–105. https://doi.org/10.1016/S0260–8774(00)00200–4
43. Martins, M. G., Martins, D. E. G., da Silva Pena, R. (2015). Drying kinetics and hygroscopic behavior of pirarucu (*Arapaima gigas*) fillet with different salt contents. *LWT-Food Science and Technology*, 62(1(1)), 144–151. https://doi.org/10.1016/j.lwt.2015.01.010
44. Park, K. J., Vohnikova, Z., Brod, F. P. R. (2002). Evaluation of drying parameters and desorption isotherms of garden mint leaves (*Mentha crispa* L.). *Journal of Food Engineering*, 51(3), 193–199. https://doi.org/10.1016/S0260–8774(01)00055–3
45. Tzompa-Sosa, D. A., Yi, L., van Valenberg, H. J.F., van Boekel, M. A.J.S., Lakemond, C. M. M. (2014). Insect lipid profile: Aqueous versus organic solvent-based extraction methods. *Food Research International*, 62, 1087–1094. https://doi.org/10.1016/j.foodres.2014.05.052
46. Ribeiro, J. C., Lima, R. C., Maia, M. R.G., Almeida, A. A., Fonseca, A. J.M., Cabrita, A. R. J. et al. (2019). Impact of defatting freeze-dried edible crickets (*Acheta domesticus* and *Gryllodes sigillatus*) on the nutritive value, overall liking and sensory profile of cereal bars. *LWT*, 113, Article 108335. https://doi.org/10.1016/j.lwt.2019.108335
47. Pathare, P. B., Opara, U. L., Al-Said, F. A. -J. (2013). Colour measurement and analysis in fresh and processed foods: a review. *Food and Bioprocess Technology*, 6(1), 36–60. https://doi.org/10.1007/s11947–012–0867–9

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BIOTECHNOLOGICAL TECHNIQUES FOR INTENSIFICATION OF PROTEIN EXTRACTION FROM THE PORCINE PANCREAS

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Abstract
Processing of secondary products after slaughter of farm animals is in demand. The pancreas is a rich source of bioactive protein substances, effective extraction of which is a serious problem today due to their aggregation. The aim of the work was to assess the extractivity of protein substances of the porcine pancreas using sodium chloride, trehalose, arginine, and combination of glycine and proline. The protein concentration was determined in the obtained extracts by the biuret reaction and their protein composition was assessed by densitometry of two-dimensional electropherograms using software ImageMaster™ 2D Platinum powered by Melanie 8.0. The results showed a positive effect of anti-aggregation agents on the release of protein substances into a solution. The highest protein concentration (33.36±0.64 g/l) was observed when adding 1M L-arginine; however, it was conditioned mainly by an increase in the content of three major protein fractions rather than by diversity of the protein composition. In general, the use of 0.9% NaCl as an extractive agent was quite effective, but selectivity to certain protein groups was observed for anti-aggregation agents such as sodium chloride, trehalose, arginine, glycine and proline, as well as their combination. The obtained results are important for intensifying extraction of protein substances including target ones with the subsequent application in different fields.

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Introduction

Many secondary products are generated during processing of agricultural products. The questions of their effective and rational use often remain to be unsolved. In animal slaughter, the yield of secondary products is quite high and accounts for about 30% of live weight [1,2], including by-products and low-value non-edible raw materials. Certainly, there are traditional approaches to the utilization and processing of such non-edible raw materials [3,4], however, the use and implementation of alternative technologies to intensify the rational environmental management are particularly in demand within the framework of the concept of circular economy [5].

One of such by-products is the porcine pancreas, which was used earlier in high quantities to produce insulin for people suffering from diabetes mellitus [6]. Nowadays, insulin is produced mainly using the technology of recombinant DNA [7], as a result, a high demand for the processing of the pancreas is absent. Nevertheless, bioinformatic analysis with the use of the UniProt database [8] shows that this type of raw materials contains quite a large quantity of biologically active substances of protein nature such as pancreatic alpha-amylase, triacylglycerol lipase, phospholipase A2, proglucagon, pancreatic elastase and so on. This allows regarding the pancreas as a potential object for extracting target protein compounds with the following use in the pharmaceutical and/or food industries as well as in the modern laboratory practice [9].

Today, effective extraction and purification of target protein substances from animal raw materials is a serious problem for researchers due to tendency of protein molecules to aggregation [10]. A degree of aggregation depends on many factors, which in a broad sense can be classified as internal (primary, secondary, tertiary or quaternary structure of proteins) and external (type of solution for extraction, conditions and type of isolation process) [11]. Protein aggregation can lead to a decrease in the biological activity of a molecule or its complete loss, an increase in the potential immunogenicity, sedimentation of protein aggregates, as well as other side undesirable effects [11,12]. There are many additives to solutions that stabilize the protein structure preventing thereby their aggregation and enhancing their extractivity from the initial raw materials. Such stabilizers (anti-aggregation agents) include several

amino acids, sugars, polyhydric alcohols, osmolytes and cosmotropic salts [13], which are characterized by safety and can be used to intensify extraction of protein substances, including target ones, with the following use with various purposes. The aim was to study an effect of anti-aggregation agents, such as sodium chloride, trehalose, several amino acids and their combination, on extractivity of protein substances from the porcine pancreas.

Objects and methods

The porcine pancreas was taken in LLC "Pushkinsky myasnoy dvor", Moscow region, Pushkino. Animal raw materials were cleaned of connective tissues, frozen at minus 18 °C, then minced in the frozen state and stored until the subsequent extraction.

The minced pancreas was thawed at a temperature of 4 °C and mixed with an extracting agent in a ratio of 1:5. Extraction was carried out on a laboratory dispersing equipment (LDU, Labotex, Russia) with a mixing speed of 400 rpm; extraction time was 150 min.

Four extractions were carried out with the following extracting agents:

1) 0.9% sodium chloride solution (LLC Gematek, Russia), (0.9% NaCl);
2) 0.9% sodium chloride solution (LLC Gematek, Russia) with addition of 1 M L-arginine (PanReac, Germany) (0.9% NaCl, 1M L-Arg);
3) 0.9% sodium chloride solution (LLC Gematek, Russia) with addition of 0.5 M trehalose (Narodnaya zdrava, Russia) (0.9% NaCl, 0.5 M trehalose);
4) 0.9% sodium chloride solution (LLC Gematek, Russia) with addition of 1% glycine (PanReac AppliChem, Germany), 0.1 M L- proline (Sigma-Aldrich, USA) (0.9% NaCl, 1% Gly, 0.1M Pro).

After the end of the extraction process, supernatant was separated by centrifugation at a speed of 3500 rpm on CM-6M centrifuge for 5 min (ELMI, Latvia). The protein concentration was measured in each sample by the biuret reaction on a semi-auto biochemistry analyzer BioChem SA (HTI, USA) using the standard total protein reagent (HTI, USA). The measurements were carried out in triplicate. The results were calculated with the use of the software STATISTICA 10.0 and presented as "mean ± SD". Significant differences were tested by non-parametric statistical Mann–Whitney *U*-tests for independent variables. Differences with P-values of <0.1 were considered statistically significant.

The proteomic composition of extracts and the pancreas was assessed by two-dimensional gel electrophoresis (2-DE). A sample (100 mg) was taken and a lysing solution (2000 µl) was added. The lysing solution consisted of 9 M urea (PanReac, Germany), 5% β-mercaptoethanol (PanReac, Germany), 2% triton X-100 (Helicon, Russia), 2% ampholines pH 3–10 (Serva, Germany). The obtained homogenate was purified by centrifugation (Centrifuge 5427 R, Eppendorf, Germany) at 14,000 rpm for 20 minutes. At the first stage, isoelectric focusing (IEF) was carried out in tube gels (2.4 mm x 160 mm) in a chamber (Bio-Rad, USA) up to reaching 3,650 volt-hours; an aliquot of the introduced samples contained 140 µg of protein. As an anode buffer and a cathode buffer, 0.01 M orthophosphoric acid (Component-Reactive, Russia) and 0.02 M sodium hydroxide (Panreac, Spain), respectively, were used. After IEF, gels were incubated during 10 min in 2.5 ml of equilibration buffer I (6 M urea (Panreac, Germany), 20% glycerol (Panreac, Germany), 2% SDS (Panreac, Spain) and 1% DTT (Panreac, Spain) in 50 mM Tris-HCl buffer, pH 8.8 (Panreac, Germany)). Then, incubation was carried out in equilibration buffer II (6 M urea (Panreac, Germany), 20% glycerol (Panreac, Germany), 2% SDS (Panreac, Spain) and 4% iodoacetamide (SIGMA, USA) in 375 mM Tris-HCl buffer, pH 8.8 (Panreac, Germany)) [14]. After that, electrophoresis was carried out in 12.5% polyacrylamide gel (170 mm × 180 mm × 1.5 mm) in a chamber VE-20 (Helicon, Russia) using buffer containing 25 mM Tris-HCl (Panreac, Germany), 192 mM glycine (Panreac, Germany) and 0.1% SDS (Panreac, Spain). The process was performed at amperage of 30 mA/gel until the dye front reached the gel edge. Two-dimensional electropherograms were obtained in triplicate for each sample.

Computer densitometry of two-dimensional electropherograms in a wet state was performed using a Bio-5000 plus scanner (Serva, Germany) at a resolution of 300 ppi 1D-Gray. The obtained images were analyzed using one-way ANOVA (between gels of different samples) and ImageMaster™ 2D Platinum software powered by Melanie 8.0 (GE Healthcare and Genebio, Switzerland). Protein fractions were compared by volume, and the fold-value, an excess of which by more than two units is generally considered statistically significant difference, was calculated. All results are presented as mean ± SD from three independent experiments.

Results and discussion

The results of the determination of the protein concentration in the extracts obtained with the use of 0.9% sodium chloride or with addition of anti-aggregation agents are presented in Table 1.

It was shown that addition of anti-aggregation agents to 0.9% NaCl facilitated release of protein substances into the extracting agent. The highest protein content was observed upon addition of 1 M L-Arg and was 33.36 ± 0.64 g/l,

Table 1. Results of the determination of the total protein concentration in the extracts

Extracting agent	0.9% NaCl	0.9% NaCl, 1 M L-Arg	0,9% NaCl, 0.5 M trehalose	0.9% NaCl, 1% Gly, 0.1 M L-Pro
Protein concentration, g/l	24.84 ± 1.08	33.36 ± 0.64*	29.47 ± 1.58*	28.22 ± 1.36*

* statistically significant difference from extraction with 0.9% NaCl ($p < 0.1$)

which exceeded the value of the total protein content in the extract obtained using 0.9% NaCl by 34.3% ($p < 0.1$). Addition of 0.5 M trehalose and a mixture of 1% Gly with 0.1 M L-Pro facilitated an increase in the protein content in the extract by 18.6% ($p < 0.1$) and 13.6% ($p < 0.1$), respectively, compared to its content in the extract when using 0.9% NaCl.

Then, the protein composition of the obtained extracts was assessed by densitometry of two-dimensional electropherograms with the same protein load. The two-dimensional electropherograms of the extracts and pancreas tissue are presented in Figure 1. The two-dimensional electropherogram of the pancreas tissue shows the presence of protein fractions P1 (47 kDa), P2 (48 kDa), P3 (27 kDa) and P4 (36 kDa), marked in Figure 1, which are not extracted by the types of used extracting agents. In addition, many protein fractions with a molecular weight of less than 15 kDa were observed in the pancreas tissue; the relative volume change of these fractions was significantly lower in the obtained extracts. It is interesting that the highest content of total protein in the extract with addition of 1 M L-Arg was conditioned mainly by an increase in the content of three major protein fractions rather than by diversity of the protein composition. Also, two-dimensional electropherograms show protein fractions D1 (16.279 kDa), D2 (28.92 kDa), D3 (29 kDa), D4 (30 kDa), D5 (26 kDa), which were not detected on other gels.

On the two-dimensional electropherograms, fraction F4 (13 kDa) located in the alkaline side was additionally detected in the extracts obtained using 0.9% NaCl with 1 M L-Arg, while the extracts obtained using 0.9% NaCl, 0.9% NaCl with 0.5 M trehalose or 1% Gly, 0.1 M L-Pro contained protein fractions F1 (16.56 kDa), F2 (14.581 kDa), F3 (17 kDa) in the alkaline area. Furthermore, upon extraction with the use of 0.9% NaCl, 0.9% NaCl with 0.5 M trehalose or 1% Gly, 0.1 M L-Pro, two protein groups were observed in the alkaline area in a range of 24–32 kDa and 37–40 kDa marked in Figure 1 with the violet color; when 0.9% NaCl was used as an extracting agent, protein fraction L1 (34 kDa) was found. These fractions were not detected in the initial pancreas tissue but were found in the extracts.

The statistically significant relative volume change of protein fractions is depicted in Figures 2 and 3.

Extraction with the use of 0.9% NaCl facilitated enrichment of the extract practically with all protein fractions, excluding S20 (28.699 kDa), S27 (35 kDa) and S29 (13.969 kDa), the relative volume change of which was 1.5, 4 and 1.7 times lower, respectively, than in pancreas tissue. The relative volume change of fractions S24 (27.419 kDa) and

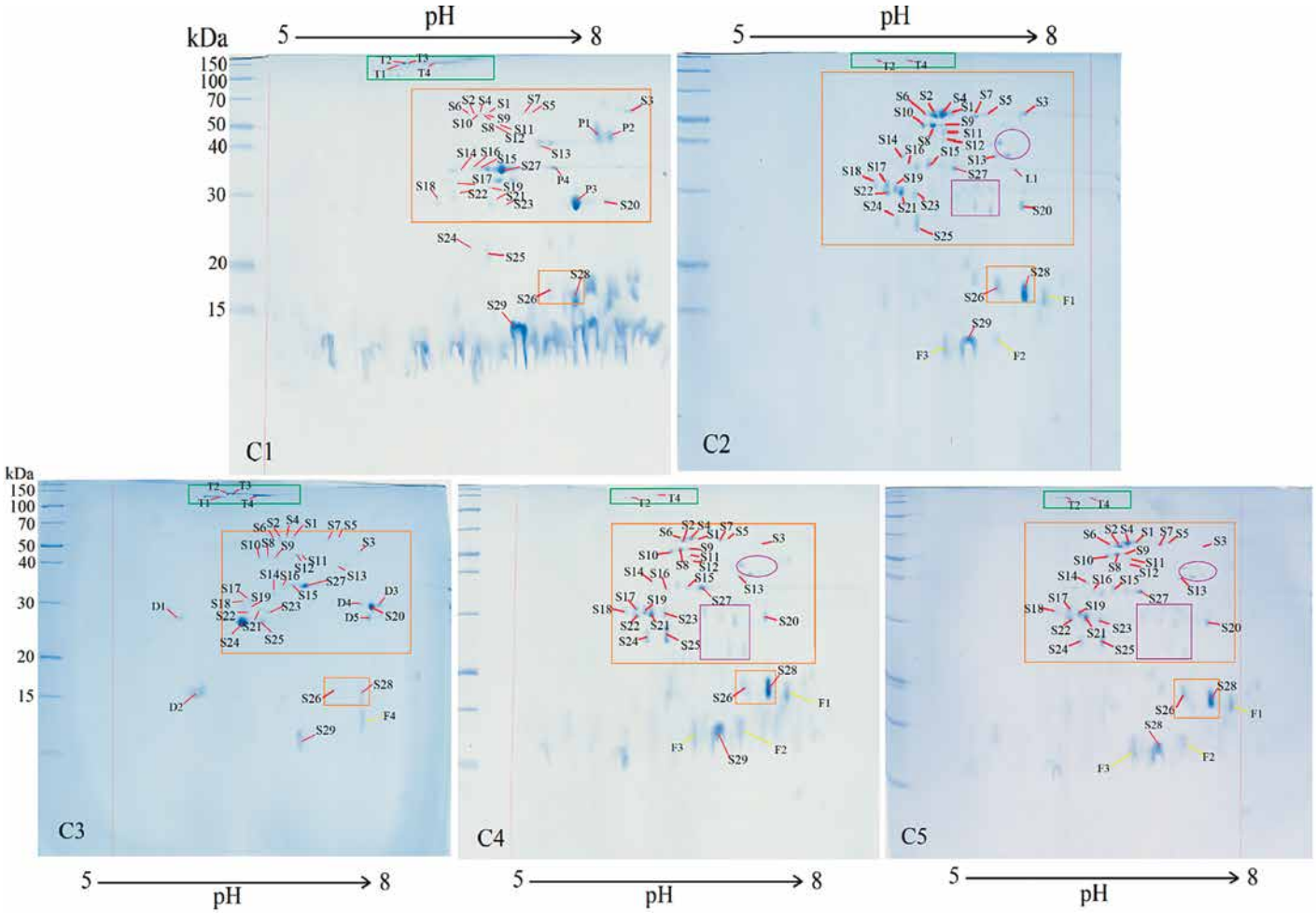

Figure 1. Two-dimensional electropherograms of extracts and pancreas tissue
C1 — pancreas; C2–0.9% NaCl; C3–0.9% NaCl, 1 M L-Arg; C4–0.9% NaCl, 0.5 M trehalose; C5–0.9% NaCl, 1% Gly, 0.1 M L-Pro.
Protein fractions, the relative volume change of which was significantly different, are marked with red arrows

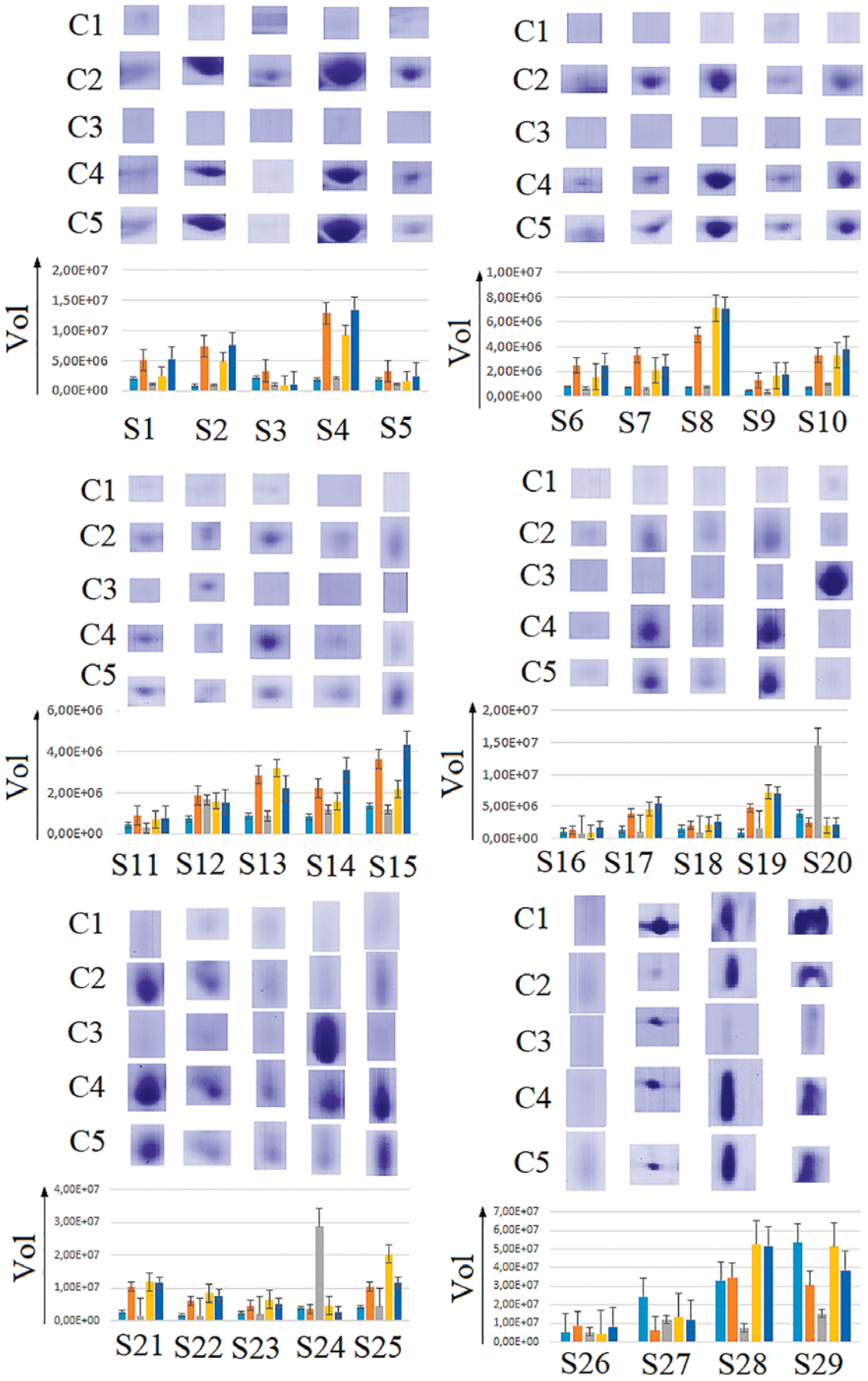

Figure 2. Relative volume change of protein fractions.
Light blue– C1, pancreas; orange– C2, 0.9% NaCl; grey– C3, 0.9% NaCl, 1 M L-Arg; yellow– C4, 0.9% NaCl, 0.5 M trehalose; dark blue– C5, 0.9% NaCl, 1% Gly, 0.1 M L-Pro; S1-S29 — protein fractions, the relative volume change of which was significantly different

Note: the spot intensity was normalized by the total valid spot intensity and the mean value for duplicate analytical gels from triplicates. The data presented are mean ± SD for three independent experiments.

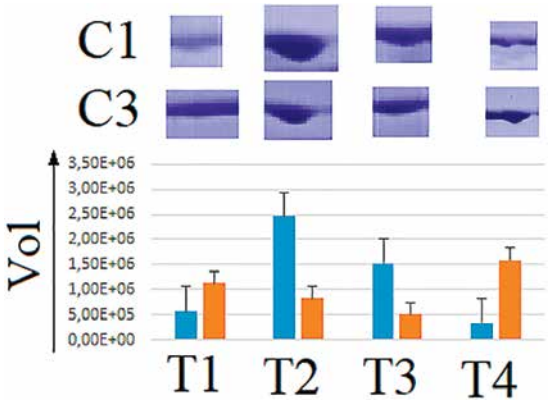

Figure 3. Relative volume change of protein fractions
Light blue– C1, pancreas; orange– C3, 0.9% NaCl, 1 M L-Arg; T1–T4 — protein fractions, the relative volume change of which was significantly different.

Note: the spot intensity was normalized by the total valid spot intensity and the mean value for duplicate analytical gels from triplicates. The data presented are mean ± SD for three independent experiments.

S28 (17.08 kDa) almost did not differ from the pancreas. On the contrary, addition of 1M L-Arg to 0.9% NaCl facilitated enrichment of the extract with fractions S20 (28.699 kDa) and S24 (27.419 kDa), the relative volume change of which was 3.7 and 7.1 times higher, respectively, than in pancreas tissue. Also, an increase by 1.4–2.3 times, on average, in the content of fractions S10 (48.25 kDa), S12 (40 kDa), S14 (36.11 kDa) and S19 (30.68 kDa), respectively, was observed. The relative volume change of other protein fractions was either significantly lower than the level of pancreas tissue or did not differ from it. Addition of 0.5 M trehalose to 0.9% NaCl led to enrichment of the extract practically with all protein fractions excluding S3 (52.168 kDa), S20 (28.699 kDa) and S27 (35 kDa), the relative volume change of which was 2.6, 2.0 and 1.8 times lower, respectively, then in pancreas tissue. The relative volume change of fractions S5 (52.306 kDa), S16 (34.908 kDa), S26 (19.203 kDa) and S29 (13.969 kDa) almost did not differ from pancreas tissue. The use of 0.9% NaCl with 1% Gly, 0.1 M L-Pro as an extracting agent also facilitated enrichment of the extract with all protein fractions excluding fractions S3 (52.168 kDa) S20 (28.699 kDa), S24 (27.419 kDa), S27 (35 kDa) and S29 (13.969 kDa), the relative volume change of which was 2.2, 1.8, 1.5, 2, and 1.3 times lower, respectively, then in pancreas tissue.

In general, the use of 0.9% NaCl as an extracting agent was quite effective. Addition of 1 M L-Arg to 0.9% NaCl significantly reduced extractivity of practically all protein fractions excluding S12 (40 kDa), which did not differ from the extract obtained using 0.9% NaCl. Also, fractions S20 (28.699 kDa) and S24 (27.419 kDa), turned to be an exclusion; their content was the highest among all types of extracting agents. Addition of 0.5 M trehalose to 0.9% NaCl facilitated enrichment of the extract with protein fraction S25 (27.172 kDa), the relative volume change of which was 2.0 times higher than in 0.9% NaCl; the content of fraction S23 (28.821 kDa) also increased by 1.4 times. Addition to the 0.9% NaCl solution of anti-aggregation agents such as 0.5 M trehalose and the mixture 1% Gly and 0.1 M L-Pro led to an increase by 1.3–1.6 times, on average, in the content of fractions S8 (49.328 kDa), S9 (48.27 kDa), S19 (30.68 kDa), S22 (30.594 kDa), S28 (17.08 kDa) and S29 (13.969 kDa). Addition of the mixture of 1% Gly and 0.1 M L-Pro facilitated enrichment of the extract with protein fractions S14 (36.11 kDa), S15 (35.29 kDa), S16 (34.91 kDa), S17 (31.63 kDa), S18 (32.74 kDa), the relative volume change of which was 1.2–1.4 times higher on average than in 0.9% NaCl.

In analysis of electropherograms, selective enrichment of the extracts with certain groups of protein fractions was noted. The use of 1 M L-Arg with 0.9% NaCl facilitated the highest enrichment of fractions T1–T4, which are presented in Figure 3. Furthermore, this extraction facilitated enrichment of the extract with fractions T1 (150 kDa) и T4 (153 kDa) — their relative volume change exceeded this value in pancreas tissue by 2 and 4.6 times, respectively. For fractions T2 (152 kDa) and T3 (154.7 kDa), the relative volume change was on average 3-fold lower compared to pancreas tissue. On the contrary, when using other extracting agents, trace amounts of only two fractions — T2 (152 kDa) и T4 (154.7 kDa) — were noted on the two-dimensional electropherograms.

In general, the use of 0.9% NaCl as an extracting agent was quite effective. Cosmotropic salts act as a protein stabilizer (usually small ions, low polarizability) and as creators of the polar water structure [15,16]. For weak cosmotropic salts such as NaCl and KCl, the recommended initial concentration is 300 mM and 200 mM, respectively; the recommended concentration range is 0–1 M. The 0.15 M NaCl solution was used in the experiment described above.

To prevent protein aggregation, sugars and polyhydric alcohols are also widely used [15,17]. Polyol and sugar osmolytes can disrupt protein hydrogen bonds influencing the protein function [18] and stabilizing the lattice structure of water [19]. Several papers report about stabilization of different biomolecules with trehalose [20]. It is assumed that trehalose induces the well-defined protein-protein distance, which can explain why it inhibits protein-protein interactions and protein aggregation associated with them. However, the excellent anti-aggregation effect of trehalose can also be linked with the fact that the local solvent structures are very important for explaining the mechanism of protein stabilization [21]. The recommended initial concentration of 0.5 M for trehalose and sucrose [15,17] was used in this work.

The use of amino acids as anti-aggregation agents is in demand in the food industry and production of biologically active substances. Amino acids and their derivatives increase the surface tension of water in a concentration of 20–500 mM [19]. It is assumed that hydrophobic surfaces that are present on proteins interact with the hydrophobic surface represented by the arginine clusters. Masking of the hydrophobic surface inhibits protein-protein aggregation [22]; however, scientists also describe other mechanisms, by which arginine prevents protein aggrega-

tion [22,23]. Even though arginine demonstrated the best results in prevention of aggregation among 15 tested amino acids [24], in this work its addition negatively affected diversity of extracted proteins. Glycine demonstrated two stages of stabilization. The first effect (at concentrations lower than 100 mM) is specific for protein and, possibly, is conditioned by multiple direct interactions with polar or charged side chains and partial charges on the peptide backbone of protein. The second stage (at concentrations higher than 100 mM) is similar to anions with the high charge density, where it is associated with competition for water in the unfolding process [25]. Proline has a closed circular structure in the side chain, which has the hydrophobic surface allowing it to interact with proteins by hydrophobic interactions [26]. It has been assumed that the multimeric forms of proline can be responsible for its inhibiting action on aggregation [22]. Combination of glycine (action via the polar part of protein) and proline (action via the hydrophobic part) is able to exert the complex action suppressing protein aggregation, which was demonstrated in the present work.

In general, the use of 0.9% NaCl as an extracting agent was quite effective; however, selectivity of anti-aggregation agents such as sodium chloride, trehalose, arginine, glycine and proline, and their combinations to certain protein groups was noticed.

Conclusion

Effective extraction and purification of target protein substances from animal raw materials is a serious problem for researchers due to the tendency of protein molecules to aggregation. The present work shows that the 0.9% sodium chloride solution was able to extract quite effectively a wide spectrum of protein substances from pancreas tissue, and addition of anti-aggregation agents was characterized by selectivity to certain protein groups. Although arginine demonstrated the best results in prevention of the development of aggregates in several scientific works, the highest content of total protein in the extract with the addition of 1 M L-arginine was conditioned by an increase in three major protein fractions rather than by diversity of the protein composition. Addition of 0.5 M trehalose to the 0.9% sodium chloride solution or a mixture of 1% glycine and 0.1 M L-proline led to an increase in the content of several protein fractions, including those with pI shifted to the alkaline area.

The obtained results are important for intensifying extraction of protein substances including target ones with the following application with various purposes. An effect of anti-aggregation agents on the processes of purification and separation of protein mixtures using membrane technologies will be studied in the subsequent work.

REFERENCES

1. Alao, B., Falowo, A., Chulayo, A., Muchenje, V. (2017). The potential of animal by-products in food systems: Production, prospects and challenges. *Sustainability (Switzerland)*, 9(7), Article 1089. https://doi.org/10.3390/su9071089
2. Marti, D. L., Johnson, R. J., Mathews, K. H. Jr. (2011). Where's the (Not) Meat? Byproducts From Beef and Pork Production. Retrieved from https://www.ers.usda.gov/webdocs/outlooks/37427/8801_ldpm20901.pdf. Accessed October 3, 2022.
3. Jayathilakan, K., Sultana, K., Radhakrishna, K., Bawa, A.S. (2012). Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. *Journal of Food Science and Technology*, 49(3), 278–293. https://doi.org/10.1007/s13197–011–0290–7
4. Nasonova, V. V. (2018). Perspective ways the use of by-products. *Theory and Practice of Meat Processing*, 3(3), 64–73. https://doi.org/10.21323/2414–438X-2018–3–3–64–73
5. Velenturf, A. P. M., Purnell, P. (2021). Principles for a sustainable circular economy. *Sustainable Production and Consumption*, 27, 1437–1457. https://doi.org/10.1016/j.spc.2021.02.018
6. Ladisch, M. R., Kohlmann, K. L. (1992). Recombinant human insulin. *Biotechnology Progress*, 8(6), 469–478. https://doi.org/10.1021/bp00018a001
7. Siew, Y. Y., Zhang, W. (2021). Downstream processing of recombinant human insulin and its analogues production from E. coli inclusion bodies. *Bioresources and Bioprocessing*, 8(1), Article 65. https://doi.org/10.1186/s40643–021–00419-w
8. UniProt Protein Database (2022). Retrieved from http://www.uniprot.org/. Accessed July 3, 2022.
9. Vasilevskaya, E. R., Aryuzina, M. A., Vetrova, E. S. (2021). Comparative study of technologies for extraction of biologically active substances from the raw material of animal origin. *Theory and Practice of Meat Processing*, 6(3), 226–235. https://doi.org/10.21323/2414–438X-2021–6–3–226–235
10. Dobson, C. M. (2004). Principles of protein folding, misfolding and aggregation. *Seminars in Cell and Developmental Biology*, 15(1), 3–16. https://doi.org/10.1016/j.semcdb.2003.12.008
11. Wang, W., Nema, S., Teagarden, D. (2010). Protein aggregation — Pathways and influencing factors. *International Journal of Pharmaceutics*, 390(2), 89–99. https://doi.org/10.1016/j.ijpharm.2010.02.025
12. Alam, P., Siddiqi, K., Chturvedi, S. K., Khan, R. H. (2017). Protein aggregation: From background to inhibition strategies. *International Journal of Biological Macromolecules*, 103, 208–219. https://doi.org/10.1016/j.ijbiomac.2017.05.048
13. Hamada, H., Arakawa, T., Shiraki, K. (2009). Effect of additives on protein aggregation. *Current Pharmaceutical Biotechnology*, 10(4),400–407.https://doi.org/10.2174/138920109788488941
14. Akhremko, A., Vasilevskaya, E. R., Fedulova, L. (2020). Adaptation of two-dimensional electrophoresis for muscle tissue analysis. *Potravinarstvo Slovak Journal of Food Sciences*, 14, 595–601. https://doi.org/10.5219/1380
15. Lebendiker, M., Danieli, T. (2014). Production of prone-to-aggregate proteins. *FEBS Letters*, 588(2), 236–246. https://doi.org/10.1016/j.febslet.2013.10.044
16. Vasilevskaya, E. R., Aryuzina, M. A., Vetrova, E. S. (2021). Saline extraction as a method of obtaining a mixture of biologically active compounds of protein nature from a porcine pancreas. *Food Systems*, 4(2), 97–105. https://doi.org/10.21323/2618–9771–2020–4–2–97–105 (In Russian)
17. Bondos, S. E., Bicknell, A. (2003). Detection and prevention of protein aggregation before, during, and after purification. *Analytical Biochemistry*, 316(2), 223–231. https://doi.org/10.1016/S0003–2697(03)00059–9
18. Li, J., Chen, J., An, L., Yuan, X., Yao, L. (2020). Polyol and sugar osmolytes can shorten protein hydrogen bonds to modulate function. *Communications Biology*, 3(1), Article 528. https://doi.org/10.1038/s42003–020–01260–1
19. Schein, C. H. (1990). Solubility as a function of protein structure and solvent components. *Nature Biotechnology*, 8(4), 308–317. https://doi.org/10.1038/nbt0490–308
20. Jain, N. K., Roy, I. (2008). Effect of trehalose on protein structure. *Protein Science*, 18(1), 24–36. https://doi.org/10.1002/pro.3
21. Olsson, C., Swenson, J. (2019). The role of disaccharides for protein–protein interactions — a SANS study. *Molecular Physics*, 117(22), 3408–3416. https://doi.org/10.1080/00268976.2019.1640400
22. Das, U., Hariprasad, G., Ethayathulla, A.S., Manral, P., Das, T.K., Pasha, S. et al. (2007). Inhibition of protein aggregation: Supramolecular assemblies of arginine hold the key. *PLoS*

ONE, 2(11), Article e1176. https://doi.org/10.1371/journal.pone.0001176
23. Tsumoto, K., Umetsu, M., Kumagai, I., Ejima, D., Philo, J.S., Arakawa, T. (2004). Role of arginine in protein refolding, solubilization, and purification. *Biotechnology Progress*, 20(5), 1301–1308. https://doi.org/10.1021/bp0498793
24. Shiraki, K., Kudou, M., Fujiwara, S., Imanaka, T., Takagi, M. (2002). Biophysical effect of amino acids on the prevention of protein aggregation. *Journal of Biochemistry*, 132(4), 591–595. https://doi.org/10.1093/oxfordjournals.jbchem.a003261
25. Platts, L., Falconer, R. J. (2015). Controlling protein stability: Mechanisms revealed using formulations of arginine, glycine and guanidinium HCl with three globular proteins. *International Journal of Pharmaceutics*, 486(1–2), 131–135. https://doi.org/10.1016/j.ijpharm.2015.03.051
26. Rajan, R., Ahmed, S., Sharma, N., Kumar, N., Debas, A., Matsumura, K. (2021). Review of the current state of protein aggregation inhibition from a materials chemistry perspective: special focus on polymeric materials. *Materials Advances*, 2(4), 1139–1176. https://doi.org/10.1039/D0MA00760A

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STUDY OF ADIPOSE TISSUE OF KEMEROVO PIGLETS: DETECTION OF BEIGE ADIPOCYTES

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Keywords: *pigs, histology, white adipocytes, beige adipocytes, localization, morphometry, Raman spectroscopy, fatty acid composition*

Abstract

Animals have two types of adipose tissue differing in structure and function: white (WAT) and brown (BAT). Beige adipose tissue (BeAT) is a result of WAT browning, when beige adipocytes appear between white adipocytes in response to exposure to cold, diet or physical activity. BeAT shares morphological and biochemical characteristics with BAT, is thermogenic and dissipates energy in the form of heat, unlike WAT, which is responsible for energy storage. Pigs do not have classic BAT, and modern breeds are sensitive to cold. There is literature information that BeAT has been found in cold-resistant pigs. The aim of the work was to study adipose tissue of piglets of the Kemerovo cold-resistant breed under growing conditions in the cold season and to identify the localization of BeAT. Histological studies have shown two types of adipocytes in subcutaneous fat samples (lateral, backfat and axillary): white, with one large fat droplet, and beige, multilocular. Larger cells were detected in backfat fat (69.36 ± 12.98 μm) compared with lateral (53.25 ± 9.27 μm) and axillary fat (45.94 ± 8.29 μm). Only WAT with a diameter of 35.69 ± 6.96 μm was present in the internal perirenal fat. Raman spectroscopy was used to evaluate the overall fatty acid profile of the tested samples. The main peaks were noted in all samples: 970 cm^{-1} (=C-H out-of-plane bend), 1266/1272 cm^{-1} (=C-H symmetrical rock) and 1655 cm^{-1} (C=C stretching) responsible for unsaturated bonds, and signals at 1297/1301 cm^{-1} (CH_2 twisting), 1430/1460 cm^{-1} (CH_2 symmetrical deformation (scissoring)) and 1735/1746 cm^{-1} (C=O stretching) corresponding to saturated bonds or ester groups. Internal perirenal fat contained the largest number of saturated fatty acid bonds, subcutaneous axillary fat — the smallest. The average intensity of the peaks was 0.4801010 and 0.639995, respectively. According to the results of gas chromatography, the largest amount of polyunsaturated fatty acids was noted in the subcutaneous fat samples: 20.199 in backfat fat, 21.749 in lateral and 20.436 in axillary fat compared to 18.636 in internal fat. Activation of beige cells in Kemerovo pigs under cold exposure, according to the authors, plays a crucial role in the heat balance, allowing them to tolerate cold without severe shivering. The study of the BeAT formation is of great practical importance for changing energy metabolism and increasing thermogenesis in newborn piglets by genome editing, as well as for improving the quality of pig's fat.

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Introduction

Adipose tissue is a metabolically active organ playing a key role in regulation of energy homeostasis of the body, including thermogenesis, takes part in the glucose metabolism, insulin secretion and regulation of immune reactions, secretes several bioactive peptides (adipokines) [1,2].

Based on the physiological functions, morphology and visual color, mammalian adipose tissue is divided into two types: white (WAT) and brown (BAT) [3]. WAT is located mainly under the skin and around internal organs. Cells contain a large unilocular lipid droplet and a small number of mitochondria. BAT is present in the embryonic period and during winter dormancy; its cells have many small multilocular lipid droplets and a large number of mitochondria. Beige adipose tissue (BeAT) is a result of WAT browning, when beige adipocytes appear between white adipocytes in response to exposure to several factors (for example, cold, diet or physical activity) [4]. Beige adipocytes have many common morphological and biochemical characteristics with brown adipocytes, including multilocular lipid droplets. Both brown and beige adipocytes are thermogenic and dissipate energy as heat, while white adipocytes are responsible for energy storage (Figure 1) [8].

Domestic pigs are an important resource in agriculture worldwide. Four main fat depots are differentiated in pigs: visceral, subcutaneous, intermuscular and intramuscular [6]; each of them has specific morphological and metabolic properties [7]. According to data from

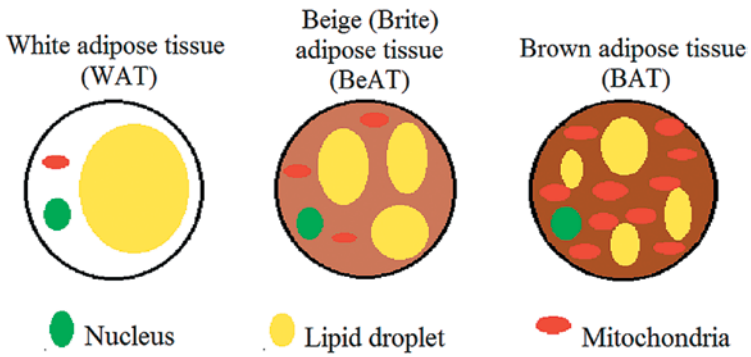

Figure 1. Comparison of three types of adipocytes [8]

literature sources, pigs do not have classic BAT despite the presence of brown adipocytes in the majority of mammals [8]. Thus, modern breeds are sensitive to cold, which is the main cause of mortality among piglets in pig husbandry [9]. However, there is information that beige adipocytes were found in axillary, inguinal subcutaneous, and perirenal WAT of cold-resistant Tibetan pigs and Min pigs [10].

The Kemerovo breed was selected in the Kemerovo region (Western Siberia) by crossing local pigs with sires of the Long-eared White, Large White, Large Black, Berkshire, Siberian North and Siberian Black-and-white breeds. The breed was approved in 1960 [11]. Nowadays, Kemerovo pigs are raised on the territory of Siberia and the Far East; however, the population is not large. It is significant that animals of this breed are distinguished by cold resistance and good adaptation to local climatic conditions, high viability and calm temper, intensive growth, sound constitution and early maturation. It is expedient to use these qualities in pig breeding to select new breeds and crosses in regions with sharp climate fluctuations and seasonal feeding base. Kemerovo pigs fall into the meat-and-lard (universal) direction of productivity and according to various literature data, a backfat size (at a level of 6^{th} –7^{th} thoracic vertebrae at a live weight of 100 kg) will be from 27–29 mm [12] to 35.8 ± 1.65 mm [13]. According to the data of Bekenev V. A. [14], backfat of Kemerovo pigs is characterized by an increased content of α-linolenic and docosahexaenoic fatty acids, a high PUFA: SFA ratio (0.12), which indicates its low fusibility, good palatability and high technological properties.

Several publications of both national and foreign authors are devoted to the study of adipose tissue of different pig breeds. The main attention in them is paid to the technological and consumer characteristics of raw materials obtained from adult (5–6 months of age) animals. Serra et al. [15] showed differences in thickness and fatty acid composition of backfat in Iberian and Landrace pigs with the same slaughter weight (105–118 kg). Iberian pigs were characterized by thicker backfat (48.1 versus 20.7 mm) that contained more saturated (SFA) and monounsaturated (MUFA) fatty acids and low concentrations of linoleic and linolenic fatty acids. Similar results but for Creole and Large White pigs are presented in [16]. In [17,18] characteristics of backfat for adult Basque and Large White pigs were compared: the Basque breed was characterized by earlier and higher development of adipose tissue (backfat depth was 26 versus 17 mm), as well as the high activity of enzymes responsible for lipid synthesis compared to the Large White animals. Nakajima et al. [19] showed that hypertrophy of adipocytes makes the highest contribution to the backfat depth and Kojima et al. [20] established differences in genome-wide expression profiles in adipose tissue in lard-type Meishan pigs compared to Landrace pigs. In [21] a relationship between the mRNA signaling pathway with subcutaneous adipogenesis and backfat thickness was shown by the example of Chinese Jiaxing pigs and White Large pigs. However, there is little information about the structure of the fat depot of difference loci and revelation of different types of adipocytes in them, especially in young individuals. Studies of functional and morphological properties of adipose tissue are especially important to extend geography of pig husbandry and prevent neonatal mortality. Therefore, it was interesting to study adipose tissue from pigs of domestic cold-resistant breeds (by the example of the Kemerovo breed) at the initial (juvenile) stage of the postembryonal development under conditions of raising in the cold season to reveal BeAT localization.

Objects and methods

Samples of adipose tissue of different localization from three-week-old Kemerovo piglets (n=3) kept under free-range conditions (private farm, Kemerovo) were studied. The samples included backfat (B), lateral (L), axillary fat (A), and internal perirenal fat (P) (Figure 2). The number of samples from one localization was no less than ten.

Animals were slaughtered according to the conventional techniques by cutting the jugular vein and the following bleeding. All manipulations were carried out according to the Directive 2010/63/EU of the European Parliament and of the Council [22], European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS No. 123) [23], Recommendations for euthanasia of experimental animals: Part 1. Laboratory Animals (1996) 30, Recommendations

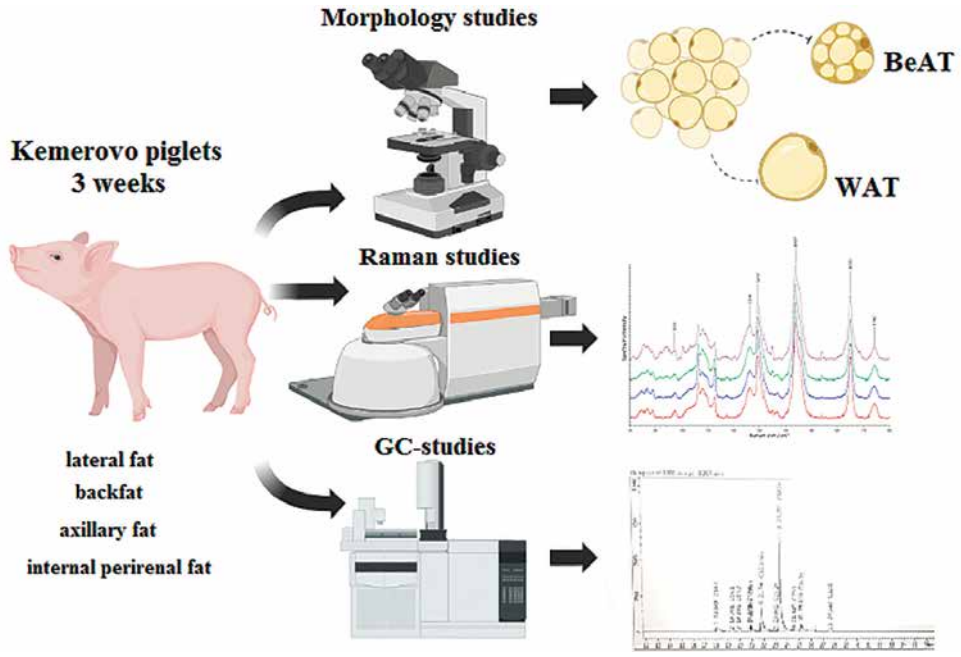

Figure 2. Graphic illustration of the performed study

for euthanasia of experimental animals: Part 2. Laboratory Animals (1997) 31, 1–32), as well as requirements of GOST 33215–2014[1]

Histological studies

To study morphology of adipose tissue, samples were fixed in the 10% neutral buffered formalin solution (BioVitrum, Russia) for 72 hour at room temperature, washed with cold running water for four hours and embedded in gelatin (AppliChem GMBH, Germany) in an ascending concentration (12.5%, 25%) at a temperature of 37 °C for 8 hours each using a thermostat TS-1/20 SPU (Smolensk SKTB-SPU, Russia). Sections with a thickness of 12 μm were made on the cryostat «MIKROM–HM525» (Thermo Scientific, USA). The obtained sections were mounted on Menzel-Glaser slides (Thermo Scientific, USA) and stained with Ehrlich's hematoxylin and 1% aqueous-alcoholic solution of eosin (BioVitrum, Russia) by the conventional method [24]. The histological preparations were studied using an Axio Imager A1 light microscope (Carl Zeiss, Germany).

To count a diameter of adipocytes, the modified method [25] was applied: sections with a thickness of 12 μm were made from the samples fixed in formalin, mounted on slides, put in a droplet of the physiological solution under a cover glass and immediately analyzed using the image analysis system AxioVision 4.7.1.0 (Carl Zeiss, Germany). No less than three sections were made from each piece. A diameter of adipocytes was measured for 100 cells in each section in the interactive mode with an accuracy of ± 0.1 μm.

Raman spectroscopy

Raman spectroscopy was used to assess the overall fatty acid profile of tested samples [26]. Collection of spectra was carried out on the confocal Raman dispersive spectrometer Renishaw (model inVia Reflex, Renishaw plc, Wotton-under-Edge, UK). A laser with a wavelength of 785 nm, power of 100 mW and exposure time of 10s was used. Power of laser radiation and time of integration were thoroughly optimized to prevent photodegradation of fat samples. Calibration of the spectrometer was performed by registration of the silicon spectrum at 520 cm^{-1}. Raman spectra were obtained directly from samples of adipose tissue with a size of 10 × 10 × 5 mm. For laser focusing, an objective with a magnification of L50× was used. Measurements were recorded in a detection range of 700–1800 cm^{-1}. No less than six spectra were recorded for each sample.

All collected spectra were subjected to preliminary treatment (cosmic ray removal, baseline correction, smoothing using the algorithm of Savitzky-Golay, normalization) and analysis using the software Renishaw WiRE 5.2 (Renishaw plc, Wotton-under-Edge, UK).

Gas chromatography

For chromatographic analysis, a sample was rendered on an IKA Hot Plate C–MAG HP 7 (IKA®-Werke GmbH & Co. KG, Germany). To obtain fatty acid methyl esters, rendered fat was taken in a quantity of 200 μl and transferred into a 15 ml centrifuge tube, 2 ml of 2M potassium hydroxide solution in methanol was added with the following addition of 4 ml of hexane; centrifugation was carried out at 3,000–5,000 rpm for 1–3 min. After centrifugation, 200 μl were taken from the upper hexane layer and transferred into a chromatographic vial; 800 μl of pure hexane was added to dilute concentrations of fatty acids. The obtained sample was analyzed on a gas chromatograph Agilent

[1] GOST 33215–2014 "Guidelines for accommodation and care of animals. Environment, housing and management" Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200127789 Accessed September 16, 2022

7890 (Agilent Technologies, Inc., USA) with a flame ionization detector using a capillary column Agilent HP 5 30 m × 0.32 mm × 0.25 μm (carrier gas: nitrogen) (Agilent Technologies, Inc., USA).

Statistical processing of results

Statistical data analysis was carried out using a package STATISTICA, version 10.0 (StatSoft, Inc., USA). The results of morphometry were presented as root mean square (S), root-mean-square (standard) deviation (±SD), minimum and maximum values [MIN MAX] of the interquartile range (P 25/75). For analysis of Raman spectra, the geometric mean (*GeoMean*) was used. Differences were considered significant and a relationship between parameters was acknowledged at a probability level of not higher than 0.05.

Results and discussion

In histological examination of samples taken from adipose tissue of different localization, it was found that two cell types are present in the samples of subcutaneous B, L and A fat: 1 — round, with one large fat droplet and flattened nucleus shifted to periphery, that correspond to white adipocytes by structure and 2 — having a polygonal shape and several fat droplets with different sizes (from 2 to 15 μm in diameter) corresponding to beige adipocytes by their structure [5] (Figures 3A, 3B). Connective tissue interlayers with the large number of fibrous elements were located between adipocytes. In the internal perirenal fat, cells were characterized by more round shape; the presence of beige adipocytes was not revealed. However, according to data [9] their formation in this locus was noticed in the cold-resistant Tibetan and Min pig breeds.

It is known that subcutaneous WAT is especially prone to the BeAT development in the periods of adaptive thermogenesis [8]. Lin et al. [9] found beige adipocytes in white adipose tissue of pigs exposed to cold. In our research, samples of adipose tissue were taken from piglets kept under free-range conditions during winter in the natural temperature conditions without additional heating, which led to the development of BeAT in WAT. Probably, this is linked with the fact that the mechanism of UCP3-dependent thermogenesis in beige adipocytes has been evolutionally developed in cold-resistant pigs [8,10].

As known, on early life stages, porcine adipose tissue grows mainly due to hyperplasia (an increase in the number) of adipocytes [6,7]. After a significant increase in the cell number, adipocytes begin to increase in size (hypertrophy) due to accumulation of triglycerides [27]. In our samples, an average diameter of adipocytes was as follows: 69.36 ± 12.98 μm in backfat, 53.25 ± 9.27 in lateral, 45.94 ± 8.29 in axillary fat, 35.69 ± 6.96 μm in internal perirenal fat. Our results agree with the results of other authors regarding sizes of adipocytes in piglets. According to [19], an average diameter of adipocytes in backfat in three-week-old piglets of the Landrace bacon breed was 53.4 ± 3.6 μm and of the lard-type Meishan breed 58.7 ± 7.4 μm.

It was established that a size of adipocytes of subcutaneous fat changes depending on the location on the animal body — larger cells were revealed in backfat fat samples compared to lateral and axillary fat (Figure 3C). It is linked with the most intensive development of fat in this locus. A wide range of adipose cell sizes and the presence of "small" adipocytes also noticed in other pig breeds [19] allow suggesting that both cell hyperplasia and hypertrophy are still active. Adipose tissue in all studied loci is in the intensive growth period and there is the potential for the following filling of cells with lipids and increase in the thickness of subcutaneous fat. A proportion of "small" adipocytes was 18–20%.

Adipose tissue in pigs is the main place of lipid synthesis, i. e. lipogenesis, during which adipocytes synthesize and accumulate triglycerides and provide no less than 80% of deposited fatty acids [6,28]. Today, quick and non-destructive methods for assessing overall fatty acid profile that allow performing analysis directly in production are in demand. We used Raman spectroscopy, which belongs to such screening methods [29].

Raman spectra of pork fat are presented by signals conditioned by vibrations of hydrocarbon chains in saturated and unsaturated structures [30]. The main peaks responsible for unsaturated bonds were 970 cm^{-1} (=C-H out-of-plane bend), 1266/1272 cm^{-1} (=C-H symmetric rock) and 1655 cm^{-1} (C=C stretching); while signals at 1297/1301 cm^{-1} (CH_2 twisting), 1430/1460 cm^{-1} (CH_2 symmetric deformation (scissoring)) and 1735/1746 cm^{-1} (C=O stretching) correspond to saturated bonds or ester groups [31,32]. The obtained spectra of adipose tissue samples are presented in Figure 1D. Differences between samples in the intensity of the main signals were revealed. To determine the relative content of the unsaturated bonds, an intensity ratio of the main signals corresponding to unsaturated bonds, and signals of saturated bonds was calculated in each sample. For calculation, nine intensity ratios were used: I_{970}/I_{1297}, I_{970}/I_{1430}, I_{970}/I_{1735}, I_{1266}/I_{1297}, I_{1266}/I_{1430}, I_{1266}/I_{1735}, I_{1655}/I_{1297}, I_{1655}/I_{1430}, I_{1655}/I_{1735} (Table 1).

Table 1. Relative content of unsaturated bonds in the tested adipose tissue samples

Intensity (I) ratio of Raman signals	Lateral fat	Backfat	Axillary fat	Internal perirenal fat
I_{970}/I_{1297}	0.130821	0.112026	0.159802	0.090597
I_{970}/I_{1430}	0.077807	0.069489	0.092581	0.062879
I_{970}/I_{1735}	0.546442	0. 75685	0.722015	0.471012
I_{1266}/I_{1297}	0.55442	0.501723	0.580229	0.40954
I_{1266}/I_{1430}	0.3333	0.311286	0.338914	0.284375
I_{1266}/I_{1735}	2.329259	2.1305	2.635744	2.129262
I_{1655}/I_{1297}	1.098959	1.03814	1.068285	0.82681
I_{1655}/I_{1430}	0.659668	0.644123	0.626257	0.573635
I_{1655}/I_{1735}	4.613256	4.408478	4.863001	4.29427
GeoMean	0.584857	0.535684	0.639995	0.480101

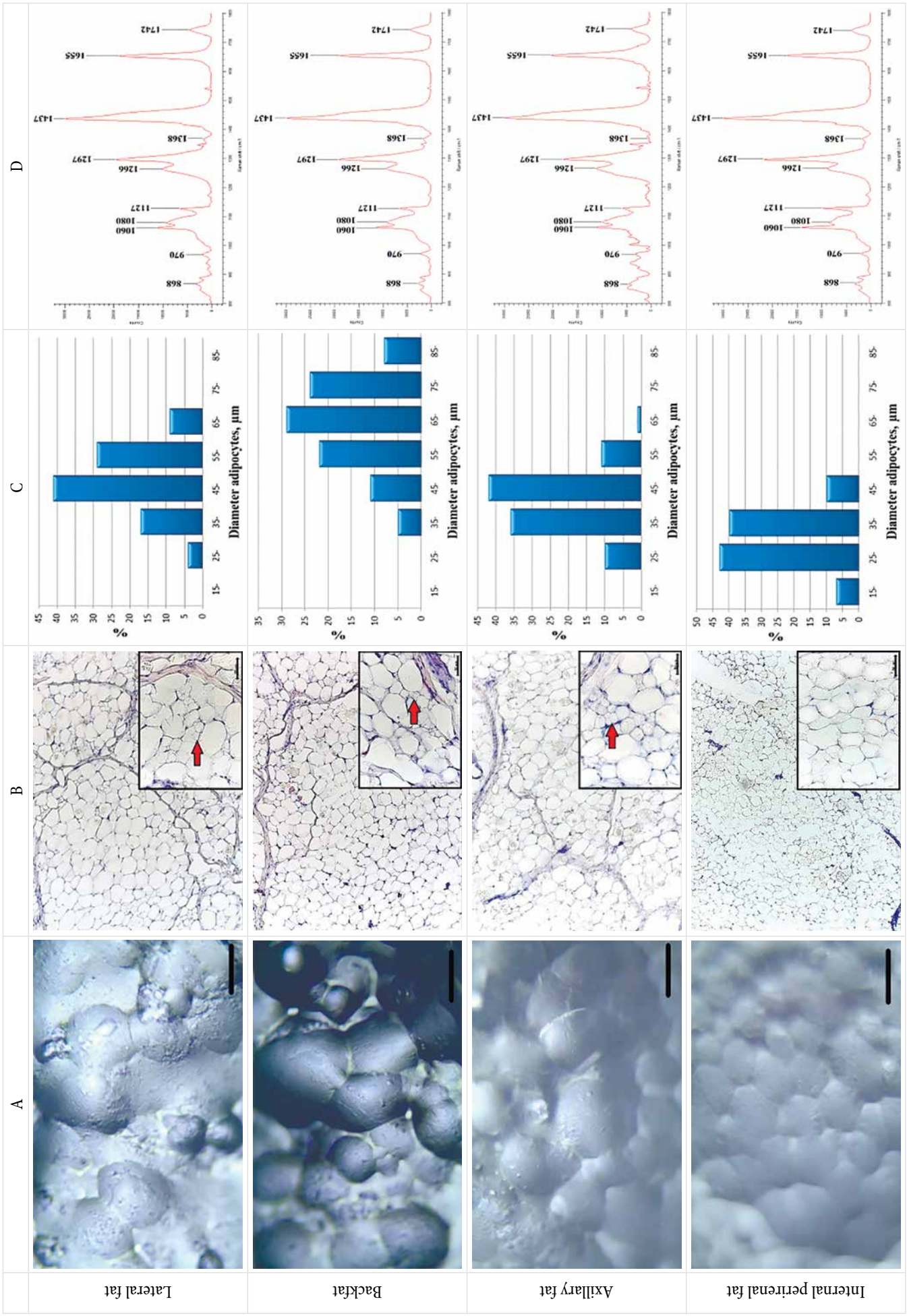

Figure 3. Effect of localization on characteristics of adipose tissue of Kemerovo piglets: a. Morphology of samples without staining (Scale bar — 50 μm); B — Morphology of samples after staining with hematoxylin and eosin (Scale bar — 50 μm). The arrow indicates beige adipocytes; C — Distribution of adipocytes by diameter; D — Raman spectra of the samples

The average intensity of peaks responsible for unsaturated bonds was lower in internal perirenal fat than in subcutaneous fat and was equal to 0.480101. This indicates that it contains lower amounts of unsaturated fatty acids and higher amounts of saturated fatty acids. Among samples of subcutaneous adipose tissue, backfat contained more saturated bonds compared to lateral and axillary fat. The highest average intensity of peaks responsible for unsaturated bonds (0.639995) was in axillary fat. As the difference between certain fatty acids resides in the length of carbon chains as well as the number and position of double bonds, they have similar Raman spectra [33,34]. Therefore, a problem of overlapping peaks from different fatty acids arises in analysis of adipose tissue samples [35]. In this connection, detection of certain fatty acids presents difficulties.

By the results of gas chromatographic analysis of the fatty acid composition, ΣSFA in all samples did not show significant differences and was 35.161 in internal perirenal fat and 35.144 in subcutaneous fat. The main detected saturated fatty acids were palmitic, stearic, margaric and myristic acids. The sum of UFA, the most important of which were oleic, palmitoleic, linoleic and linolenic acids, was 60.923 in the samples of internal perirenal fat and 61.095 in backfat. With that, the highest amounts of polyunsaturated fatty acids were found in the samples of subcutaneous fat: 20.199 in backfat, 21.749 in lateral and 20.436 in axillary fat compared to 18.636 in internal perirenal fat.

It is known that the fatty acid composition depends on fat localization in the animal body [7]. The results on the fatty acid composition in the fatty tissue samples of different localization that we have obtained are in agreement with the results of other authors. It is shown in [36] that the unsaturated fatty acid content in pigs changes according to the gradient: the highest content is in the external layer of subcutaneous adipose tissue, where less mature adipocytes are located followed by the internal layer, intermuscular and internal fat, where adipocytes are more mature. According to the opinion of Jiang et al. [37], this fact is linked with a higher Δ-9-desaturase activity index in external fat depots compared to internal and replacement of stearic fatty acid with oleic in them. Lee et al. [38] obtained similar results for three steer breeds and concluded that mature fat is more saturated than immature; therefore, a degree of unsaturation can be an index of its maturity.

Conclusion

The Kemerovo breed of pigs was selected in Siberia and, consequently, is adapted to local cold climate conditions. Histological analysis of adipose tissue of different localization taken from three-week-old piglets exposed to cold showed the presence of two types of adipocytes in subcutaneous fat (backfat, lateral and axillary): white having one large fat droplet and beige having several fat droplets with various sizes (multilocular). Larger cells were revealed in backfat samples compared to lateral and axillary fat. Only white adipocytes were found in the internal perirenal fat. In comparison of the fatty acid profile in the samples by the results of Raman spectroscopy, a larger quantity of saturated bonds was noticed in internal fat, while in subcutaneous fat, especially axillary, a larger quantity of unsaturated bonds was found. This finding was confirmed by the results of gas chromatography. Polyunsaturated fatty acids, linoleic and linolenic, dominated in the samples of subcutaneous fat.

Activation of beige cells in Kemerovo pigs on exposure to cold can play an important role in the heat balance allowing them to tolerate cold without severe shivering. Studying the development of beige adipocytes as well as mechanisms and factors inducing it can be of great importance for increasing thermogenesis in newborn piglets by genome editing. Beige adipogenesis in cold-resistant pigs opens a possibility of changing energy metabolism and improving quality of porcine adipose tissue.

REFERENCES

1. Ali, A. T., Hochfeld, W. E., Myburgh, R., Pepper, M. S. (2013). Adipocyte and adipogenesis. *European Journal of Cell Biology*, 92(6–7), 229–236. https://doi.org/10.1016/j.ejcb.2013.06.001
2. Cheng, L., Wang, J., Dai, H., Duan, Y., An, Y., Shi, L. et al. (2021). Brown and beige adipose tissue: a novel therapeutic strategy for obesity and type 2 diabetes mellitus. *Adipocyte*, 10(1), 48–65. https://doi.org/10.1080/21623945.2020.1870060
3. Chu, D.-T., Gawronska-Kozak, B. (2017). Brown and brite adipocytes: Same function, but different origin and response. *Biochimie*, 138, 102–105. https://doi.org/10.1016/j.biochi.2017.04.017
4. Pilkington, A.-C., Paz, H.A., Wankhade, U.D. (2021). Beige adipose tissue identification and marker specificity — Overview. *Frontiers in Endocrinology*, 12, Article 599134. https://doi.org/10.3389/fendo.2021.599134
5. Cohen, P., Kajimura, S. (2021). The cellular and functional complexity of thermogenic fat. *Nature Reviews Molecular Cell Biology*, 22(6), 393–409. https://doi.org/10.1038/s41580–021–00350–0
6. Poklukar, K., Čandek-Potokar, M., Lukač, N.B., Tomažin, U., Škrlep, M. (2020). Lipid deposition and metabolism in local and modern pig breeds: A review. *Animals,* 10(3), Article 424. https://doi.org/10.3390/ani10030424
7. Monziols, M., Bonneau, M., Davenel, A., Kouba, M. (2007). Comparison of the lipid content and fatty acid composition of intermuscular and subcutaneous adipose tissues in pig carcasses. *Meat Science*, 76(1), 54–60. https://doi.org/10.1016/j.meatsci.2006.10.013
8. Chernukha, I.M., Fedulova, L.V., Kotenkova, E.A. (2022). White, beige and brown adipose tissue: structure, function, specific features and possibility formation and divergence in pigs. *Foods and Raw Materials*, 10(1), 10–18. https://doi.org/10.21603/2308–4057–2022–1–10–18
9. Lin, J., Cao, C, Tao, C., Ye, R., Dong, M., Zheng, Q. et al. (2017). Cold adaptation in pigs depends on UCP3 in beige adipocytes. *Journal of Molecular Cell Biology*, 9(5), 364–375. https://doi.org/10.1093/jmcb/mjx018
10. Zhao, J., Tao, C., Chen, C., Wang, Y., Liu, T. (2021). Formation of thermogenic adipocytes: What we have learned from pigs. *Fundamental Research*, 1(4), 495–502. https://doi.org/10.1016/j.fmre.2021.05.004
11. Kharzinova, V.R., Zhuchaev, K.V., Kostyunina, O.V., Kochneva, M.L., Chydym, S.M., Zinovieva, N.A. (2017). Molecular-genetic identification and certification of Kemerovo breed of pigs based on STR- analysis. *Achievements of Science and Technology of the Agro-Industrial Complex,* 31(6), 62–64. (In Russian)
12. Comparative table for pig breeds. Meatinfo Retrieved from: https://meatinfo.ru/info/show?id=58. Accessed September 20, 2022 (In Russian)
13. Bekenev, V.A., Arishin, A.A., Mager, S.N., Bolshakova, I.V., Tretyakova, N.L., Kashtanova, E.V. at al. (2021). Lipid profile of pig tissues contrasting in meat production. *The Natural Products Journal*, 11(1), 108–118. https://doi.org/10.2174/2210315509666191203124902
14. Bekenev, V.A., Deeva, V.S., Arishin, A.A., Chernukha, I.M., Botan, I.V., Tretyakova, N.L. (2016). Usage of pig breeding bioresources in quality of pork. *Vestnik NSAU (Novosibirsk State Agrarian University)*, 3(40), 176–184 (In Russian)
15. Serra, X., Gil, F., Pérez-Enciso, M., Oliver, M.A., Vázquez, J.M., Gispert, M. et al. (1998). A comparison of carcass, meat quality and histochemical characteristics of Iberian (Guadyerbas line) and Landrace pigs. *Livestock Production Science*, 56(3), 215–223. https://doi.org/10.1016/S0301–6226(98)00151–1
16. Renaudeau, D., Mourot, J. (2007). A comparison of carcass and meat quality characteristics of Creole and Large White pigs slaughtered at 90kg BW. *Meat Science*, 76(1), 165–171. https://doi.org/10.1016/j.meatsci.2006.10.024
17. Alfonso, L., Mourot, J., Insausti, K., Mendizabal, J. A., Arana, A. (2005). Comparative description of growth, fat deposition, carcass and meat quality characteristics of Basque and Large White pigs. *Animal Research*, 54(1) 33–42. https://doi.org/10.1051/animres:2005001
18. Lebret, B., Dourmad, J.Y., Mourot, J., Pollet, P.Y., Gondret, F. (2014). Production performance, carcass composition, and adipose tissue traits of heavy pigs: Influence of breed and production system. *Journal of Animal Science,* 92(8), 3543–3556. https://doi.org/10.2527/jas.2013–7398
19. Nakajima, I., Oe, M., Ojima, K., Muroya, S., Shibata, M., Chikuni, K. (2011). Cellularity of developing subcutaneous adipose tissue in Landrace and Meishan pigs: Adipocyte size differences between two breeds. *Animal Science Journal*, 82(1), 144–149. https://doi.org/10.1111/j.1740–0929.2010.00810.x
20. Kojima, M., Nakajima, I., Arakawa, A., Mikawa, S., Matsumoto, T., Uenishi, H. et al. (2018). Differences in gene expression profiles for subcutaneous adipose, liver, and skeletal muscle tissues between Meishan and Landrace pigs with different backfat thicknesses. *PloS ONE*, 13(9), Article e0204135. https://doi.org/10.1371/journal.pone.0204135
21. Zhang, D., Wu, W., Huang, X., Xu, K., Zheng, C., Zhang, J. (2021). Comparative analysis of gene expression profiles in differentiated subcutaneous adipocytes between Jiaxing Black and Large White pigs. *BMC Genomics*, 22(1), Article 61. https://doi.org/10.1186/s12864–020–07361–9
22. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union, L 276/33 Retrieved from https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ: L:2010:276:0033:0079: en: PDF Accessed September 16, 2022
23. European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. European Treaty Series — No. 123. Retrieved from https://rm.coe.int/168007a67b Accessed September 16, 2022
24. Romeis B. (1989). Microscopic technique. München: Urban und Schwarzenberg, 1989. (In German)
25. Velotto, S., Claudia, V., Crasto, A. (2012). Muscle fibre types, fat deposition and fatty acid profile of Casertana versus Large White pig. *Animal Science Papers and Reports*, 30(1), 35–44.
26. Lee, J.-Y., Park, J.-H., Mun, H., Shim, W.-B., Lim, S.-H., Kim, M.-G. (2018). Quantitative analysis of lard in animal fat mixture using visible Raman spectroscopy. *Food Chemistry*, 254, 109–114. https://doi.org/10.1016/j.foodchem.2018.01.185
27. Louveau, I., Perruchot, M.-H., Bonnet, M., Gondret, F. (2016). Invited review: Pre- and postnatal adipose tissue development in farm animals: From stem cells to adipocyte physiology. *Animal,* 10(11), 1839–1847. https://doi.org/10.1017/S1751731116000872
28. Kloareg, M., Noblet, J., van Milgen, J. (2007). Deposition of dietary fatty acids, de novo synthesis and anatomical partitioning of fatty acids in finishing pigs. *British Journal of Nutrition,* 97(1), 35–44. https://doi.org/10.1017/S0007114507205793
29. Pchelkina, V.A., Chernukha, I.M., Fedulova, L.V., Ilyin, N.A. (2022). Raman spectroscopic techniques for meat analysis: A review. *Theory and Practice of Meat Processing*, 7(2), 97–111. https://doi.org/10.21323/2414–438X-2022–7–2–97–111
30. Beattie, J.R., Bell, S.E.J, Borgaard, C., Fearon, A., Moss, B.W. (2006). Prediction of adipose tissue composition using Raman spectroscopy: Average properties and individual fatty acids. *Lipids*, 41(3), 287–294. https://doi.org/10.1007/s11745–006–5099–1
31. Berhe, D.T., Eskildsen, C.E., Lametsch, R., Hviid, M.S., van den Berg, F., Engelsen, S.B. (2016). Prediction of total fatty acid parameters and individual fatty acids in pork backfat using Raman spectroscopy and chemometrics: Understanding the cage of covariance between highly correlated fat parameters. *Meat Science*, 111, 18–26. https://doi.org/10.1016/j.meatsci.2015.08.009
32. Saleem, M., Amin, A., Irfan, M. (2021). Raman spectroscopy based characterization of cow, goat and buffalo fats. *Journal of Food Science and Technology*, 58(1), 234–243. https://doi.org/10.1007/s13197–020–04535-x
33. Czamara, K., Majzner, K., Pacia, M., Kochan, K., Kaczor, A., Baranska, M. (2014). Raman spectroscopy of lipids: A review. *Journal of Raman Spectroscopy*, 46, 4–20. https://doi.org/10.1002/jrs.4607
34. De Gelder, J., De Gussem, K., Vandenabeele, P., Moens, L. (2007). Reference database of Raman spectra of biological molecules. *Journal of Raman Spectroscopy*, 38, 1133–1147. https://doi.org/10.1002/jrs.1734
35. Afseth, N.K., Dankel, K., Andersen, P.V., Difford, G.F., Horn, S.S., Sonesson, A. et al. (2022). Raman and near Infrared Spectroscopy for quantification of fatty acids in muscle tissue –A salmon case study. *Foods*, 11(7), Article 962. https://doi.org/10.3390/foods11070962
36. de Tonnac, A., Guillevic, M., Mourot, J. (2018). Fatty acid composition of several muscles and adipose tissues of pigs fed n-3 PUFA rich diets. *Meat Science*, 140, 1–8. https://doi.org/10.1016/j.meatsci.2017.11.023
37. Jiang, Q., Li, C., Yu, Y., Xing, Y., Xiao, D., Zhang, B. (2018). Comparison of fatty acid profile of three adipose tissues in Ningxiang pigs. *Animal Nutrition*, 4(3), 256–259. https://doi.org/10.1016/j.aninu.2018.05.006
38. Lee, J.-H., Yamamoto, I., Jeong, J.-S., Nade, T., Arai, T., Kimura, N. (2011). Relationship between adipose maturity and fatty acid composition in various adipose tissues of Japanese Black, Holstein and Crossbred (F1) steers. *Animal Science Journal*, 82, 689–697. https://doi.org/10.1111/j.1740–0929.2011.00893.x

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THE WAYS TO IMPROVE THE BIOLOGICAL AND MORPHOLOGICAL PARAMETERS OF YOUNG FATTENING PIGS

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Abstract
Studies were carried out to find the new ways to improve the efficiency of iodine use for the young pigs. During the scientific and economic experiment it was found that bringing iodine to the physiological norm, in combination with introduction of 3% bentonite clay into the diets of young fattening pigs, provided a positive effect on the morphological composition of carcasses, slaughter parameters and meat quality of the pigs. So fattening the yelts with the diets with a physiological norm of iodine in combination with 3% bentonite clay allowed young pigs of the 3rd experimental group to reach a slaughter weight of 78.95 kg at the age of 8 months, which is 10.1% more than in the control group, and 4.2% and 2.5% more in comparison with the 1st and 2nd experimental groups. The slaughter yield in yelts of the 3rd experimental group increased by 2.43% (P < 0.05) compared to the control group. The largest (30.17 cm²) area of the "rib eye" was recorded in the 3rd experimental group, which is 5.60% more than the control one, while the carcasses of pigs of the 3rd experimental group contained muscle tissue by 4.74% (P < 0.05) more than the same in the control group. Analysis of the Musculus longissimus showed a significant increase in protein content in the 3rd experimental group by 1.22% compared to the control group (P < 0.05). The protein-quality parameter was significantly higher in the muscle tissue of young pigs of the 3rd experimental group and amounted to 10.90 (P < 0.05), which is 1.12% higher than in the muscle tissue of the control group yelts. The maximum content of oleic acid was found in the lard of animals of the 3rd experimental group — 49.59, which is 1.28% (P < 0.05) higher than in the control group.

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Introduction

Protection of the national interests is becoming a national priority in terms of increasing international competition and the globalization of the world economy. This is defined in the new Strategy of Economic Security of the Russian Federation for the period up to 2030. In the current conditions, while the country is exposed to the sanctions policy of the Western countries, food security issues are the basis of national security and have acquired the special relevance [1–4].

The modern strategy of the state agrarian policy is to increase the volume of food products and enhance the efficiency of cattle husbandry [5, 6]. Swine breeding in Russia is the leading sphere of this industry in terms of increasing the volume of meat production. In 2020, farms of all categories produced 4,286.7 thousand tons of pork in slaughter weight. The main growth in production is provided by the large agricultural enterprises, which share accounts for 89.5% of the total volume of meat production. In 2020, the volume of pork imports amounted to 8.41 thousand tons, which indicates the achievement of full self-sufficiency in the national domestic market for this type of meat. By 2025 pork production in Russia presumable shall reach 6 million tons in live weight, which is than 500 thousand tons more than in 2020. The main goal of the industry is to increase domestic product consumption, increase competitiveness in the world market and improve the quality of the products [5, 7–9].

To assess the state of food security in the Russian Federation, we have taken into account the dynamics of meat consumption per capita. So, in 2021 in Russia, this figure reached 77 kg. This figure was achieved by a steady increase in consumers' demand for pork. It should be noted that over the past three years, pork has had a very significant impact on the dynamics of total meat consumption, in particular, the average pork consumption in 2021 reached 28.3 kg/person [10].

Efficient production of livestock products, and pig breeding in particular, is possible only with the application of scientifically based systems and methods of conducting the industry, first of all, full-fledged feeding that satisfies the needs of animals in all nutrients, including minerals [11, 12]. One of the most efficient methods is the introduction of high-energy feed additives and enzyme preparations into the diet [13, 14]. Ivanov S. A. found that the productivity of sows gets higher under the conditions of chelate compounds consumption [15]. It was found that the use of silicon-containing zeolite in the compound feed in an amount of 3% by weight contributes significantly to an increase in the dynamics of live weight gain and improvement of the metabolic processes of pigs [16]. A group of authors revealed the efficiency of bacterial strains *Bifidobacterium Teenagelis*, *Lactobacillus acidophilus* and oak bark extract as biologically active substances, added in the diet. The feed additive contributed to an increase in the antioxidant activity of the animal organism and the antimicrobial components of blood plasma [17]. Probiotics and phytobiotics show their efficiency too [18, 19]. Thus, the application of yeast probiotic supplements positively affects the content of erythrocytes, total protein, calcium and phosphorus in the blood of animals, as well as the number of lactobacteria and bifidobacteria in the intestine, increases live weight and increases rate of multiple pregnancy [20, 21]. Studies were conducted on the effect of including cellulose into the pigs feed in the amount of 1% and 2% of the total mass [22]. Pigs are most sensitive animals to the level of minerals in their diet. Among the vital elements of micro-mineral nutrition is iodine, which biological role in the animal body is exceptionally high and which is closely related to the function of thyroid gland, to the synthesis and metabolism of thyroid hormones, which in their turn regulate many physiological functions [23, 24].

Studies on the etiology of endemic goiter (hypothyroidism) in our country and abroad have shown that iodine does not act simply on its own. Its effect on the body does not follow a plain formula: iodine deficiency brings on endemic goiter, but it acts in rather difficult conditions, when other (additional) factors play an important role in the emergence, spread and strength of endemia [25]. In case of insufficient intake of iodine in the body of an animal, the processes of growth and development are violated; reproductive functions and productivity are reduced, though iodine excess in the diet leads to violation of the functional activity of the thyroid gland [26].

The role of iodine is especially great in those geographical areas where a deficiency of this trace element in soils, feed, and water is observed. Therefore, the use of iodine preparations in the diets of pigs is not only desirable, but quite necessary. Currently, in the practice of feeding farm animals, various methods of replenishing iodine deficiency are used [27–29]. Thus, studies have shown that the use of selenium and iodine preparations in combination with a probiotic for feeding the young pigs ensures optimal secretory activity of the thyroid gland [30]. The use of the preparation "Ioddar" provides a positive effect on the increase in the animals live weight [31].

Almost the entire preparations introduction is reduced to oral administration; however, the use of inorganic iodine salts is sometimes inefficient due to the high volatility of the element being added. In addition, when administered orally, iodine-containing drugs are exposed to an acidic environment in the gastrointestinal tract and are converted into indigestible forms. In this regard, it is necessary to find new ways to increase the efficiency of iodine digestion by young pigs. In our opinion, the binding, sorption and ion-exchange properties of bentonite clays can be used for this task.

The purpose of the research is to study the effect of potassium iodide and bentonite clay on the slaughter parameters and meat qualities of the young fattening pigs.

Materials and Methods

Ethical statement

This study is an integral part of scientific research conducted at the Department of Technology of Storage and Processing of Animal Products of the Federal State Budgetary Educational Institution of Higher Education "Kurgan State Agricultural Academy named after T. S. Maltsev" (state registration number AAAA-A16–116020210398–1).

Animals, Study Design

In order to accomplish the goal of research at the educational and scientific base of the Federal State Budgetary Educational Institution of Higher Education Kurgan State Agricultural Academy, a scientific and economic experiment was run on young white pigs. 4 groups of 4-month-old piglets were formed, 8 heads each. The animals were selected for the groups according to the principle of homogeneity, taking into account their age, live weight and origin.

Animal management was the same for all the pigs, and met the sanitary and hygienic and zootechnical requirements (GOST 28839–2017[1]). All animals were clinically healthy and were under the supervision of a veterinarian.

The conditions of the pigs feeding and managing were the same too. Feeding rations for young pigs were normalized taking into account the chemical composition and nutritional value of the feed based on the norms recommended by the Russian Academy of Sciences [32]. The only difference in feeding was that the yelts of the control group received the main diet with a natural level of iodine, consisting at the age of 4–6 months of the following (% by weight): barley — 50.5, wheat — 20, peas — 15, sunflower meal — 6, fodder yeast — 3, meat and bone meal — 3, fluorine-free phosphate — 2, table salt — 0.5; at the age of 7–8 months as follows: barley — 64.5, wheat — 15, peas —

[1] GOST 28839–2017 "Agricultural animals. Pigs. Zootechnical requirements for fattening". Moscow: Standartinform, 2017. Retrieved from https://docs.cntd.ru/document/1200146267 Accessed June 20, 2022. (In Russian)

11, sunflower meal — 3, fodder yeast — 3, meat and bone meal — 2, fluorine-free phosphate — 2, table salt — 0.5. The pigs of the 1st experimental group, in addition to the main diet, got potassium iodide with bringing the level of iodine to the physiological norm. The 2nd experimental groups got 97% of the main diet and 3% bentonite clay by weight of the feed. The 3rd experimental groups got 97% of the main diet, 3% bentonite and potassium iodide, with bringing iodine up to physiological norm.

Data collection

At the end of the scientific and economic experiment on fattening the pigs, part of them were transferred to control slaughter in order to determine the meat productivity (meat yield) of young pigs (3 pigs in each group) according to generally accepted methods. The slaughter parameters of pigs were determined in accordance with GOST 31476–2012[2] and GOST 31778–2012[3].

The slaughter yield was determined by the ratio of the slaughter weight to the pre-slaughter weight (slaughter weight is the weight of the carcass with the head, legs, internal fat, without liver and intestines; pre-slaughter weight is the weight of a live pig after 12 hours starvation).

The length of the carcass was measured from the anterior edge of the first cervical vertebra to the anterior edge of the pubic bone fusion. The thickness of the fat was determined over the 6–7th thoracic vertebra.

"Rib eye" parameters were determined as the area of the transverse section of the longest muscle of the back between the thoracic and lumbar regions (along the last rib). The weight of the rear third of the half-carcass was determined on the right half-carcass by a cut between the last and penultimate sacral vertebrae.

The composition of individual natural anatomical parts and the carcass as a whole was established by deboning the right side of the carcass, which had been preliminary chilled for 24 hours at a temperature of –2 to +4 °C. Based on the deboning, the absolute and relative content of bone and muscle tissue, as well as subcutaneous fat, was determined.

For chemical analysis and qualitative characteristics of the meat, a sample was taken from the *musculus longissimus* in the area of 10–13 thoracic and 1–2 lumbar vertebrae, bacon was sampled at the level of 6–7 ribs.

Laboratory studies were carried out on the basis of an accredited testing laboratory "Veles" of the individual entrepreneur Iltyakova D. V. (Chostoozerye village, Kurgan region, Russia) and in the laboratories of the Department of Storage and Processing Technologies of Livestock Products of the Kurgan State Agricultural Academy named after T. S. Maltsev (Lesnikovo village, Kurgan region, Russia). Chemical and biochemical parameters of the muscle tissue quality were established on the basis of these analyzes: moisture content was determined according to GOST 33319–2015[4] — by drying the sample to a constant weight at a temperature of 103 ± 2 °C; fat content was determined according to GOST 23042–2015[5] — by extracting a dry sample with ether in a Soclet apparatus; protein content was determined according to GOST 25011–2017[6] — by the method of determining total nitrogen according to Kjeldahl; mineral substances (ash) content was determined according to GOST 31727–2012[7] — by dry mineralization of the samples in a muffle furnace at a temperature of 450–600 °C.

The amino acid composition of the *musculus longissimus* was determined on the LC-20 *Prominence* device (Shimadzu, Japan) according to the method M-02–902–142–07 "Method for measuring the mass fraction of amino acids by high-performance liquid chromatography".

The fatty acid composition of bacon was analyzed on a gas chromatograph device "Crystal-2000M" (LLC NPF "Meta-chrome", Russia) in the laboratory of the Kurgan branch of the Federal Budgetary Institution "Tyumen Center for Standardization and Metrology" (Kurgan region, Kurgan, Russia) according to GOST R55483–2013[8].

The content of iodine in feed was determined according to the method developed by A. M. Bulgakov. The technique of determination consists in preparing the material for its ashing (grinding, mixing with potash, drying at 105–110 °C, ashing at 500–550 °C) followed by extraction, filtration, evaporation, and drying of the precipitate [33].

The growth intensity of fattened young pigs was controlled by monthly weighing, and based on the obtained data the average daily gains in live weight of pigs were calculated.

Statistical analysis

Scales for weighing carcasses and deboned meat; tape measures and rulers for measuring carcass length, other dimensions, fat thickness, rib eye area, etc. were supplied under a contract with LLC "Pribor-Service" (Tyumen,

[2] GOST 31476–2012 "Pigs for slaughter. Pork carcasses and semi-carcasses. Specifications". Moscow: Standartinform, 2013. Retrieved from https://docs.cntd.ru/document/1200095684 Accessed September 5, 2022. (In Russian)

[3] GOST 31778–2012 "Meat. Cutting pork into cuts. Specifications". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200096913 Accessed September 5, 2022. (In Russian)

[4] GOST 33319–2015 "Meat and meat products. Method for determining the mass fraction of moisture". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200123927 Accessed September 12, 2022. (In Russian)

[5] GOST 23042–2015 "Meat and meat products. Methods for determining fats". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200133107 Accessed September 12, 2022. (In Russian)

[6] GOST 25011–2017 "Meat and meat products. Protein determination methods". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200146783 Accessed September 12, 2022. (In Russian)

[7] GOST 31727–2012 "Meat and meat products. Method for determining the mass fraction of total ash". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200098742 Accessed September 12, 2022. (In Russian)

[8] GOST R55483–2013 "Meat and meat products. Determination of fatty acid composition by gas chromatography". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200103852 Accessed September 12, 2022. (In Russian)

Russia). During the research, measuring instruments were used that were verified and calibrated at the Kurgan branch of the Federal Budgetary Institution "Tyumen Center for Standardization and Metrology" (Kurgan Region, Kurgan, Russia).

The obtained digital data were processed by variation statistics methods. The obtained digital data were statistically processed using a computer with an Intel Core2 Quad processor (USA), licensed software package Microsoft Office 2007 (USA). Student's t-test was used to assess the significance of differences between the two means. Differences were considered statistically significant at $P<0.05$; $P<0.01$; $P<0.001$.

Results and discussion

Feeding provides a great influence on the quantitative and qualitative parameters of the meat productivity of pigs. The final stage of the technological process of fattening pigs is the slaughter of animals. Conducting a control slaughter of animals allows establishing the features of the development of the main tissues, internal organs, their chemical composition, and makes it possible to calculate the conversion of feed nutrients into the meat products. Data on the yield of the main slaughter products are presented in Table 1.

An analysis of the data of the control slaughter of animals indicates that the live weight of yelts before slaughter after starvation in the experimental groups significantly exceeded the weight of pigs in the control group, respectively, in the 1st experimental group by 2.94% ($P<0.05$), in the 2nd experimental group by 4.23% ($P<0.05$) and in the 3rd experimental group by 6.63% ($P<0.01$).

The animals of the 3rd experimental group featured the highest slaughter weight (78.95 kg), which is 10.1% more than in the control group, 4.2% and 2.5% more than the 1st experimental and 2nd experimental groups. Head weight in all groups was almost the same. In the 3rd experimental group the weight of the legs and skin exceeded the weight of the same parts in the control group by 7.9 and 20.8%, respectively. The mass of kidney fat of pigs in the 1st and 2nd experimental groups was almost the same, while in the 3rd experimental group it was 1.57 kg only, which is 0.23 kg less than in the control group. The mass of the hot carcass in the control group was 55.73 kg, in the 1st experimental group it was 3.9 kg more, and in the 2nd and 3rd groups it exceeded the weight of pigs from the control group by 5.17 and 6.4 kg, respectively.

An important parameter characterizing the slaughter parameters of fattened pigs is the slaughter yield, which exceeded by 1.96 and 2.3%, respectively, the yield in the 1st in the 2nd experimental groups in comparison with the control group. The animals of the 3rd experimental group featured the highest (75.07%) slaughter yield (2.43% more than in the control group).

The introduction of potassium iodide into the diet in combination with bentonite contributed to the increase and improvement of the meat qualities of pigs. The animals of the 3rd experimental group had the greatest carcass length, which exceeded the control group by 2.16% (Table 2).

The meatiness of pigs carcasses can be judged quite accurately by the thickness of the fat. The fat thickness over the 6th-7th thoracic vertebrae was the highest in the control group and amounted to 33.03 mm, in the 1st and 2nd experimental groups — 32.30 and 31.40 mm, which is more than in the 3rd experimental group, respectively, by 8.04; 5.66 and 2.71%.

Table 1. Yield of the main slaughter products ($\bar{X} \pm S\bar{x}$)

Parameter	Group			
	Control	1st experimental	2nd experimental	3rd experimental
Live weight before slaughter after starvation, kg	98.63±0.65	101.53±0.46*	102.80±0.38*	105.17±0.50**
Slaughter weight (carcass weight with skin, head, legs and kidney fat), kg	71.65±0.81	75.75±0.91*	77.05±0.94*	78.95±0.74**
Including weight of, kg:				
Head	5.57±0.28	5.43±0.14	5.55±0.19	5.82±0.17
Skin	6.97±0.22	7.05±0.13	7.10±0.16	7.52±0.14
Legs	1.58±0.07	1.93±0.13	1.75±0.12	1.91±0.11
Kidney fat	1.80±0.12	1.71±0.13	1.75±0.12	1.57±0.05
Hot carcass	55.73±1.07	59.63±0.92*	60.90±0.67*	62.13±0.43**
Slaughter yield, %	72.64±0.40	74.60±0.56*	74.94±0.64*	75.07±0.37*

* P<0,05; ** P<0,01

Table 2. Parameters of the pork qualities ($\bar{X} \pm S\bar{x}$)

Parameter	Group			
	Control	1st experimental	2nd experimental	3rd experimental
Carcass length, cm	95.73±0.71	96.93±0.73	97.07±0.95	97.80±0.81
The thickness of the fat over the 6th-7th thoracic vertebra, mm	33.03±0.67	32.30±0.81	31.40±0.61	30.57±0.43*
The area of the "rib eye", cm^2	28.57±0.35	29.07±0.59	29.20±0.74	30.17±0.43*
Weight of rear third of half-carcass, kg	10.27±0.38	11.77±0.35*	11.90±0.36*	12.10±0.32*

* P < 0,05

The area of the "rib eye" increases most intensively in the first 5–6 months (especially within the period from the birth till the age of three months), and the area of fat over the "rib eye" begins to grow intensively from four months. The area of the "rib eye" was significantly larger in the pigs of the 3rd experimental group — 30.17 cm2 ($P < 0.05$), which was more than in the control group, the 1st and 2nd experimental groups by 5.60%, 3.78 and 3.32% respectively. The most valuable part of the pork half carcass is the rear third. Therefore, the quality of the carcass itself depends significantly on its mass and morphological composition. This parameter had significant differences. The yelts of the 3rd experimental group featured the largest weight of the rear third. In this group, compared with animals of the control group, of the 1st and 2nd experimental groups, the weight of the rear third of the half carcass was more by 17.82%, 2.8 and 1.68%, respectively.

The quality of pork depends on numerous factors, among which the most significant are genetic heredity and method of feeding. High-quality and rational feeding can influence not only on the weight and size of the animal, but also the ratio of tissues in its body.

The performed deboning of half-carcasses of pigs of the control group and experimental groups made it possible to define the absolute and relative amount of the main tissues of the body. Deboning results are shown below in Table 3.

Along with an increase in pre-slaughter weight, the ratio of meat, fat and bones undergoes major changes: the share of muscle tissue decreases when yelts are slaughtered at 60 kg from 66.22% down to 55.92% when yelts are slaughtered at 140 kg. The proportion of bones in the carcass does not change (it does not exceed 1%). Weighing before deboning the chilled carcasses showed a significant difference in all experimental groups. Thus, the weight of a carcass in the 1st experimental group exceeded the weight of a carcass from the control group by 3.97% ($P < 0.05$), the 2nd experimental group — by 5.57% ($P < 0.05$), the 3rd experimental group — by 7, 27% ($P < 0.01$). The analysis of soft tissues separated from bones showed that the content of muscle tissue in the carcasses of yelts from the control group was 30.36 kg, and in the 2nd, 3rd experimental groups this value was significantly higher — by 4.05 kg ($P < 0.05$) and 4.74 kg ($P < 0.05$), respectively. The carcasses of the experimental groups featured high percentage of fat and exceeded the control group: in the 1st experimental group by 0.91%, in the 2nd experimental group by 1.18%, in the 3rd experimental group by 2.01%. There were no significant differences between the groups in terms of the amount of bone tissue in the carcasses, although a tendency to increasing this parameter in the experimental groups was recorded.

The growth and development of an animal, the quantitative content of the main tissues in its body largely depends on the development of the main parenchymal organs that ran the metabolism in the body. The mass of internal organs represent the rate of the animal development. While the control slaughter, we took into account the absolute mass of the main organs, the results are presented below in Table 4.

The chemical composition of the muscle tissue of the body is greatly influenced by the full-fledged feeding of pigs, the provision of the diet with basic nutrients, while the balance of macro- and microelements has a significant impact.

Animals that received iodine in combination with bentonite had heavier internal organs than the animals in the control group.

The lungs of all slaughtered animals were pink, without pathology, with well-defined lobes. The alveolar tissue was well developed. The weight of the lungs in the experimental groups did not have a significant difference, but exceeded the control one: in the 1st experimental group the lungs were heavier by 1.12%; in the 2nd experimental — by 1.98%; in the 3rd experimental group — by 4.73%.

The heart of the pigs had dense texture, the color was dark red. The state of the endocardium and valves was normal. The mass of the heart of the animals of the experi-

Table 3. Composition of pig carcasses ($\bar{X} \pm S\bar{x}$)

Group	Weight of chilled carcass, kg	Meat		Fat		Bones	
		kg	%	kg	%	kg	%
Control group	54.10 ± 1.00	30.36 ± 0.96	56.10 ± 0.81	15.67 ± 0.26	28.40 ± 0.73	8.07 ± 0.48	14.92 ± 0.90
1st experimental group	58.07 ± 0.84*	32.75 ± 0.25	56.42 ± 0.60	16.53 ± 0.43	29.31 ± 0.98	8.79 ± 0.66	15.11 ± 0.92
2nd experimental group	59.67 ± 0.99*	34.41 ± 0.70*	56.89 ± 0.83	16.56 ± 0.44	29.58 ± 0.48	8.70 ± 1.19	14.52 ± 1.74
3rd experimental group	61.37 ± 0.41**	35.10 ± 0.64*	57.18 ± 0.67	17.38 ± 0.59	30.41 ± 1.09	8.89 ± 0.61	14.49 ± 1.05

*P < 0,05; **P < 0,01

Table 4. Weight of internal organs of the yelts, g ($\bar{X} \pm S\bar{x}$)

Parameter	Group			
	Control	1st experimental	2nd experimental	3rd experimental
Lungs	1163.67 ± 114.64	1176.67 ± 111.44	1186.67 ± 90.89	1218.67 ± 89.91
Heart	350.67 ± 40.13	349.33 ± 33.27	351.67 ± 32.64	358.33 ± 27.79
Liver	1468.67 ± 38.91	1552.00 ± 54.59	1591.33 ± 69.74	1628.67 ± 38.88*
Kidneys	328.33 ± 48.36	330.67 ± 24.70	323.33 ± 46.82	338.67 ± 41.58
Spleen	151.67 ± 9.06	156.33 ± 8.37	158.67 ± 3.48	168.33 ± 13.97

* P < 0,05

mental groups was almost the same and did not significantly exceed the control group.

The liver of all animals was dark red, the capsule was shiny, smooth, of dense texture, the edges of the liver were sharp. The weight of the liver was significantly higher in the animals of the 3rd experimental group and exceeded the weight of the liver of the control yelts by 160 g, or 10.89%.

The kidneys were dark brown in color, and had dense texture. The maximum mass of this organ was observed in the animals of the 3rd experimental group — 338.67 g, and the minimum in yelts of the 2nd experimental group — 323.33 g. The difference between them amounted to 15.34 g, or 4.74%.

The spleen of the animals was dark red. Lymph nodes had no visible differences. This organ did not have a significant difference and was larger in animals of the 3rd experimental group by 10.98% in comparison with the control group.

Thus in the animals, in whose diets iodine was brought to the physiological norm, the main internal organs were better developed, which ensured the enhancement of redox processes in the body of the pigs and the function of hematopoiesis.

To assess the meat qualities of pigs, the chemical composition of the longest back muscle was analysed (Table 5).

Table 5. Chemical composition of the *Musculus longissimus*, % ($\bar{X} \pm S\bar{x}$)

Parameter	Group			
	Control	1st experimental	2nd experimental	3rd experimental
Dry matter	27.63±0.43	27.72±0.39	28.14±0.60	28.26±0.33
Protein	18.35±0.34	19.12±0.25	19.24±0.54	19.57±0.27*
Fat	6.46±0.38	6.54±0.32	6.38±0.25	6.52±0.47
Ash	1.06±0.10	1.14±0.12	1.17±0.08	1.19±0.12

* P < 0,05

The chemical composition of the *musculus longissimus* showed that the dry matter content in all experimental animals was practically the same. The amount of protein in the muscle tissue was significantly higher in the 3rd experimental group by 1.22% (P < 0.05) in comparison with the same of the control group.

Fat tissue is desirable in a certain ratio to muscle tissue, since with a high fat content the relative amount of proteins in meat decreases and the digestibility of meat decreases also. The content of fat in muscle tissue in all groups was almost the same and amounted to 6.46%, 6.54, 6.38, 6.52%, respectively. The ash content of muscle tissue varied from 1.06% in the control group to 1.19% in the 3rd experimental group.

Considering that we studied the mineral supplement in the form of potassium iodide and bentonite, the question arises about its effect on the content of macro- and microelements in the muscle tissue of animals. The content of the main macronutrients in the *musculus longissimus* is presented below in the Table 6.

Table 6. The content of macronutrients in the muscle tissue of pigs, g/kg ($\bar{X} \pm S\bar{x}$)

Parameter	Group			
	Control	1st experimental	2nd experimental	3rd experimental
Calcium	0.19±0.02	0.22±0.06	0.26±0.04	0.29±0.02*
Phosphorus	1.90±0.10	2.03±0.18	2.10±0.12	2.23±0.09
Potassium	3.60±0.27	3.86±0.12	4.20±0.14	4.22±0.14
Sodium	0.42±0.01	0.43±0.02	0.43±0.03	0.46±0.05
Magnesium	0.26±0.02	0.27±0.02	0.31±0.02	0.33±0.03

* P < 0,05

It should be noted that the calcium content in the muscle tissue of yelts in the 1st experimental group was 0.03%, in the 2nd experimental group by 0.07% and in the 3rd experimental group by 0.10% (P < 0.05) more, in comparison with the control group. The amount of phosphorus in the muscle tissue of yelts of the 1st experimental group is 0.13% higher than in the control group, respectively, in the 2nd experimental group by 0.20% and in the 3rd experimental group by 0.33%, compared with the control group. The maximum (4.22 g/kg) content of potassium was recorded in the muscle tissue of young pigs of the 3rd experimental group, which is 0.62% more than in the control group. The content of sodium and magnesium in the muscle tissue of the experimental animals did not differ significantly, although there was a tendency for increase of this parameter in the experimental groups.

The content of the main trace elements in the *musculus longissimus* is presented below in the Table 7.

Table 7. The content of trace elements in the muscle tissue of pigs, mg/kg ($\bar{X} \pm S\bar{x}$)

Parameter	Group			
	Control	1st experimental	2nd experimental	3rd experimental
Iron	10.73±1.63	11.50±1.25	13.17±1.19	16.40±2.55
Manganese	0.23±0.03	0.23±0.03	0.23±0.03	0.27±0.07
Copper	0.13±0.03	0.13±0.03	0.17±0.07	0.17±0.03
Zinc	14.47±0.39	14.53±0.48	15.90±0.32*	17.32±0.46**

* P < 0,05; ** P < 0,01

The micro-mineral composition showed that the content of iron in the muscle tissue of pigs in the 1st experimental group was 0.77% higher, in the 2nd experimental group it was higher by 2.44% and in the 3rd experimental group it was higher by 5.67%, in comparison with the control group. The content of manganese in the muscle tissue of the 3rd experimental group was 0.27 mg/kg, which was 0.04% more than in the control group. Copper content in all groups was almost the same. The muscle tissue of the yelts of the control group contained 14.47 mg/kg of zinc, which was 0.06% less than in the 1st experimental group, by 1.43% (P < 0.05) in comparison with the 2nd experimental group, and by 2.85% (P < 0.01) than in the 3rd experimental group.

Amino acids are a whole class of organic compounds, represented in nature by more than 20 species with a range of properties. The main function of amino acids is their

participation in the structure of proteins. Amino acids, absorbed into the blood from the intestines, are then transported to the cells of the body.

The table 8 below shows the content of amino acids in the muscle tissue of pigs.

Table 8. The content of amino acids in the muscle tissue of pigs, % ($\bar{X} \pm S\bar{x}$)

Parameter	Group			
	Control	1st experimental	2nd experimental	3rd experimental
Isoleucine	0.99±0.05	1.04±0.04	1.03±0.01	1.04±0.04
Threonine	0.91±0.03	0.88±0.01	0.91±0.03	0.94±0.04
Serene	0.75±0.02	0.73±0.00	0.74±0.01	0.77±0.03
Glycine	0.79±0.02	0.76±0.00	0.78±0.01	0.81±0.03
Alanine	1.38±0.04	1.33±0.01	1.35±0.03	1.41±0.06
Valine	1.21±0.04	1.17±0.01	1.19±0.02	1.25±0.06
Methionine	0.51±0.02	0.49±0.00	0.51±0.01	0.54±0.03
Leucine	1.41±0.08	1.49±0.06	1.46±0.01	1.47±0.05
Glutamine	3.95±0.12	3.82±0.03	3.88±0.07	4.06±0.17
Proline	0.56±0.03	0.53±0.01	0.54±0.02	0.59±0.05
Phenyl-alanine	0.75±0.03	0.72±0.01	0.74±0.02	0.77±0.03
Lysine	1.78±0.06	1.71±0.01	1.74±0.04	1.83±0.09
Arginine	1.23±0.04	1.20±0.02	1.21±0.02	1.25±0.05
Tryptophan	0.30±0.01	0.32±.01	0.32±0.01	0.33±0.02
Oxyproline	0.031±0.001	0.030±0.001	0.031±0.001	0.030±0.002
БКП	9.78±0.11	10.80±0.42	10.44±0.30	10.90±0.33*

* P < 0,05

From the data in the table above it can be seen that in the composition of the muscle tissue of the 3rd experimental group, the largest share of such amino acids belongs to methionine, lysine, tryptophan, which exceeded the control group by 0.03%, 0.05, 0.03%, respectively. In the muscle tissue of pigs, the protein-quality index exceeded the control group: in the 1st experimental group by 1.02, in the 2nd experimental — by 0.66 and in the 3rd experimental — by 1.12%.

The biological role of lysine, methionine, tryptophan, leucine, isoleucine, threonine, phenylalanine, histidine, valine and sometimes arginine is determined by the fact that they are included in all the most important proteins of the animal body, but are neither synthesized in the body, nor they can be replaced by other amino acids. Lack of one or more of the 10 essential amino acids in the diet adversely affects the condition of animals: the young animals stop growing and developing, while adult animals lose weight, eat food reluctantly, and are susceptible to various diseases. This is explained by the fact that with a lack of essential amino acids in the body, proteins of cells, blood and lymph cannot be synthesized, as these amino acids necessarily participate there.

There was no significant difference in the content of amino acids in the muscle tissue of yelts. However, the content of isoleucine, trionine and valine was maximal in the animals of the 3rd experimental group and exceeded the control by 0.05, 0.03 and 0.04%, respectively. In terms of the amount of methionine, the muscle of the 3rd experimental group exceeded the control group, the 2nd experimental group by 0.03%, and the 1st experimental group by 0.05%. The greatest amount of lysine was found in the muscle tissue of the 3rd experimental group. The control group featured less content of lysine by 0.05% than the 3rd experimental group, less than in the 1st experimental group — by 0.12% and in the 2nd experimental group — by 0.09%.

The chemical composition of fat is influenced by breed, age and degree of fatness. The main part of fat tissue is fats. Sometimes fats make up to 98% of its mass. Unlike other tissues, there is little water and protein in fat tissue. Table 9 below shows the chemical composition of pork fat.

Table 9. Chemical composition of fat (%), $\bar{X} \pm S\bar{x}$

Parameter	Group			
	Control	1st experimental	2nd experimental	3rd experimental
Dry matter	92.67±0.26	93.11±0.19	93.41±0.20	93.57±0.17*
Protein	1.45±0.05	1.65±0.08	1.63±0.04	1.77±0.10*
Fat	90.93±0.18	91.17±0.19	91.45±0.12	91.45±0.16
Ash	0.28±0.03	0.29±0.02	0.33±0.05	0.35±0.06

* P < 0,05

The data in the table above shows, that the pig fat of animals of the 3rd experimental group contained significantly more dry matter, fat and ash, respectively, by 0.90, 0.32, 0.07%, than in the control group. The greatest amount of protein was found in the fat of the 2nd and 3rd experimental groups, and exceeded the control group by 0.52%. The biological value of fat is determined by the content of polyunsaturated fatty acids, which are not synthesized in the human body, but play an important role in the physiological and metabolic processes of the body.

Pork is not only a source of complete protein, but also source of fatty acids that are not synthesized in the human body. The fatty acid composition of yelts' fat is presented below in the Table 10.

Table 10. Fatty acids composition of pork fat (%), $\bar{X} \pm S\bar{x}$

Acid	Group			
	Control	1st experimental	2nd experimental	3rd experimental
Lauric	1.53±0.03	1.55±0.01	1.58±0.02	1.60±0.02
Palmitic	14.04±0.73	14.27±1.03	14.86±1.11	15.93±0.65
Palmitoleic	18.18±0.47	18.89±0.60	18.82±0.70	18.92±0.49
Stearic	16.33±0.11	16.54±0.25	16.41±0.05	16.63±0.19
Oleic	48.31±0.32	49.04±0.63	48.70±0.30	49.59±0.27*
Linoleic	19.03±0.47	20.37±0.60	20.53±0.49	20.65±0.87

* P < 0,05

The data in the table above shows that the content of lauric acid in the yelts' fat in all groups was practically at the same level. The back fat of yelts of the control group contained 14.04% of palmitic acid, which is 0.23% less than in the 1st experimental group, by 0.82% less than in the 2nd experimental group and by 0.89% less than in the

3rd experimental group. The maximum content of oleic acid was found in the fat of animals of the 3rd experimental group — 49.59%, which is 1.28% ($P < 0.05$) more than in the control group. The content of linoleic acid in the fat of yelts of the 1st experimental group was 1.34% higher than in the control group. In the 2nd and 3rd experimental groups the content of linoleic acid was higher by 1.50% and 1.62%, respectively than in the control group.

Conclusion

Summarizing the results of the studies, we can conclude that bringing iodine to the physiological norm in combination with addition of 3% of bentonite clay into the diets of young fattening pigs provided a positive effect on the morphological composition of carcasses, slaughter parameters and meat qualities of the pigs.

So feeding the yelts with a physiological norm of iodine in combination with 3% bentonite clay allowed the young pigs of the 3rd experimental group to reach a slaughter weight of 78.95 kg at the age of 8 months, which is 10.1% more than in control group and 4.2% and 2.5% in comparison with the 1st and 2nd experimental groups. The slaughter yield in yelts of the 3rd experimental group increased by 2.43% ($P < 0.05$) compared to the control group. The largest (30.17 cm2) area of the "rib eye" was observed in the 3rd experimental group, which is 5.60% more than the control group, while the carcasses of pigs from the 3rd experimental group contained muscle tissue by 4.74% ($P < 0.05$) more than the same of the control group.

Analysis of the *musculus longissimus* showed a significant increase in protein content in the 3rd experimental group by 1.22% compared to the control group ($P < 0.05$). The protein-quality parameter was significantly higher in the muscle tissue of young pigs of the 3rd experimental group and amounted to 10.90 ($P < 0.05$), which is 1.12% higher than in the muscle tissue of the yelts from the control group. The maximum content of oleic acid was observed in the fat of animals of the 3rd experimental group. It amounted to 49.59, which is 1.28% ($P < 0.05$) more than in the control group.

REFERENCES

1. Minakov, A.V., Lapina, S.B. (2021). Ensuring the economic security of the state in the context of western sanctions. *Vestnik of Economic Security*, 2, 305–312. https://doi.org/10.24412/2414-3995-2021-2-305-312 (In Russian)
2. Barikaeva, A.F. (2019). Improvement of certain elements of the risk management system of food security in Russia. *Vestnik of Moscow University of the Ministry of Internal Affairs of Russia,* 1, 218–221. https://doi.org/10.24411/2073-0454-2019-10052 (In Russian)
3. Zhilina, E.V., Khunafina, E.A., Kuzyashev, A.N. (2020). Analysis and forecasting of the consumer market of meat and meat products in the Russian Federation. *Economics and Management: Research and Practice Journal*, 3(153), 41–47. https://doi.org/10.34773/EU.2020.3.8 (In Russian)
4. Tikhomirov, A. I. (2021). Development of pig breeding in Russia under the food embargo: Modern challenges and opportunities. *Pig Breeding*, 1, 4–7. https://doi.org/10.37925/0039-713X-2021-1-4-7 (In Russian)
5. Plaksin, I.E., Plaksin, S.I., Trifanov, A.V. (2022). Trends and prospects of pig breeding development in Russia. *AgroEcoEngineering*, 1(110), 155–168. https://doi.org/10.24412/2713-2641-2022-1110-155-168 (In Russian)
6. Butolina, E. A. (2021). *Pig production development trends.* In the collection: Youth science 2021: technology, innovation. Materials of the All-Russian scientific and practical conference of young scientists, graduate students and students, dedicated to the Year of Science and Technology in the Russian Federation. Perm, 275–277. (In Russian)
7. Minakov, I. A., Kuvshinov, V. A. (2021). Development of pig breeding in Russia: Trends and performance. *Science and Education*, 4(1), Article 254. (In Russian)
8. Demakova, E.A. (2021). Forecast for the development of the pig production market in Russia. *Economy, Labor, Management in Agriculture,* 7(76), 3–11. (In Russian)
9. Kovalev, Yu. I. (2021). *Pig breeding in Russia: Current situation and development prospects until 2025.* Proceedings of the All-Russian Research Institute of Experimental Veterinary Medicine. Ya. R. Kovalenko, 82, 324–328. (In Russian)
10. Khayrullina, O.I (2021). Trends in the production and consumption of the main types of meat in Russia. *Creative Economy*, 15(5), 2245–2260. https://doi.org/10.18334/ce.15.5.112098 (In Russian)
11. Frame, C. A., Huff-Lonergan, E., Kerr, B. J., Serao, M. R. (2021). Feeding oxidized chicken byproduct meal impacts digestibility more than performance and oxidative status in nursery pigs. *Journal of Animal Science,* 99(2). Article skab029. https://doi.org/10.1093/jas/skab029
12. Ellner, C., Martínez-Vallespín, B., Saliu, E.-M., Zentek, J., Röhe, I. (2021). Effects of cereal and protein source on performance, apparent ileal protein digestibility and intestinal characteristics in weaner piglets. *Archives of Animal Nutrition,* 75(4), 263–277. https://doi.org/10.1080/1745039X.2021.1958647
13. Morozova, L., Mikolaychik, I., Rebezov, M., Fedoseeva, N., Derkho, M., Safronov, S. et al. (2020). Improving the physiological and biochemical status of high-yielding cows through complete feeding. *International Journal of Pharmaceutical Research*, 12, 2181–2190. https://doi.org/10.31838/ijpr/2020.SP1.319
14. Mikhailova, L. R., Lavrentiev, A. Yu. (2022). *The use of an enzyme preparation in feeding young pigs.* In the collection: Scientific and educational environment as the basis for the development of the intellectual potential of agriculture in the regions of Russia. Materials of the II International Scientific and Practical Conference. Cheboksary, 276–278. (In Russian)
15. Ivanov, S.A. (2014). Effect of feed composition with chelated trace elements on productivity of sows. *Collection of Scientific Papers of the Stavropol Scientific Research Institute of Animal Husbandry and Feed Production*, 3(7), 323–326. (In Russian)
16. Mikhaylova, L.R., Zhestyanova, L.V., Lavrentiev, A. Yu., Sherne, V.S. (2021). Application of natural zeolites in mixed feeds of young pigs. *Agrarian Science*, 3, 43–47. https://doi.org/10.32634/0869-8155-2021-346-3-43-47 (In Russian)
17. Duskaev, G.K., Rakhmatullin, S.G., Kazachkova, N.M., Sheida, Y.V., Mikolaychik, I.N., Morozova, L.A. (2018). Effect of the combined action of *Quercus cortex* extract and probiotic substances on the immunity and productivity of broiler chickens. *Veterinary World*, 11(10), 1416–1422. https://doi.org/10.14202/vetworld.2018.1416-1422
18. Belookov, A.A., Belookova, O.V., Chukhutin, E.V., Gorelik, O.V. (2022). The efficiency of probiotics in industrial pig breeding. *Agrarian Science*, 7–8, 98–101. https://doi.org/10.32634/0869-8155-2022-361-7-8-98-101
19. Bagno, O. A., Prokhorov, O. N., Shevchenko, S. A., Shevchenko, A. I., Dyadichkina, T. V. (2018). Use of phytobioticts in farm animal feeding. *Sel'Skokhozyaistvennaya Biologiya,* 53(4), 687–697. https://doi.org/10.15389/agrobiology.2018.4.687eng
20. 20. Mikolaychik, I.N., Morozova, L.A., Kahikalo, V.G., Ovchinnikova, L. Yu., Yarmots, L.P., Karmatskikh, Yu.A. et al. (2020). Microbiological supplements for the metabolic rate correction in calves. *International Transaction Journal of Engineering, Management and Applied Sciences and Technologies*, 11(1), Article 11A02S. https://doi.org/10.14456/ITJEMAST.2020.39
21. Belookov, A.A., Belookova, O.V., Chukhutin, E.V. (2021). Experience in the use of probiotic drug in pig breeding. *Feeding*

of Agricultural Animals and Feed Production, 9(194), 45–50. https://doi.org/10.33920/sel-05–2109–04 (In Russian)
22. Cho, H. M., Kim, E., Wickramasuriya, S. S., Shin, T. K., Heo, J. M. (2021). Growth and gut performance of young pigs in response to different dietary cellulose concentration and rearing condition. *Animal Bioscience,* 34(10), 1653–1662. https://doi.org/10.5713/AB.20.0721
23. Mikolaychik, I.N., Morozova, L.A., Iltyakov, A.V. (2015). New in the production of environmentally safe pork. *Head of Animal Breeding,* 2, 21–28. (In Russian)
24. Chabaev, M., Nekrasov, R., Nadeev, V. (2013). Organic trace elements for industrial pig farming. *Compound Feed*, 6, 77–79. (In Russian)
25. Andersson, M., Karumbunathan, V. Zimmcrmann, M.B. (2012). Global iodine status in 2011 and trends over the past decade. *Journal of Nutrition*. (142), 744–750 https://doi.org/10.3945/jn.111.149393
26. Lazarus, J. (2014). Iodine status in Europe in 2014. *European Thyroid Journal*, 3, 3–6. https://doi.org/10.1159/000358873
27. Gerasimov, G.A., Haar, F., Lazarus, J.H. (2017). Overiew of iodine deficiency prevention strategies in the South-Eastern Europe and Central Asia Region: 2009–2016. *Clinical and Experimental Thyroidology*, 13(4), 16–22. https://doi.org/10.14341.ket9531
28. Haldimann, M. Alt A., Blanc, A., Bloundeau, K. (2005). Iodine content of food groups. *Journal of Food Composition and Analysis*, 18(6), 461–471. https://doi.org//10.1016/j.jfca.2004.06.003
29. Zimmcrmann, MB., Andersson, M. (2012). Assessment of iodine nutrition in populations: past, present, and future. *Nutrition Reviews*, 70(10), 553–570. https://doi.org//10.1111/j.1753–4887 2012.00528.x
30. Rassolov, S.N., Voroshilin, R.A. (2019). Dynamics of thyroid hormones in pigs when immunonutrients are introduced into their diet. *Bulletin of the Russian Academy of Natural Sciences. West Siberian Branch,* 22, 120–127. (In Russian)
31. Ryzhova, D.D. (2019). Veterinary and sanitary evaluation of mutton after using biologically active feed additive "Yoddar". *International Research Journal,* 5(83), 115–118. https://doi.org/10.23670/IRJ.2019.83.5.023 (In Russian)
32. Nekrasov, R. V., Golovin, A. V., Makhaev, E. A., Anikin, A. S., Pervov, N. G., Strekozov, N. I. et al. (2018). Nutrient requirements for dairy cattle and pigs. Moscow: FGBNU FNTs VIZH im. L. K. Ernst, 2018. (In Russian)
33. Bulgakov, A. M. (2005). Improved method for the determination of iodine in animal feed and tissues. *Zootechnics,* 8, 16. (In Russian)

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MUTAGENIC AND/OR CARCINOGENIC COMPOUNDS IN MEAT AND MEAT PRODUCTS: POLYCYCLIC AROMATIC HYDROCARBONS PERSPECTIVE

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Keywords: *meat, thermal processes, polycyclic aromatic hydrocarbons, process parameters*

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants posing a great risk to human health due to their mutagenic and/or carcinogenic properties. They are produced from incomplete combustion of a heat source, pyrolysis of organic components, and fat-induced flame formation. Meat and meat products are one of the major sources of PAH exposure. Since PAH intake increases the risk of cancer, understanding the factors affecting PAH formation in meat and meat products is very important within the scope of PAH exposure reduction strategies. In this study, the features and formation of PAHs, the factors affecting the formation of PAH compounds and their reduction/inhibition pathways were reviewed in order to provide a perspective on the presence of PAHs in meat and meat products.

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of chemicals that contain 2–7 aromatic rings. PAHs are non-polar compounds of lipophilic nature [1,2,3]. They are formed through incomplete combustion or pyrolysis of organic matter [4]. Epidemiological studies have shown that PAH compounds can negatively affect the organism by interfering with the normal functioning of the cellular membrane and the enzyme system [5]. Storelli et al. [6] reported that PAHs bind to DNA and cause mutations that initiate the carcinogenic process. PAHs are considered common environmental pollutants due to their long-range transport and bioaccumulation [7]. Industrial emissions, agricultural resources, air, water, soil and foodstuffs are the main sources of PAHs [5]. However, it is reported that the most important PAH exposure source for humans is foodstuffs [7] and foodstuffs are responsible for approximately 88–98% of PAH pollution [8].

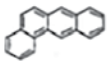
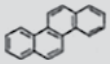
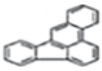
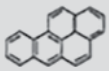
Early studies of PAHs focused on the analysis of industrial sources of PAHs. However, further studies have shown that environmental PAHs can be transferred to food such as fruits and vegetables, seafood, oils, etc [9,10]. On the other hand, food production processes based on high temperature application such as baking, frying and smoking also cause high levels of PAH formation [11,12]. Pyrolysis and oxidation of fat, protein and carbohydrates are induced during high temperature application, which can result in the production of high levels of PAHs. In addition, fat dripping onto the flame and returning to the meat as smoke can also lead to PAH production [2]. Considering that meat with its rich protein and fat content is cooked by various methods before consumption, it is understood that cooked meat and meat products play an important role in PAH exposure through food. Especially, thermal processes such as smoking, grilling, barbecue, frying, roasting can cause high levels of PAH formation in meat due to high temperature, pyrolysis and intense smoke generation [8]. Indeed, it has been reported that meat and meat products contain high concentrations of PAH and are important sources for PAH exposure through foodstuffs [13,14].

Therefore, it is essential to understand the properties and occurrence of PAHs and the factors that influence PAH formation in order to develop strategies reducing exposure to PAHs, which have adverse health effects. The present review focuses on the features and formation of PAHs, the factors affecting the formation of PAH compounds in cooked meat and meat products and their reduction/inhibition pathways.

The structure, toxicity and health hazards of PAHs

PAH compounds exhibit toxic, mutagenic, and carcinogenic effects on humans [15]. They are easily absorbed by the body and can participate in metabolism due to their lipophilic nature [16]. In addition, PAHs can cause toxicity to organisms by interfering with the normal function of biological cell membranes and membrane-associated enzyme systems [17,18]. To date, approximately 160 different PAH compounds have been identified in nature and authorities such as the European Commission Scientific Committee on Food and the United States Environmental

Table 1. Some characteristics of individual PAHs used as markers in the assessment of PAH contamination

Compound name	Abbreviation	Molecule structure	Chemical formula	Molecular weight	LARC classification*	Genotoxity
Benz(a)anthracene	BaA		$C_{18}H_{12}$	228.3	2B	Positive
Chrysene	Chry		$C_{18}H_{12}$	228.3	2B	Positive
Benzo(b)fluoranthene	BbF		$C_{20}H_{12}$	252.3	2B	Positive
Benzo(a)pyrene	BaP		$C_{20}H_{12}$	252.3	1	Positive

* Carcinogenic; 2B: Possibly carcinogenic

Protection Agency have determined 16 of these compounds as priority contaminants, considering their mutagenic and carcinogenic properties [3,14]. Benzo[a]pyrene (BaP) is classified as a Group 1 carcinogen, while the majority of other high molecular weight PAHs are largely classified in Group 2A (probably carcinogenic to humans) or Group 2B (possibly carcinogenic to humans) [19] (Table 1).

Laboratory studies have revealed that prolonged exposure to PAH compounds causes lung cancer by inhalation, gastric cancer by ingestion with food, and skin cancer by contact, in animals [20,21]. Daniel et al. [22] declared that the risk of renal cell carcinoma increases with grilled meat consumption. Alomirah et al. [23] reported that the cancer risk associated with consumption of foods of animal origin by children/adolescents and adults is $2.63/10^7$ and $9.3/10^7$ BaP equivalents, respectively.

Several regulations have been declared to protect consumers against PAH intake from diet due to the negative effects of PAHs on health [24]. In this context, Benzo(a) pyrene (BaP) and ∑PAH4 [BaP, Chrysene (Chry), Benz[a] anthracene (BaA) and Benzo[b]fluoranthene (BbF)] have been used as markers for the PAH contamination in food [25]. The legal limits of BaP and ∑PAH4 were determined as 5 μg/kg and 30μg/kg for heat treated meat products, respectively [26].

Factors affecting the formation of PAH compounds in meat and meat products

Many factors such as cooking method, cooking duration and distance, heat source and fuel type, direct/indirect heat application, pre-cooking processes and fat content of meat affect the formation of PAH compounds in meat and meat products (Figure 1).

Frying, grilling, baking, smoking, steaming are techniques commonly used in cooking of meat and meat products [2,24]. There is a strong correlation between PAH formation in meat and cooking methods. On the other hand, the level of PAHs formed varies widely depending on the cooking method [27,28,29,30]. In a study examining the effects of different cooking methods on PAH formation in beef, it was reported that the BaP and ∑PAH4 content were lower in pan-fried samples compared to barbecue-cooked samples [31]. Chung et al. [28] reported that among beef and pork samples cooked by grilling and roasting methods, the highest BaP and ∑PAH4 content was observed in the grilled samples. In addition, the researchers stated that this may be related to the pyrolysis of meat fat dripping onto the charcoal during grilling. In another study examining the effects of different cooking processes (smoking, grilling and boiling) on PAH formation in different meat products, the highest BaP content was found in the smoked samples while the lowest BaP content was detected in boiled samples [29]. As a result of the studies examining the effect of different cooking methods on the level of PAH formed in meat and meat products, it was reported that meat samples cooked by grilling, smoking and roasting methods carry a high health risk due to their high PAH content [28].

Another factor affecting the PAH level formed during the cooking of meat; is the practice of cooking by direct or indirect method. While the direct method is based on the principle of direct contact of meat with a thermal agent, in the indirect method, a thermal agent does not affect the cooked meat through direct contact. Many studies confirm that direct contact with heat source increases PAH formation and that food retains PAH compounds through adsorption and absorption [32]. In [33], the ∑PAH16 content

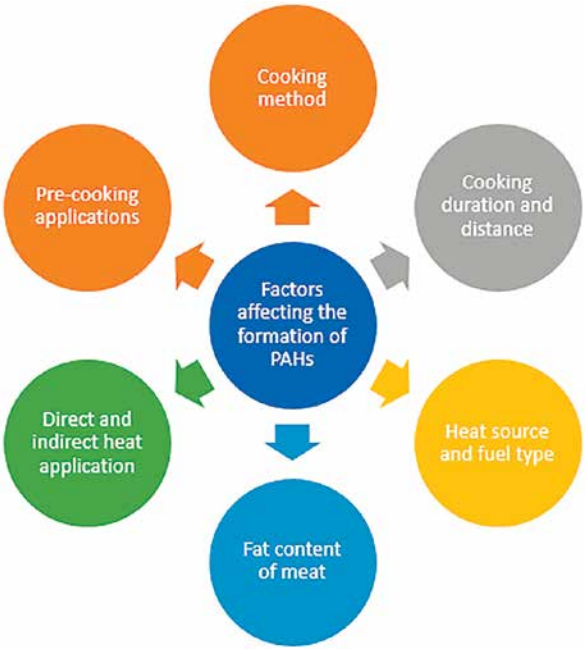


Figure 1. Factors affecting the formation of PAH compounds

of beef cooked with the electric oven grill, which is an indirect cooking technique, was determined as 2.01 μg/kg, while this value was reported to be 17.88 μg/kg in the samples cooked with the charcoal grill method, a direct cooking technique. El-Badry [34] found that direct contact with the gas flame promoted the formation of PAH in the chicken. The author [34] also reported that cooking chicken meat with the direct gas grill cooking technique should be avoided. Wretling et al. [35] stated that the BaP and ∑PAH15 content of the various meat samples smoked by the traditional "sauna" method, in which the samples are exposed directly to the hot smoke from a burning fire, were above the legal limits. Akpambang et al. [36] reported that the use of the indirect smoking technique instead of the direct smoking method is quite effective in reducing PAH formation in smoked meat products.

Free radicals formed when high temperatures are applied to foods undergo recombination to form light PAHs. Heavy PAHs that migrate to the hydrophobic portions of the food following this formation eventually attach to the fat-rich food portions [32]. In this context, the level of PAH formation and accumulation in meat may vary depending on the lipid level of meat. Wegrzyn et al. [37] reported that the fat content is an important parameter that can affect the safety of meat in terms of PAH compounds. Particularly, in meat and meat products where grill and barbecue cooking methods are applied, PAH compounds can be formed that are carried back to the meat surface as a result of the pyrolysis of meat fat or dripping into the flame [13]. The fat content of meat is an important factor influencing the level of PAH formed when this type of cooking method is used. In a study examining the effect of fat content on PAH formation in various grilled meat samples, it was determined that the BaP content of lamb steak, which had the highest fat content, was higher than that of beef steak, pork chop and ham. It has been reported that this result may be related to the formation of more lipid oxidation and degradation products in lamb steak with the high fat content [38]. Pöhlmann et al. [39] conducted a study to determine an effect of the fat content on PAH formation in smoked frankfurter sausages and found that the ∑PAH4 level of sausages with different fat contents (10, 20, 30 and 39%) increased with an increase in the fat content. They also reported that PAH formation in smoked frankfurter sausages could be reduced by reducing the fat content in the sausage formulation. In addition to an amount of fat, a type of fat can also affect the formation of PAH. As a matter of fact, Chen and Chen [40] examined the PAH level of smoke formed as a result of heat treatment of soybean oil, canola and sunflower oils and determined that the highest total PAH amount was in soybean oil. Researchers reported that this result was related to the higher levels of linolenic acid in soybean oil compared to canola and sunflower oils. Similarly, in a study examining the PAH level of meatballs cooked in barbecue using different types of fat (meat fat, sheep tail fat and a mixture of meat fat and tail fat), it was determined that the highest total PAH8 level belonged to the meatballs formulated with tail fat, which had the highest linolenic acid content [3].

The cooking process, including temperature and time, and a distance between meat and a heat source are the factors affecting the PAH formation. Literature data indicate that the level of PAH formed in meat increases in parallel with an increase in cooking temperature and time. According to Kao et al. [41] reported that high temperature and long cooking time cause more fat loss from the meat surface, which may cause a heat source to produce more PAH. In a study, in which an effect of cooking time on the PAH content of grilled pork was determined, the BaP contents of the samples cooked for 2, 3 and 4 minutes were found to be 2.4 μg/kg, 4.5 μg/kg and 10.2 μg/kg, respectively. In addition, it has been reported that the BaP level formed as a result of cooking for 4 minutes has reached the legal limit specified by the European Commission [42]. Oz and Yuzer [30] found that the total PAH8 content of well-done and very well-done beef samples in wire barbecue was 0.8 and 0.9 mg/kg, respectively, while they could not detect any of the individual PAH compounds that make up PAH8 in rare and medium cooked beef. In another study examining the PAH level of a traditional Malaysian beef product, cooking was done at different temperatures (150 °C, 200 °C, 250 °C, 300 °C and 350 °C). As a result of the research, it was reported that the lowest ∑PAH15 level was detected in the samples grilled at 150 °C and an increase in the ∑PAH15 level was observed as the temperature increased [43]. Szterk [44] reported that PAH formation increased as a result of high temperature application due to the pyrolysis of nitrogen-containing organic compounds such as amino acids and protein. In a study examining an effect of smoking time on PAH formation in meat products, it was found that both BaP and ∑PAH4 amounts were higher in meat samples with high smoking time [45]. On the other hand, studies on a distance between food and a heat source, which is another factor affecting PAH formation, show that there is a negative correlation between distance and PAH formation in general. Roseiro et al. [46] examined an effect of smoking distance on PAH formation in sausages and reported that as the distance decreased PAH penetrated into the central point of the sausages. In [11] Rose et al. determined that the ∑PAH4 content of beef burgers cooked at a distance of 9 cm from the heat source was 75% of the ∑PAH4 content of the samples cooked at a distance of 4 cm from the heat source. This relationship between distance and PAH formation is probably related to an increase in the rate of PAH formation and penetration into the sample that comes into contact with hot and intense heat more quickly in short distance.

PAH formation occurs due to the acetylene addition mechanism (HACA) with the release of hydrogen during the combustion of the fuel used in the thermal process. On the other hand, the level of PAH formed may vary depending on a type of fuel used [10]. In the report published

by the Codex Alimentarius Commission in 2009, it was recommended not to use resinous or chemically treated woods, waste or diesel oils as fuel in cooking process [47]. In [48] Viegas et al. examined an effect of fuel type on the PAH level of grilled salmon and reported that the samples grilled with coconut shell charcoal produced less PAH than the samples grilled with usual wood charcoal. The authors stated that this result is related to the ability of coconut charcoal to absorb dripping oil without creating smoke, since it is flameless and smokeless [48]. Oz [12] found that amounts of ∑PAH8 were higher in fish samples barbecued with charcoal briquette compared to those barbecued with wood charcoal, and suggested the use of wood charcoal in barbecuing. In another study examining the PAH levels of chicken meat cooked using charcoal and electric grill, it was determined that the average ∑PAH16 level of chicken meat cooked with charcoal and electric grill was 1.6 μg/kg, and 0.038 μg/kg, respectively [49]. Similarly, it was determined that the BaP content of doner kebabs cooked on charcoal and gas grills was 24.2 μg/kg and 5.7 μg/kg, respectively, and gas grilling caused less BaP formation [50]. Oz [2] reported that a type of smoke flavoring wood chips affected the level of PAH formation in barbecued salmon fillets, and bourbon-soaked oak, cherry and hickory wood chips were the wood chips types that could reduce the content of PAHs in barbecued salmon fillets. Stumpe-Viksna et al. [51] reported that a type of sawdust is a critical parameter to be controlled in the smoking process, and that softwood shavings cause higher PAH formation than hardwood shavings, possibly due to the high resin content they contain.

Pre-cooking is one of the strategies applied to reduce PAH formation in cooked meat. Ohmic heating is a widely used technique in recent years due to its advantages such as short processing time, high efficiency and preservation of the nutritional value compared to conventional heating methods [52]. Sengun et al. studied an effect of ohmic preheating on the PAH level of half-cooked meatballs and found that the PAH levels of the meatballs were within acceptable limits, and therefore the ohmic cooking method was safe in terms of PAH formation [53]. In [54] Kendirci et al. determined an effect of ohmic preheating before infrared cooking on PAH formation in beef patties. They reported that the BaP and ∑PAH4 contents formed as a result of the combination of preheating and infrared cooking did not exceed the legal limits [54]. Farhadian et al. [55] investigated an effect of preheating processes (steam and microwave) on PAH levels in grilled chicken and beef. They established that preheating processes applied to the samples strongly affected PAH levels and BaP could not be detected in pretreated samples. The authors stated that this situation may be related to a decrease in the pyrolysis rate as a result of preheating and less penetration of the smoke components into the meat surface and suggested to expand the use of preheating processes in homes and restaurants in order to reduce PAH exposure [55].

Marinating is a technique commonly used to improve the sensory properties of meat products such as texture, color and flavor. Studies on the effect of the marination on the formation of PAH in meat reveal that the effect varies depending on a type of marinade used. Farhadian et al. [56] reported that the PAH content in grilled beef marinated with basic marinades varied between 45.19–56.09 μg/kg, while this value varied between 74.0–80.6 μg/kg in the unmarinated control samples. On the other hand, researchers mentioned that adding 40 mL of oil into basic marination increased the PAH content of grilled beef meat (98.9–109 μg/kg). Similarly, it has been shown in some studies that oil and fatty components are major precursors of PAHs and that cooking oils can be contaminated with PAHs during various preparation processes [57,58]. In a study examining the level of PAH in grilled chicken, the ∑PAH16 amount of unmarinated, palm oil-marinated and sunflower oil-marinated samples was determined as 190.1μg/kg, 457.6 μg/kg and 376.6 μg/kg, respectively. In addition, it has been reported that an increase in the PAH content observed as a result of marinating with oil is related to the fact that oils are the main precursors of PAH components [59]. In [31] Büyükkurt et al. used sage and thyme extracts prepared at 0.5–2.0 °Brix concentration as a marination agent and reported that these marinades showed a reducing effect on PAH formation in barbecued beef. Viegas et al. [60] determined that marinating with different types of beer reduced the amount of ∑PAH8 formed in grilled pork by 13–53%, depending on a type of beer used. They also reported that the greatest decrease in the PAH content was observed in dark beer marinated samples, which had the highest antiradical activity. Janoszka [61] found that the use of onion (30/100 g meat) caused a 60% reduction in the total PAH content of pan-fired meat, while the use of garlic (15/100 g meat) resulted in a 54% reduction. It has been declared that the PAH-lowering effect of onion and garlic may be caused by components with sulfhydryl groups, and polyphenols responsible for the antioxidant activity [61,62]. In a study examining an effect of using a spice mixture containing cumin, coriander, black pepper, rosemary and garlic on the formation of carcinogenic PAHs in chicken meat, it was determined that the spice mixture caused a significant decrease in the level of carcinogenic PAHs. In addition, it has been suggested to use the specified mixture before cooking chicken meat in order to reduce the level of PAHs [34].

Conclusion

PAHs are a large group of environmental pollutants with mutagenic and/or carcinogenic properties. Foodstuffs are the main sources of PAH exposure. Among food groups, meat and meat products being rich in protein and fat contribute significantly to PAH exposure due to the frequent use of thermal processes and increased risk depending on consumption habits. Many factors such as cooking method, thermal process duration, fuel/wood type, pre-cooking applications, marination, additives and

fat content of meat affect the level of PAH in meat and meat products. In this context, in order to reduce the amount of PAH formed in meat, it can be recommended to apply moderate and indirect heat processes in cooking, to use non-smoke fuel types, to add antioxidants and to reduce the fat content of meat.

REFERENCES

1. Plaza-Bolaños, P., Frenich, A. G., Vidal, J. L. M. (2010). Polycyclic aromatic hydrocarbons in food and beverages. Analytical methods and trends. *Journal of Chromatography A*, 1217(41), 6303–6326. https://doi.org/10.1016/j.chroma.2010.07.079
2. Oz, E. (2020). Effects of smoke flavoring using different wood chips and barbecuing on the formation of polycyclic aromatic hydrocarbons and heterocyclic aromatic amines in salmon fillets. *PLoS One*, 15(1), Article e0227508. https://doi.org/10.1371/journal.pone.0227508
3. Oz, E. (2021). The presence of polycyclic aromatic hydrocarbons and heterocyclic aromatic amines in barbecued meatballs formulated with different animal fats. *Food Chemistry*, 352, Article 129378. https://doi.org/10.1016/j.foodchem.2021.129378
4. Purcaro, G., Moret, S., Conte, L. S. (2013). Overview on polycyclic aromatic hydrocarbons: Occurrence, legislation and innovative determination in foods. *Talanta,* 105, 292–305. https://doi.org/10.1016/j.talanta.2012.10.041
5. Bogdanović, T., Pleadin, J., Petričević, S., Listeš, E., Sokolić, D., Marković, K. et al. (2019). The occurrence of polycyclic aromatic hydrocarbons in fish and meat products of Croatia and dietary exposure. *Journal of Food Composition and Analysis*, 75, 49–60. https://doi.org/10.1016/j.jfca.2018.09.017
6. Storelli, M. M., Stuffler, R. G., Marcotrigiano, G. O. (2003). Polycyclic aromatic hydrocarbons, polychlorinated biphenyls, chlorinated pesticides (DDTs), hexachlorocyclohexane, and hexachlorobenzene residues in smoked seafood. *Journal of Food Protection*, 66(6), 1095–1099. https://doi.org/10.4315/0362-028X-66.6.1095
7. Li, Y., Wang, C., Zou, X., Feng, Z., Yao, Y., Wang, T. et al. (2019). Occurrence of polycyclic aromatic hydrocarbons (PAHs) in coral reef fish from the South China Sea. *Marine Pollution Bulletin*, 139, 339–345. https://doi.org/10.1016/j.marpolbul.2019.01.001
8. Singh, L., Agarwal, T., Simal-Gandara, J. (2020). PAHs, diet and cancer prevention: Cooking process driven-strategies. *Trends in Food Science and Technology*, 99, 487–506. https://doi.org/10.1016/j.tifs.2020.03.030
9. Wang, L., Li, C., Jiao, B., Li, Q., Su, H., Wang, J. et al. (2018). Halogenated and parent polycyclic aromatic hydrocarbons in vegetables: levels, dietary intakes, and health risk assessments. *Science of the Total Environment*, 616–617, 288–295. https://doi.org/10.1016/j.scitotenv.2017.10.336
10. Sun, Y., Wu, S., Gong, G. (2019). Trends of research on polycyclic aromatic hydrocarbons in food: A 20-year perspective from 1997 to 2017. *Trends in Food Science and Technology*, 83, 86–98. https://doi.org/10.1016/j.tifs.2018.11.015
11. Rose, M., Holland, J., Dowding, A., Petch, S. R. G., White, S., Fernandes, A. et al. (2015). Investigation into the formation of PAHs in foods prepared in the home to determine the effects of frying, grilling, barbecuing, toasting and roasting. *Food and Chemical Toxicology*, 78, 1–9. https://doi.org/10.1016/j.fct.2014.12.018
12. Oz, E. (2021). The impact of fat content and charcoal types on quality and the development of carcinogenic polycyclic aromatic hydrocarbons and heterocyclic aromatic amines formation of barbecued fish. *International Journal of Food Science and Technology*, 56(2), 954–964. https://doi.org/10.1111/ijfs.14748
13. Zelinkova, Z., Wenzl, T. (2015). The occurrence of 16 EPA PAHs in food — A review. *Polycyclic Aromatic Compounds*, 35(2–4), 248–284. https://doi.org/10.1080/10406638.2014.918550
14. Duedahl-Olesen, L., Ionas, A. C. (2022). Formation and mitigation of PAHs in barbecued meat — a review. *Critical Reviews in Food Science and Nutrition*, 62(13), 3553–3568. https://doi.org/10.1080/10408398.2020.1867056
15. Onopiuk, A., Kołodziejczak, K., Szpicer, A., Wojtasik-Kalinowska, I., Wierzbicka, A., Półtorak, A. (2021). Analysis of factors that influence the PAH profile and amount in meat products subjected to thermal processing. *Trends in Food Science and Technology*, 115, 366–379. https://doi.org/10.1016/j.tifs.2021.06.043
16. Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B., Rajendran, P., Nishigaki, I. (2015). Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pacific Journal of Tropical Biomedicine*, 5(3), 182–189. https://doi.org/10.1016/S2221-1691(15)30003-4
17. Cross, A. J., Freedman, N. D., Ren, J., Ward, M. H., Hollenbeck, A. R., Schatzkin, A. et al. (2011). Meat consumption and risk of esophageal and gastric cancer in a large prospective study. *The American Journal of Gastroenterology*, 106(3), 432–442. https://doi.org/10.1038/ajg.2010.415
18. Zhu, Z., Xu, Y., Huang, T., Yu, Y., Bassey, A. P., Huang, M. (2022). The contamination, formation, determination and control of polycyclic aromatic hydrocarbons in meat products. *Food Control*, 141, Article 109194. https://doi.org/10.1016/j.foodcont.2022.109194
19. IARC (International Agency for Research on Cancer). (2010). Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, France, 2010.
20. EPA (Environmental Protection Agency). (2008). Polycyclic aromatic hydrocarbons (PAHs) — EPA fact sheet. Washington (DC): National Center for Environmental Assessment, Office of Research and Development, 2008.
21. Abdel-Shafy, H. I., Mansour, M. S. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, 25(1), 107–123. https://doi.org/10.1016/j.ejpe.2015.03.011
22. Daniel, C. R., Schwartz, K. L., Colt, J. S., Dong, L. M., Ruterbusch, J. J., Purdue, M. P. et al. (2011). Meat-cooking mutagens and risk of renal cell carcinoma. *British Journal of Cancer,* 105(7), 1096–1104. https://doi.org/10.1038/bjc.2011.343
23. Alomirah, H., Al-Zenki, S., Al-Hooti, S., Zaghloul, S., Sawaya, W., Ahmed, N. et al. (2011). Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (PAHs) from grilled and smoked foods. *Food Control*, 22(12), 2028–2035. https://doi.org/10.1016/j.foodcont.2011.05.024
24. Ledesma, E., Rendueles, M., Díaz, M. J. F. C. (2016). Contamination of meat products during smoking by polycyclic aromatic hydrocarbons: Processes and prevention. *Food Control*, 60, 64–87. https://doi.org/10.1016/j.foodcont.2015.07.016
25. EFSA. (2008). Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food. *The EFSA Journal,* 724, 1–114.
26. Commission Regulation (EU). (2011). No: 835/2011 of 19 August 2011 amending Regulation (EC) No: 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuff.
27. Perelló, G., Martí-Cid, R., Castell, V., Llobet, J. M., Domingo, J. L. (2009). Concentrations of polybrominated diphenyl ethers, hexachlorobenzene and polycyclic aromatic hydrocarbons in various foodstuffs before and after cooking. *Food and Chemical Toxicology,* 47(4), 709–715. https://doi.org/10.1016/j.fct.2008.12.030
28. Chung, S. Y., Yettella, R. R., Kim, J. S., Kwon, K., Kim, M. C., Min, D. B. (2011). Effects of grilling and roasting on the levels of polycyclic aromatic hydrocarbons in beef and pork. *Food Chemistry*, 129(4), 1420–1426. https://doi.org/10.1016/j.foodchem.2011.05.092
29. Olatunji, O. S., Fatoki, O. S., Ximba, B. J., Opeolu, B. O. (2014). Polycyclic aromatic hydrocarbons (PAHs) in edible oil: temperature effect on recovery from base hydrolysis product and health risk factor. *Food and Public Health*, 4(2), 23–30. https://doi.org/10.5923/j.fph.20140402.02
30. Oz, F., Yuzer, M. O. (2016). The effects of cooking on wire and stone barbecue at different cooking levels on the formation of heterocyclic aromatic amines and polycyclic aromatic hydrocarbons in beef steak. *Food Chemistry*, 203, 59–66. https://doi.org/10.1016/j.foodchem.2016.02.041
31. Büyükkurt, O. K., Dinçer, E. A., Çam, I. B., Candal, C., Erbaş, M. (2017). The influence of cooking methods and some marinades on polycyclic aromatic hydrocarbon formation in beef meat. *Polycyclic Aromatic Compounds*, 40(2), 195–205. https://doi.org/10.1080/10406638.2017.1392328
32. Singh, L., Varshney, J. G., Agarwal, T. (2016). Polycyclic aromatic hydrocarbons' formation and occurrence in processed food. *Food Chemistry*, 199, 768–781. https://doi.org/10.1016/j.foodchem.2015.12.074

33. Onyango, A. A., Lalah, J. O., Wandiga, S. O. (2012). The effect of local cooking methods on polycyclic aromatic hydrocarbons (PAHs) contents in beef, goat meat, and pork as potential sources of human exposure in Kisumu city, Kenya. *Polycyclic Aromatic Compounds*, 32(5), 656–668. https://doi.org/10.1080/10406638.2012.725195
34. El-Badry, N. (2010). Effect of household cooking methods and some food additives on polycyclic aromatic hydrocarbons (PAHs) formation in chicken meat. *World Applied Sciences Journal*, 9(9), 963–974.
35. Wretling, S., Eriksson, A., Eskhult, G. A., Larsson, B. (2010). Polycyclic aromatic hydrocarbons (PAHs) in Swedish smoked meat and fish. *Journal of Food Composition and Analysis*, 23(3), 264–272. https://doi.org/10.1016/j.jfca.2009.10.003
36. Akpambang, V. O. E., Purcaro, G., Lajide, L., Amoo, I. A., Conte, L. S., Moret, S. (2009). Determination of polycyclic aromatic hydrocarbons (PAHs) in commonly consumed Nigerian smoked/grilled fish and meat. *Food Additives and Contaminants: Part A*, 26(7), 1096–1103. https://doi.org/10.1080/02652030902855406
37. Wegrzyn, E., Grzeskiewicz, S., Poplawska, W., Glod, B. K. (2006). Modified analytical method for polycyclic aromatic hydrocarbons, using sec for sample preparation and RP-HPLC with fluorescence detection. Application to different food samples. *Acta Chromatographica*, 17, 233.
38. Kao, T. H., Chen, S., Huang, C. W., Chen, C. J., Chen, B. H. (2014). Occurrence and exposure to polycyclic aromatic hydrocarbons in kindling-free-charcoal grilled meat products in Taiwan. *Food and Chemical Toxicology*, 71, 149–158. https://doi.org/10.1016/j.fct.2014.05.033
39. Pöhlmann, M., Hitzel, A., Schwägele, F., Speer, K., Jira, W. (2013). Polycyclic aromatic hydrocarbons (PAH) and phenolic substances in smoked Frankfurter-type sausages depending on type of casing and fat content. *Food Control*, 31(1), 136–144. https://doi.org/10.1016/j.foodcont.2012.09.030
40. Chen, B. H., Chen, Y. C. (2001). Formation of polycyclic aromatic hydrocarbons in the smoke from heated model lipids and food lipids. *Journal of Agricultural and Food Chemistry,* 49(11), 5238–5243. https://doi.org/10.1021/jf0106906
41. Kao, T. H., Chen, S., Chen, C. J., Huang, C. W., Chen, B. H. (2012). Evaluation of analysis of polycyclic aromatic hydrocarbons by the QuEChERS method and gas chromatography–mass spectrometry and their formation in poultry meat as affected by marinating and frying. *Journal of Agricultural and Food Chemistry*, 60(6), 1380–1389. https://doi.org/10.1021/jf204650u
42. Pan, H., Cao, Y. (2010). Optimization of pretreatment procedures for analysis of polycyclic aromatic hydrocarbons in charcoal-grilled pork. *Analytical Letters*, 43(1), 97–109. https://doi.org/10.1080/00032710903276497
43. Ahmad Kamal, N. H., Selamat, J., Sanny, M. (2018). Simultaneous formation of polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines (HCAs) in gas-grilled beef satay at different temperatures. *Food Additives and Contaminants: Part A*, 35(5), 848–869. https://doi.org/10.1080/19440049.2018.1425553
44. Szterk, A. (2015). Acridine derivatives (PANHs, azaarenes) in raw, fried or grilled pork from different origins, and PANH formation during pork thermal processing. *Journal of Food Composition and Analysis*, 43, 18–24. https://doi.org/10.1016/j.jfca.2015.04.011
45. Hokkanen, M., Luhtasela, U., Kostamo, P., Ritvanen, T., Peltonen, K., Jestoi, M. (2018). Critical effects of smoking parameters on the levels of polycyclic aromatic hydrocarbons in traditionally smoked fish and meat products in Finland. *Journal of Chemistry*, 2018, Article 2160958. https://doi.org/10.1155/2018/2160958
46. Roseiro, L. C., Gomes, A., Santos, C. (2011). Influence of processing in the prevalence of polycyclic aromatic hydrocarbons in a Portuguese traditional meat product. *Food and Chemical Toxicology*, 49(6), 1340–1345. https://doi.org/10.1016/j.fct.2011.03.017
47. CAC/RCP 68 (2009). Codex alimentarius commission (CAC). Code of practice for the reduction of contamination of food with polycyclic aromatic hydrocarbons (PAH) from smoking and direct drying processes.
48. Viegas, O., Novo, P., Pinto, E., Pinho, O., Ferreira, I. M. P. L. V. O. (2012). Effect of charcoal types and grilling conditions on formation of heterocyclic aromatic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs) in grilled muscle foods. *Food and Chemical Toxicology*, 50(6), 2128–2134. https://doi.org/10.1016/j.fct.2012.03.051
49. Hamzawy, A. H., Khorshid, M., Elmarsafy, A. M., Souaya, E. R. (2016). Estimated daily intake and health risk of polycyclic aromatic hydrocarbon by consumption of grilled meat and chicken in Egypt. *International Journal of Current Microbiology and Applied Sciences,* 5(2), 435–448. http://doi.org/10.20546/ijcmas.2016.502.049
50. Terzi, G., Celik, T. H., Nisbet, C. (2008). Determination of benzo[a] pyrene in Turkish döner kebab samples cooked with charcoal or gas fire. *Irish Journal of Agricultural and Food Research,* 47(2), 187–193.
51. Stumpe-Vīksna, I., Bartkevičs, V., Kukāre, A., Morozovs, A. (2008). Polycyclic aromatic hydrocarbons in meat smoked with different types of wood. *Food Chemistry*, 110(3), 794–797. http://doi.org/10.1016/j.foodchem.2008.03.004
52. Yildiz-Turp, G., Sengun, I. Y., Kendirci, P., Icier, F. (2013). Effect of ohmic treatment on quality characteristic of meat: A review. *Meat Science*, 93(3), 441–448. http://doi.org/10.1016/j.meatsci.2012.10.013
53. Sengun, I. Y., Yildiz-Turp, G., Icier, F., Kendirci, P., Kor, G. (2014). Effects of ohmic heating for pre-cooking of meatballs on some quality and safety attributes. *LWT-Food Science and Technology*, 55(1), 232–239. http://doi.org/10.1016/j.lwt.2013.08.005
54. Kendirci, P., Icier, F., Kor, G., Onogur, T. A. (2014). Influence of infrared final cooking on polycyclic aromatic hydrocarbon formation in ohmically pre-cooked beef meatballs. *Meat Science*, 97(2), 123–129. http://doi.org/10.1016/j.meatsci.2014.01.020
55. Farhadian, A., Jinap, S., Hanifah, H. N., Zaidul, I. S. (2011). Effects of meat preheating and wrapping on the levels of polycyclic aromatic hydrocarbons in charcoal-grilled meat. *Food Chemistry*, 124(1), 141–146. http://doi.org/10.1016/j.foodchem.2010.05.116
56. Farhadian, A., Jinap, S., Faridah, A., Zaidul, I. S. M. (2012). Effects of marinating on the formation of polycyclic aromatic hydrocarbons (benzo[a]pyrene, benzo[b]fluoranthene and fluoranthene) in grilled beef meat. *Food Control*, 28(2), 420–425. http://doi.org/10.1016/j.foodcont.2012.04.034
57. McGrath, T. E., Wooten, J. B., Chan, W. G., Hajaligol, M. R. (2007). Formation of polycyclic aromatic hydrocarbons from tobacco: the link between low temperature residual solid (char) and PAH formation. *Food and Chemical Toxicology*, 45(6), 1039–1050. http://doi.org/10.1016/j.fct.2006.12.010
58. Rojo Camargo, M. C., Antoniolli, P. R., Vicente, E., Tfouni, S. A. V. (2011). Polycyclic aromatic hydrocarbons in Brazilian commercial soybean oils and dietary exposure. *Food Additives and Contaminants: Part B*, 4(2), 152–159. https://doi.org/10.1080/19393210.2011.585244
59. Wongmaneepratip, W., Vangnai, K. (2017). Effects of oil types and pH on carcinogenic polycyclic aromatic hydrocarbons (PAHs) in grilled chicken. *Food Control*, 79, 119–125. https://doi.org/10.1016/j.foodcont.2017.03.029
60. Viegas, O., Yebra-Pimentel, I., Martinez-Carballo, E., Simal-Gandara, J., Ferreira, I. M. P. L. V. O. (2014). Effect of beer marinades on formation of polycyclic aromatic hydrocarbons in charcoal-grilled pork. *Journal of Agricultural and Food Chemistry*, 62(12), 2638–2643. https://doi.org/10.1021/jf404966w
61. Janoszka, B. (2011). HPLC-fluorescence analysis of polycyclic aromatic hydrocarbons (PAHs) in pork meat and its gravy fried without additives and in the presence of onion and garlic. *Food Chemistry*, 126(3), 1344–1353. https://doi.org/10.1016/j.foodchem.2010.11.097
62. Bianchini, F., Vainio, H. (2001). Allium vegetables and organosulfur compounds: do they help prevent cancer? *Environmental Health Perspectives*, 109(9), 893–902. https://doi.org/10.1289/ehp.01109893

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GENOME-WIDE ANALYSIS IN THE SEARCH FOR CANDIDATE GENES ASSOCIATED WITH MEAT PRODUCTIVITY TRAITS IN MEAT-AND-DAIRY GOATS

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Abstract
The development of the tourism cluster in the North Caucasus causes the expansion of product range with high consumer characteristics, in particular, a sustainable offer of dairy and meat products labeled as environmentally friendly. In the range of such products with high dietary properties, a special role may be played by goat meat obtained from Karachay goats, which are the most common meat-and-dairy goats in the region. The aim of the work was to search for candidate genes associated with live weight and meat productivity of Karachay goats. GWAS analysis using Goat 50K BeadChip high-density DNA microarray determined a genome-wide level of significance for six SNPs located on chromosomes 5, 6, 10 and 16 associated with the live weight of young animals (4 and 8 months old). Three of the six SNPs within the ± 200 kb region were localized to HMGA2, CRADD, and MAX genes. These genes were selected to study the meat productivity traits of young goats with different genotypes. It was found that in the locus linked with HMGA2 gene, young goats with GG genotype were characterized by the best indicators of meat productivity. Compared to AA genotype animals, they had superiority in pre-slaughter weight, slaughter carcass weight, slaughter yield, boneless meat weight and loin eye area by 8.9%, 13.6%, 4.3% (P <0.05), 10.5% (P <0.01), and 5.2% (P <0.05) respectively. Young goat meat of this genotype was characterized by the high protein content of 22.56% and low fat content of 9.12%. For the CRADD gene, animals with GG genotype had a higher pre-slaughter weight, slaughter weight, slaughter carcass yield, boneless meat weight and loin eye area. Animals with AG genotype were characterized by the lowest indicators. According to the above characteristics, the difference between the compared genotypes was 15.8%, 25.7% (P <0.01), 8.4% (P <0.05), 18.3%, and 15.7% (P <0.01) respectively. There were no significant differences in the chemical composition of muscle tissue between animals of different genotypes. HMGA2 and CRADD genes are promising for further research of Karachay goats breeding to increase meat productivity and meat quality.

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Introduction

Goats are one of the earliest domesticated ruminants. They are traditional sources of meat, milk, wool, raw leather and other animal products. The unique abilities of goats, such as unpretentiousness in maintenance and nutrition, the ability to adapt to almost any environmental conditions, made their distribution ubiquitous. More than 250 breeds of goats exist in 197 countries all over the world with total number about 1 billion 200 million animals. The number of goats in Russia is about 2 million [1,2]. It should be noted that in the last two decades, there has been an increase in the number of dairy and meat-and-dairy goats and an increase in the production of goat milk and meat. Meat-and-dairy goats are mainly represented by local breeds, which are bred mainly in the foothills and mountainous regions of Altai, Tyva, Khakassia and North Caucasus [3].

In the North Caucasus, Karachay goats are ones of the most common. They are bred in alpine and subalpine pastures, which are characterized by an exceptional rich flora often not accessible to other types of domestic animals due to steep slopes and rocky ledges [4]. This fact makes it possible to obtain environmentally friendly products from Karachay goats. Moreover, the development of tourism in the North Caucasus stimulates their intensive breeding. At the same time, along with excellent biological and productive indicators, Karachay goats (of different populations) are characterized by high variability in live weight and meat productivity, which makes it relevant to improve them by breeding.

Along with traditional methods of goat breeding, marker-assisted breeding based on the use of molecular genetic methods has become increasingly important in recent years [5,6]. Progress in genotyping technologies has made

it possible to conduct genome-wide association studies (GWAS) to identify new single nucleotide polymorphisms (SNP) associated with indicators of body size that may affect the meat productivity of goats.

Genotyping of seven goat breeds in Pakistan using Goat 50K BeadChip DNA microarray and GWAS showed that *DKK2, TBCK, FGF, ANK2* genes were associated with body size. Functional annotation of the identified genes showed that *DKK2* (dickkopf WNT signaling pathway inhibitor 2) plays a role in embryonic development, while *TBCK* (TBC1 domain containing kinase), *FGF* (epidermal growth factor), and *ANK2* (ankyrin 2) are involved in the regulation of cell proliferation and growth [7].

Several studies performed on large populations of white cashmere goats (n=1038; 1953; 1405; 1759) genotyped with Goat 50K BeadChip DNA microarray and GWAS established a highly significant relationship between 16-bp indel mutations in prolactin (*PRLR*) and lysine-specific demethylase 6A (*KDM6A*) genes, 13-bp indel mutations in A-kinase anchor protein (*AKAP12*) gene, 17-bp and 21-bp indel mutations in sorting nexin 29 (*SNX29*) gene and height, body length, chest width and depth and live weight. The data obtained allowed the authors to recommend the selection of animal with desirable genotypes in the *PRLR, KDM6A, AKAP12, and SNX29* genes to increase goat meat production [8,9,10,11]. At the same time, it was noted that the most promising candidate gene for the live weight trait is the *SNX29* gene, the functional role of which is well known and consists in the regulation of the differentiation and proliferation of muscle tissue cells, myocytes [11].

GWAS for traits associated with body weight and meat productivity was performed for meat goats, in particular Chinese Dazu black goat. In this study, genotypes were determined by sequencing the entire genome of 30 individuals. It was found that *PSTPIP2* (proline-serine-threonine phosphatase-interacting protein 1), *CCL19 C–C* (motif ligand 2), *FGF9* (fibroblast growth factor) and *SIPA1L* (signal-induced proliferation-associated 1-like protein 1) genes were associated with body size and weight. Functional annotation of the identified genes showed that they are involved in the regulation of skeletal muscle development. The authors believe that these genes may be used as candidate genes for the meat productivity of Dazu black goats [12].

Another study performed on goats (n=1044) of the same breed, but genotyped using Goat 50K BeadChip DNA microarray revealed that 12-bp deletion in PR domain zinc finger protein (*PRDM6*) gene is associated with the height and length of the body, chest width and depth, hips width, and the live weight of young animals. Thus, selection for this gene is profitable for increasing the meat productivity of goats [13].

Given the relevance of genome-wide association analysis and the lack of such studies for goats in national selection, the aim of the work was to search for candidate genes associated with the live weight of Karachay goats, as well as to study meat productivity traits for different genotypes, taking into account the identified significant SNPs and functional annotation.

Objects and methods

The material for the study was genome-wide SNP genotypes of 281 young Karachay goats obtained using Goat 50K BeadChip high-density DNA microarray (Illumina, San Diego, CA, USA), as well as live weight indicators of genotyped animals at the age of 4 and 8 months old. Quality control and filtering of genotyping data for each SNP and each sample were performed using PLINK 1.9 software package [14].

Genome-wide association studies were performed using multiple linear regression analysis in Plink 1.90 software with preliminarily population adjustment according to its structure (–-genome, —covar). To confirm the significant influence of SNPs and identify significant regions in the goat genome, Bonferroni test was used to test null hypothesis: threshold value $P < 1.53\times10^{-6}$; 0.05/32629 SNP. Data visualization was carried out in the qqman software package using the R programming language [15].

Identified genome-wide SNPs associated with body weight at both 4 and 8 months old were selected for the list of total SNPs. This list was used for structural annotation of genes located within ± 0.2-Mb region from the identified SNP. Genes were identified using the 11.1 ARS1.2 genome assembly and Ensembl Genes release 103 database [16]. For functional annotation, DAVID software was used [17]. To simultaneously test several independent hypotheses aimed at controlling the level of false rejections defined as the expected ratio of false rejections to the total number of rejections, Benjamini-Hochberg procedures were used [18].

Three young goats of different genotypes were selected based on the results of GWAS, identified genome-wide SNPs, and functional annotation of genes, within which SNP localization was determined. Their live weight corresponded to the average value of all young goats of the same genotype from the entire sample. Meat productivity for young goats of different genotypes was studied based on the results of slaughter at the age of 8 months old.

Preslaughter live weight was determined by weighing after 24 hours of starvation with an accuracy of 0.1 kg on VET-150–20/50–1S-DBSK balance (Mekhelectron-M LLC, Russia). Carcass yield was calculated by the ratio of carcass weight with kidneys and suet to preslaughter live weight. Slaughter weight was calculated by summing the weight of exsanguinated carcass without a head, skin, tail, internal organs, limbs cut to the hock and carpal joints and internal fat. Internal fat weight was determined by the total amount of pelvic, renal, intestinal, gastric, and diaphragmatic fat. Slaughter yield was calculated as the ratio of slaughter weight to preslaughter weight. Meatiness was determined by the ratio of boneless meat weight to bones weight. Boneless meat weight was determined with an accuracy of

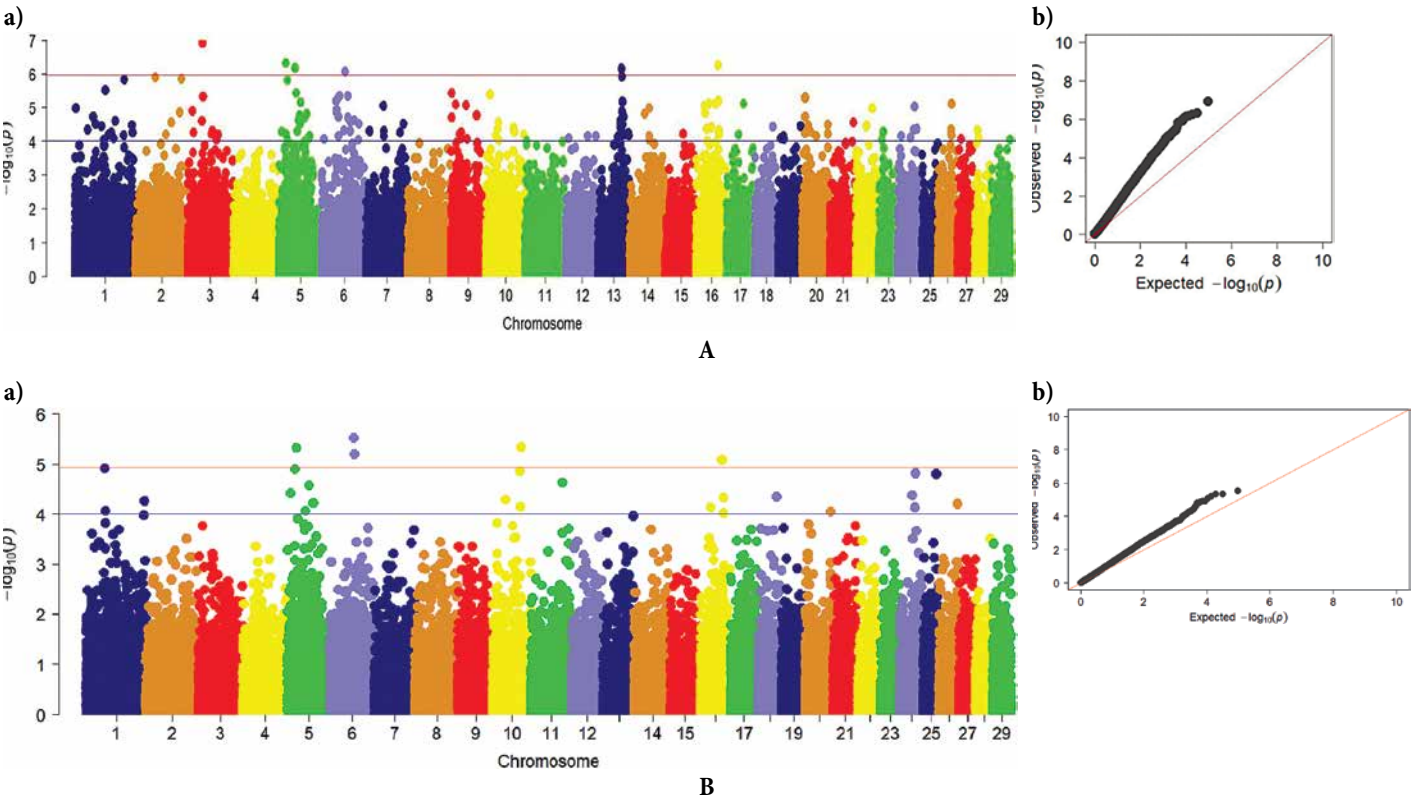

Figure 1. a) location of statistically significant SNPs in 29 autosomes of Karachay goats for the live weight trait: **A** — at the age of 4 months old; **B** — at the age of 8 months old; the negative logarithm of q value (Y-axis) is plotted for each chromosome (X-axis); on the Y-axis — the lower line corresponds to the significance level $p \leq 0.00001$, the upper line corresponds to the significance level $p \leq 0.000001$; **b)** quartile of the probability distribution of the expected and observed deviations from the normal distribution for the significance values

0.05 kg by deboning the carcass on SW-10 balance (CAS, South Korea).

During the control slaughter, meat samples were taken from the main areas of the carcass for the preparation of an average sample and chemical analysis. Moisture content in the average sample of minced meat was determined according to GOST 9793–2016[1]. Fat content was determined according to GOST 23042–2015[2], protein content was determined according to GOST 25011–2017[3] (by the Kjeldahl method; calorie content was determined by calculation using the equation of V. M. Aleksandrov: K = [D — (F + A)] x4.1 + (F × 9.3), where K is calorie content, kcal; D, A, F are dry matter, ash, and fat contents respectively.

Slaughter of experimental animals was carried out in accordance with the requirements of GOST 33215–2014[4] by cutting the jugular vein and exsanguination. During slaughter, the recommendations of the Directive 2010/63/EU of the European Parliament and the Council of the European Union [19], the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123) [20] were strictly followed.

The resulting raw data were subjected to biometric processing using Microsoft Office and BIOSTAT software. Based on the mean values and standard errors, the significance of the difference in the mean values were calculated using Student's t-test.

Results and discussion

For GWAS, a sample of 281 young animals was used. Average values and phenotypic variability in the live weight at the age of 4 and 8 months old were 24.8 kg and 14.5%, 36.3 kg and 11.1% respectively.

The results of GWAS analysis are presented in Figure 1.

Visualization of the genome-wide analysis results allows to state that GWAS patterns for live weight at the age of 4 and 8 months old are generally similar. A match was found for 18 SNPs located on chromosomes 5, 6, 10, 16, 18, 20, and 24 (Figure 1a). At the same time, genome-wide level of significance was established for 6 SNPs located on chromosomes 5, 6, 10, and 16, the localization and description of polymorphism for which are presented in Table 1. It should be noted that the distribution of deviations from the normal distribution for the significance values of the live weight both at the age of 4 and 8 months old were close to expected (Figure 1b).

[1] GOST 9793–2016. "Meat and meat products. Method for determination of moisture content". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200144231 Accessed September 16, 2022 (In Russian)

[2] GOST 23042–2015 "Meat and meat products. Methods of fat determination". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200133107 Accessed September 16, 2022 (In Russian)

[3] GOST 25011–2017 "Meat and meat products. Protein determination methods". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200146783 Accessed September 16, 2022 (In Russian)

[4] GOST 33215–2014 "Guidelines for accommodation and care of animals. Environment, housing and management" Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200127789 Accessed September 16, 2022 (In Russian)

Analysis of six selected SNPs showed the presence of genes localized within the ± 200-kb region for two SNPs: T47480416C (rs268269710 A/G) on chromosome 5 and A25854668G (rs268234545 A/G) on chromosome 10, and directly within the gene for one SNP, A23345368G (rs268270492 G/A) on chromosome 5 (Table 2). These genes were selected as candidate genes associated with the live weight trait in young Karachay goats.

Analysis of scientific information sources for the previously described functions of the genes presented in Table 2 showed that they were associated with growth and development rates in animals of other species (mice and pigs).

Thus, it was found that *CRADD* gene, along with *SOCS2* and *PLXNC1* genes, is localized within the so-called "high-growing region" on chromosome 10 in mice. These genes are associated not only with the phenotype of high growth in mice, but also with no obesity [21]. Another study demonstrated a significant relationship between the "high-growing region" mouse genes, *CRADD*, *SOCS2* and *PLXNC1*, as well as two closely located genes, *ATP2B1* and *DUSP6*, with the growth rate in pigs, as well as the quality of meat and fat [22].

HMGA2 gene has also been identified as a candidate gene associated with the growth and development of pigs [23]. *HMGA2* gene is activated only during early postnatal development and controls the total number of cells in the animal. Its expression level was determined to be proportional to the body weight in pigs [24,25].

The protein encoded by the *MAX* gene is a member of *bHLHZ* family of transcription factors. *MAX* as a partner of *MYC* is involved in the control of cell proliferation [26].

Our own data and the results of studies by other authors substantiated the feasibility for conducting studies on meat productivity traits and chemical analysis of muscle tissue

Table 1. Candidate SNPs that were associated with body weight at the age of 4 and 8 months old

SNP name	SNP	Address A_ID	AlleleA_ProbeSeq	Chr	MapInfo	Source Strand	SourceSeq
snp14251-scaffold157-188734	[T/C]	60796360	CCAAAACACCAAGTCTGC TGGCTCCAGGTAATCTG AAGACTCAATATGCT	5	19 934 148 (Manifest) / 19 499 085 (GWAS)	TOP	CCCACAGAGGGTGGGAGAGACAGG AGGTGGGCGTTGCGGTTAGGAGGGC ACATTAGCAGC[A/G]AGCATATTGA GTCT
snp38426-scaffold486-2412676	[A/G]	15705507	AGCTGTTTTAAATCAGATTGT GTCTTTCAGCTTAAGCTATGT TCTGAGAC	5	23 345 368 (Manifest) / 22 879 856 (GWAS)	BOT	CCCTGCAATTACACACTATGGATTTT CAATAACACCTATCAGATTTTTTGTC CATTTATG[T/C]GTCTCAGAACATAG CTTAAGCTGAAAGACACAATCTGAT TTAAAACAGCTAGGGGCTGAA
snp37630-scaffold463-64670	[T/C]	25802440	CCTTGTTTAACGGATAGAGTA AGTCACATTTCCTGTTTTCCTC TTAGTCA	5	47 480 416 (Manifest) / 46 630 429 (GWAS)	TOP	TTAGCATTTGTCTTGAGGGTTTGAG GCCTTAGAAAATATTGTTTTTATCCT ACAGAGTAC[A/G]TGACTAAGAGGA AAACAGGAAATGTGACTTACTCTAT CCGTTAAACAAGGAATTTTTTTT
snp40083-scaffold511-2344051	[A/G]	18716406	ATCTGTTCAAACTTTGTTCAT GACATACAAAAGGACTGGGA GTGGGAGGT	6	62 949 329 (Manifest) / 60 553 915 (GWAS)	BOT	CTAATACCTATTTTGAGACTGGAGC TAAACTGAATACAGAGGATGACCTA AATCAGAATG[T/C]ACCTCCCACTCC CAGTCCTTTTGTATGTCATGAACAA AGTTTGAACAGATAAAAAACAAA
snp1448-scaffold104-1147808	[T/C]	43609359	CTAGATGTCAGGTGTTGGGAC AGGGGTGTAGAAGGGAGATT TGAGAGCCA	10	25 854 668 (Manifest) / 73 347 268 (GWAS)	TOP	GCTTGAGCTACCCAGGTGTGATCCT CGCTCCACGGCATGAGCTCAGAGGT GCTGCAAACC[A/G]TGGCTCTCAAAT CTCCCTTCTACACCCCTGTCCCAACA CCTGACATCTAGACCAAGAAGG
snp8624-scaffold131-2001386	[A/C]	52698321	GTGATCCTTCGGAGGTTGTTC TTAAAATTCACATTTCCACTC GAAGTTAT	16	57 408 452 (Manifest) / 56 416 291 (GWAS)	BOT	GAACTAACAGTACGTCTTTTAACAT ACTTGTACATGTGTAAGTCAGAACC ATTAAAATGC[T/G]ATAACTTCGAGT GGAAATGTGAATTTTAAGAACAACC TCCGAAGGATCACGGGAAGATGG

Table 2. Characteristics of candidate SNPs associated with live weight of Karachay goats

SNP	Polymorphism	Chromosome	Name of		Localization in the reference sequence of NCBI: NC_030812.1
			gene	protein	
snp37630-scaffold463–64670	T47480416C, rs268269710 A/G	5	*HMGA2* — high-mobility group AT-hook 2	high mobility group protein *HMGI-C*	47,162,168 … 47,306,445
snp38426-scaffold486-2412676	A23345368G, rs268270492 G/A	5	*CRADD* — CASP2 and RIPK1 domain containing adaptor with death domain	death domain-containing protein *CRADD*	23,232,979 … 23,425,649
snp1448-scaffold104–1147808	A25854668G, rs268234545 A/G	10	*MAX* — MYC associated factor X	*MYC*-associated factor X protein *MAX*	25,900,233 … 25,924,465

in young Karachay goats at the age of 8 months old with different genotypes according to the identified SNPs in *HMGA2*, *CRADD*, and *MAX* genes.

This age was used due to the fact that in previous studies it was found that the most intensive growth of young Karachay goats is observed before this age period. Until this age, animals are most efficient at converting the nutrients they consume into meat products. Subsequently, the synthesis of bone and muscle tissues slows down and the synthesis of adipose tissue increases, which is not effective from an economic point of view [27].

An analysis of the data obtained made it possible to establish that in *HMGA2* gene, the best meat productivity traits were observed in young goats with GG genotype. So, compared to animals with AA genotype, they had a significant advantage in preslaughter weight, slaughter carcass weight, slaughter yield, boneless meat weight and loin eye area by 8.9%, 13.6%, 4.3% ($P < 0.05$), 10.5% ($P < 0.01$), and 5.2% ($P < 0.05$) respectively. Animals with AG genotype also demonstrated superiority over young goats with AA genotype. However, the difference between the studied meat productivity traits was not significant (Table 3).

Comparison of *CRADD* gene for meat productivity traits in young goats with different genotypes revealed that animals with GG genotype had a higher preslaughter weight, slaughter weight, carcass yield, boneless meat weight and loin eye area. Animals with AG genotype were characterized by the lowest indicators. The difference between the compared genotypes according to the above characteristics was 15.8%, 25.7% ($P < 0.01$), 8.4% ($P < 0.05$), 18.3%, and 15.7% ($P < 0.01$) respectively.

Comparison of animals with different genotypes in *MAX* gene did not reveal a significant advantage in meat productivity traits of any of the established genotypes. Animals with AA genotype had a tendency to superiority in most indicators.

It should be noted that the difference in the studied meat productivity traits between the compared genotypes in *CRADD* gene was more significant than between the genotypes in *HMGA2* gene. It is also necessary to emphasize animals with GG genotypes in *HMGA2* and *CRADD* genes according to the content of boneless meat, which is the most valuable part of the carcass. It makes carriers of these genotypes the most preferable for breeding in terms of obtaining more meat products.

Comparison of chemical composition indicators in young goat meat did not reveal a significant superiority between the compared genotypes in *HMGA2, CRADD*, and *MAX* genes. At the same time, it should be noted that the meat of young goats with all genotypes at the age of 8 months old had high nutritional properties due to its rather high protein content of 20.9 to 22.56% and low fat content of 7.65 to 9.62%. At the same time, animals with GG genotype in HMGA2 gene had not only the best slaughter properties, but also the best ratio of protein to fat in meat from the functional nutrition point of view. So, in the average sample of minced meat from animals with this genotype, 1.0 gram of fat accounted for 2.47 grams of protein, while in samples of meat from AA and AG genotypes, 1.0 gram of fat accounted for 2.38 and 2.29 grams of protein respectively. No such dependence was found for other genes. A greater amount of protein in meat in relation to fat was in animals with AG genotype in *CRADD* gene, and with GG genotype in *MAX* gene, i. e. 2.65 and 2.71 grams, while in animals with other genotypes this value was 2.33 to 2.46 grams. At the same time, the carriers of these genotypes had no best slaughter properties (Table 3).

The generalization of the results obtained allows to conclude that the identified SNPs and candidate genes, *HMGA2*, *CRADD* and *MAX*, associated with the live weight of young Karachay goats are to some extent involved in their meat productivity. The most promising for further research and use in Karachay goat breeding to increase meat productivity, in our opinion, is the SNP in *HMGA2* gene. GG genotype in rs268269710 position is associated not only with a higher live weight, carcass yield, and boneless meat content, but also with the ratio of protein to fat in meat that is preferable for functional nutrition. At the same time, it should be noted that in order to confirm the revealed patterns, it is necessary to test the results obtained on a larger sample of animals with additional researches to study the meat properties from animals of different genotypes.

In discussing the results obtained in this study, it should be noted that of the three genes that were associated with body weight of goats at both 4 and 8 months old, two, *HMGA2* and *CRADD*, were significantly associated with postmortem meat parameters. Functional annotation of these genes showed that they control cell proliferation at early stages of embryogenesis and also regulate the growth of muscle tissue cells, myocytes [17].

In a number of studies, it was also found that *SNX29* and *FGF* genes associated with the live weight of goats had a pronounced function of regulating the differentiation and proliferation of muscle and connective tissue cells, myocytes and fibroblasts [11].

When studying 11 signs of growth and meat productivity (birth weight, weight at the age of 4 and 6 months old, body weight gain before and after 4 months, daily weight gain for the entire growing period, loin eye area, fat thickness, height at the withers, chest circumference and shank circumference) in sheep, the influence of *MEF2B, RFXANK, CAMKMT, TRHDE, RIPK2, GRM1, POL, MBD5, UBR2, RPL7* and *SMC2* genes on the formation of these traits was established. Gene annotation showed that these identified genes are transcription factors that regulate myocyte proliferation and fatty acid metabolism [28].

In another study, GWAS analysis revealed that *AADACL3, VGF, NPC1* and *SERPINA12* genes were also significantly associated with the live weight and meat productivity of sheep. Ontology analysis and signaling pathways study showed that these genes are involved in the development of skeletal muscles and lipid metabolism [29].

Table 3. Meat productivity traits of young goats with different genotypes for SNPs in *HMGA2, CRADD* and *MAX* genes (8 months)

Indicator	Gene/Polymorphism/Genotype								
	HMGA2 / rs268269710 A/G			*CRADD* / rs268270492 G/A			*MAX* / rs268234545 A/G		
	AA	AG	GG	AA	AG	GG	AA	AG	GG
Slaughter indicators, morphological composition of carcasses									
Preslaughter weight, kg	32.81 ± 0.59	34.16 ± 0.37	35.73 ± 0.42*	34.64 ± 0.33	31.22 ± 0.54	36.15 ± 0.48**	34.92 ± 0.37	34.83 ± 0.21	33.82 ± 0.46
Hot carcass weight, kg	14.24 ± 0.38	15.12 ± 0.24	16.07 ± 0.29	15.88 ± 0.21	12.65 ± 0.32	15.86 ± 0.29	15.85 ± 0.22	15.02 ± 0.14	14.42 ± 0.27
Internal fat weight, kg	0.96 ± 0.06	1.12 ± 0.12	1.20 ± 0.17	1.00 ± 0.04	0.89 ± 0.06	1.17 ± 0.09	1.15 ± 0.10	1.10 ± 0.04	1.00 ± 0.07
Slaughter weight, kg	15.20 ± 0.32	16.24 ± 0.19	17.27 ± 0.24*	16.88 ± 0.12	13.54 ± 0.24	17.03 ± 0.12**	17.00 ± 0.20	16.12 ± 0.10	15.42 ± 0.18
Slaughter yield, %	46.32 ± 0.44	47.54 ± 0.22	48.33 ± 0.32*	48.73 ± 0.28	43.37 ± 0.32	47.11 ± 0.44*	48.86 ± 0.25	46.28 ± 0.12	45.59 ± 0.21
Boneless meat weight, kg	10.38 ± 0.13	11.28 ± 0.06	12.46 ± 0.10**	12.22 ± 0.05	10.32 ± 0.10	12.21 ± 0.08**	12.04 ± 0.06	11.96 ± 0.03	11.42 ± 0.09
Bone and tendon weight, kg	3.32 ± 0.16	3.64 ± 0.07	3.70 ± 0.09	3.61 ± 0.08	3.35 ± 0.05	3.59 ± 0.04	3.45 ± 0.06	3.49 ± 0.04	3.37 ± 0.04
Ratio of boneless meat to bones and tendons	3.13 ± 0.12	3.10 ± 0.07	3.37 ± 0.10	3.38 ± 0.06	3.08 ± 0.09	3.40 ± 0.11	3.49 ± 0.11	3.42 ± 0.08	3.39 ± 0.09
Loin eye area, cm^2	12.44 ± 0.17	12.46 ± 0.09	13.09 ± 0.14*	13.01 ± 0.04	11.47 ± 0.10	13.21 ± 0.12**	12.26 ± 0.12	12.08 ± 0.06	11.95 ± 0.10
The content in the average sample of minced meat, %									
Moisture	69.40 ± 0.68	68.55 ± 0.72	67.20 ± 0.67	69.14 ± 0.59	69.21 ± 0.72	66.90 ± 0.41	68.72 ± 0.68	68.25 ± 0.72	69.05 ± 0.67
Fat	8.75 ± 0.21	9.23 ± 0.26	9.12 ± 0.18	8.65 ± 0.19	8.41 ± 0.26	9.62 ± 0.18	9.10 ± 0.17	8.88 ± 0.26	8.06 ± 0.18
Ash	1.0 ± 0.04	1.0 ± 0.05	1.1 ± 0.07	1.0 ± 0.05	1.0 ± 0.05	1.1 ± 0.06	1.0 ± 0.05	1.0 ± 0.06	1.0 ± 0.05
Protein	20.90 ± 0.21	21.20 ± 0.34	22.56 ± 0.17	21.21 ± 0.27	22.34 ± 0.43	22.48 ± 0.52	21.16 ± 0.23	21.87 ± 0.43	21.87 ± 0.27
Calorie content, kcal/100 g	167.06 ± 0.39	172.94 ± 0.47	177.38 ± 0.55	167.40 ± 0.40	170.01 ± 0.34	181.63 ± 0.63	171.47 ± 0.39	172.24 ± 0.47	164.71 ± 0.32

In a study performed on the Rendena cattle bred in the Italian Alps important for beef production, it was found that *SLC12A1, CGNL1, PRTG* genes were associated with average daily body weight gain, *LOC513941* gene was associated with carcass yield, *CDC155* gene was associated with the content of boneless meat in the carcass, *NLRP2* gene was associated with both indicators of carcass quality. Metabolic pathways analysis showed that some of the genes were associated with neurogenesis and synaptic signaling in cells, and some were associated with actin synthesis and transmembrane transport [30].

Thus, the results of our research and researches by other authors show that GWAS is the most commonly used and informative method of searching for candidate genes associated with body size indicators and affecting the meat productivity of animals. Despite the fact that different studies have identified different genes, in most cases they were transcription factors that control the differentiation and proliferation of muscle and connective tissue cells, and regulate signaling transmembrane cell pathways.

Conclusion

GWAS analysis using Goat 50K BeadChip high-density DNA microarray determined a genome-wide level of significance for six SNPs located on chromosomes 5, 6, 10, and 16 associated with the body weight of young Karachay goats at the age of 4 and 8 months old.

Of the six SNPs, one was determined to be localized within *CRADD* gene, and other two were localized within the ± 200-kb region in *HMGA2* and *MAX* genes. These SNPs were selected to study the meat productivity traits of young Karachay goats.

It was found that the best meat productivity traits were in young goats with GG genotype in *HMGA2* gene. Compared to animals with AA genotype, they had superiority in preslaughter weight, slaughter carcass weight, slaughter yield, boneless meat weight and loin eye area by 8.9%, 13.6%, 4.3% (P <0.05), 10.5% (P <0.01), and 5.2% (P <0.05) respectively. The meat of young goats with this genotype was characterized by a high protein content of 22.56% and low fat content of 9.12%.

Animals with GG genotype in *CRADD* gene had a higher preslaughter weight, slaughter weight, carcass slaughter yield, boneless meat weight, and loin eye area. Animals with AG genotype were characterized by the lowest indicators. The difference between the compared genotypes in the above characteristics was 15.8%, 25.7% (P <0.01), 8.4% (P <0.05), 18.3%, and 15.7% (P <0.01) respectively. There were no significant differences in the chemical composition of muscle tissue between animals of different genotypes.

HMGA2 and *CRADD* genes are promising for further research, accumulation of more experimental data, approbation of the results obtained with the aim of subsequent use in breeding the Karachay goats to increase meat productivity and meat quality.

REFERENCES

1. Miller, B.A., Lu, C.D. (2019). Current status of global dairy goat production: An overview. *Asian-Australasian Journal of Animal Sciences,* 32(8), 1219–1232. https://doi.org/10.5713/ajas.19.0253
2. Erokhin, A.I., Karasev, E.A., Erokhin, S.A. (2020). Dynamics of goat population and production goat's milk and meat in the world and in Russia. *Sheep, Goats, Wool Business*, 4, 22–25. https://doi.org/10.26897/2074–0840–2020–4–22–25 (In Russian)
3. Erokhin, A.I., Karasev, E.A., Erokhin, S.A. (2018). The state and dynamics of the goat population and goat meat production in the world and Russia. *Sheep, Goats, Wool Business*, 1, 29–31. (In Russian)
4. Aybazov, M.M., Selionova, M.I., Mamontova, T.V. (2019). Exterior and some biological indices of Karachai goats. *Zootechniya*, 12, 5–9. https://doi.org/10.25708/ZT.2019.51.83.002 (In Russian)
5. Selionova, M.I., Trukhachev, V.I, Aybazov, A-M.M., Stolpovsky, Yu.A., Zinovieva, N.A. (2021). Genetic markers of goats (Review). *Agricultural Biology*, 56(6), 1031–1048. https://doi.org/10.15389/agrobiology.2021.6.1031rus (In Russian)
6. Zonaed Siddiki, A.M.A.M., Miah, G., Islam, M.S., Kumkum, M., Rumi, M.H., Baten, A. et al. (2020). Goat genomic resources: The search for genes associated with its economic traits. *International Journal of Genomics*, 2020, Article 5940205. https://doi.org/10.1155/2020/5940205
7. Moaeen-ud-Din, M., Danish Muner, R., Khan, M.S. (2022). Genome wide association study identifies novel candidate genes for growth and body conformation traits in goats. *Science Reports*, 12(1), Article 9891. https://doi.org/10.1038/s41598–022–14018-y
8. Liu, X., Ma, L., Wang M., Wang, K, Li, J., Yan, H., Zhu, H., Lan, X. (2020). Two indel variants of prolactin receptor (PRLR) gene are associated with growth traits in goat. *Animal Biotechnology*, 31(4), 314–323, https://doi.org/10.1080/10495398.2019.1594863
9. Wang, K., Cui, Y., Wang, Z., Yan, H., Meng, Z., Zhu, H. et al. (2019). One 16 bp insertion/deletion (indel) within the KDM6A gene revealing strong associations with growth traits in goat. *Gene,* 686, 16–20. https://doi.org/10.1016/j.gene.2018.11.010
10. Bai, Y., Yuan, R., Luo, Y., Kang, Z., Zhu, H., Qu, L. et al. (2021). Exploration of genetic variants within the goat a-kinase anchoring protein 12 (Akap12) gene and their effects on growth traits. *Animals*, 11(7), Article 2090. https://doi.org/10.3390/ani11072090
11. Bi, Y., Chen, Y., Xin, D., Liu, T., He, L., Kang, Y. et al. (2022). Effect of indel variants within the sorting nexin 29 (SNX29) gene on growth traits of goats. *Animal Biotechnology,* 33(5), 914–919. https://doi.org/10.1080/10495398.2020.1846547
12. Wang, Z., Wang, C., Guo, Y., She, S., Wang, B., Jiang, Y. et al. (2020). Screening of deletion variants within the goat PRDM6 gene and its effects on growth traits. *Animals*, 10(2), Article 208. https://doi.org/10.3390/ani10020208
13. Gu, B., Sun, R., Fang, X., Zhang, J., Zhao, Z., Huang, D. et al. (2022). Genome-wide association study of body conformation traits by whole genome sequencing in Dazu Black Goats. *Animals,* 12(5), Article 548. https://doi.org/10.3390/ani12050548
14. PLINK 1.9 and 2.0 Retrieved from https://zzz.bwh.harvard.edu/plink/plink2.shtml Accessed June 5, 2022.
15. R Development Core Team. (2018). R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria. Retrieved from https://www.R-project.org. Accessed July 17, 2022.
16. Kinsella, R.J., Kähäri, A., Haider, S., Zamora, J., Proctor, G., Spudich, G. et al. (2011). Ensembl BioMarts: A hub for data retrieval across taxonomic space. *Database*, 2011, Article bar030. https://doi.org/10.1093/database/bar030
17. Huang, D.W., Sherman, B.T., Lempicki, R.A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols*, 4(1), 44–57. https://doi.org/10.1038/nprot.2008.211
18. Benjamini, Y., Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological),* 57(1), 289–300. https://doi.org/10.1111/j.2517–6161.1995.tb02031.x
19. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union, L 276/33 Retrieved from https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ: L:2010:276:0033:0079: en: PDF Accessed October 10, 2022.
20. European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. European Treaty Series — No. 123. Retrieved from https://rm.coe.int/168007a67b Accessed October 10, 2022.
21. Wong, M., Islas-Trejo, A., Medrano, J.F. (2002). Structural characterization of the mouse high growth deletion and discovery of a novel fusion transcript between suppressor of cytokine signaling-2 (Socs-2) and viral encoded semaphorin receptor (Plexin C1). *Gene*, 299(1–2), 153–163. https://doi.org/10.1016/s0378–1119(02)01052–1
22. Ramos, A.M., Pita, R.H., Malek, M., Lopes, P.S., Guimara, S.E.F., Rothschild M. F. (2009). Analysis of the mouse high-growth region in pigs. *Journal of Animal Breeding and Genetics,* 126(5), 404–412. https://doi.org/10.1111/j.1439–0388.2009.00801.x
23. Quan, J., Ding, R., Wang, X., Yang, M., Yang, Y., Zheng, E. et al. (2018). Genome-wide association study reveals genetic loci and candidate genes for average daily gain in Duroc pigs. *Asian-Australasian Journal of Animal Sciences,* 31(4), 480–488. https://doi.org/10.5713/ajas.17.0356
24. Li, X., Huang, Q., Feng, L., Guo, Y., Liang, J., Lan, G. (2021). Sequence and expression differences of BMP2 and, FGFR3 genes in Guangxi Bama mini pig and Landrace pig. *Journal of Southern Agriculture*, 52(6), 1709–1718. https://doi.org/10.3969/j.issn.2095–1191.2021.06.032
25. Pan, B., Long, H., Yuan, Y., Zhang, H., Peng, Y., Zhou, D. et al. (2022). Identification of body size determination related candidate genes in domestic pig using genome-wide selection signal analysis. *Animals*, 12(14), Article 1839. https://doi.org/10.3390/ani12141839
26. Blaževitš, O., Bolshette, N., Vecchio, D., Guijarro, A., Croci, O., Campaner, S. et al. (2020). MYC-associated factor MAX is an essential regulator of the clock core network. *International Journal of Molecular Sciences,* 21(7), Article 2294. https://doi.org/10.3390/ijms21072294
27. Selionova, M.I., Mamontova, T. V., Aybazov, M.M., Petrovic V. C., Petrovic, M.P. (October 6–8, 2021). *Quality of Aborigenous Karachay Goat Meat Under Different Conditions.* Article Conference at the Proceedings of the 13th International Symposium Modern Trends in Livestock Production, Belgrade, Serbia, 117–125.
28. Zhang, L., Liu, J., Zhao, F., Ren, H., Xu, L., Lu, J. et al. (2013). Genome-wide association studies for growth and meat production traits in sheep. *PLoS ONE,* 8(6), Article e66569. https://doi.org/10.1371/journal.pone.0066569
29. Lu, Z., Yue, Y., Yuan, C., Liu, J., Chen, Z., Niu, C. et al. (2020). Genome-wide association study of body weight traits in Chinese fine-wool sheep. *Animals*, 10(1), Article 170. https://doi.org/10.3390/ani10010170
30. Mancin, E., Tuliozi, B., Pegolo, S., Sartori, C., Mantovani, R. (2022). Genome Wide Association Study of Beef Traits in Local Alpine Breed Reveals the Diversity of the Pathways Involved and the Role of Time Stratification. *Frontiers Genetics*, 12, Article 746665. https://doi.org/10.3389/fgene.2021.746665

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

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Keywords: *mutton, trace elements, proteins, Ovis aries, myocardium, cortical substance of the kidneys*

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

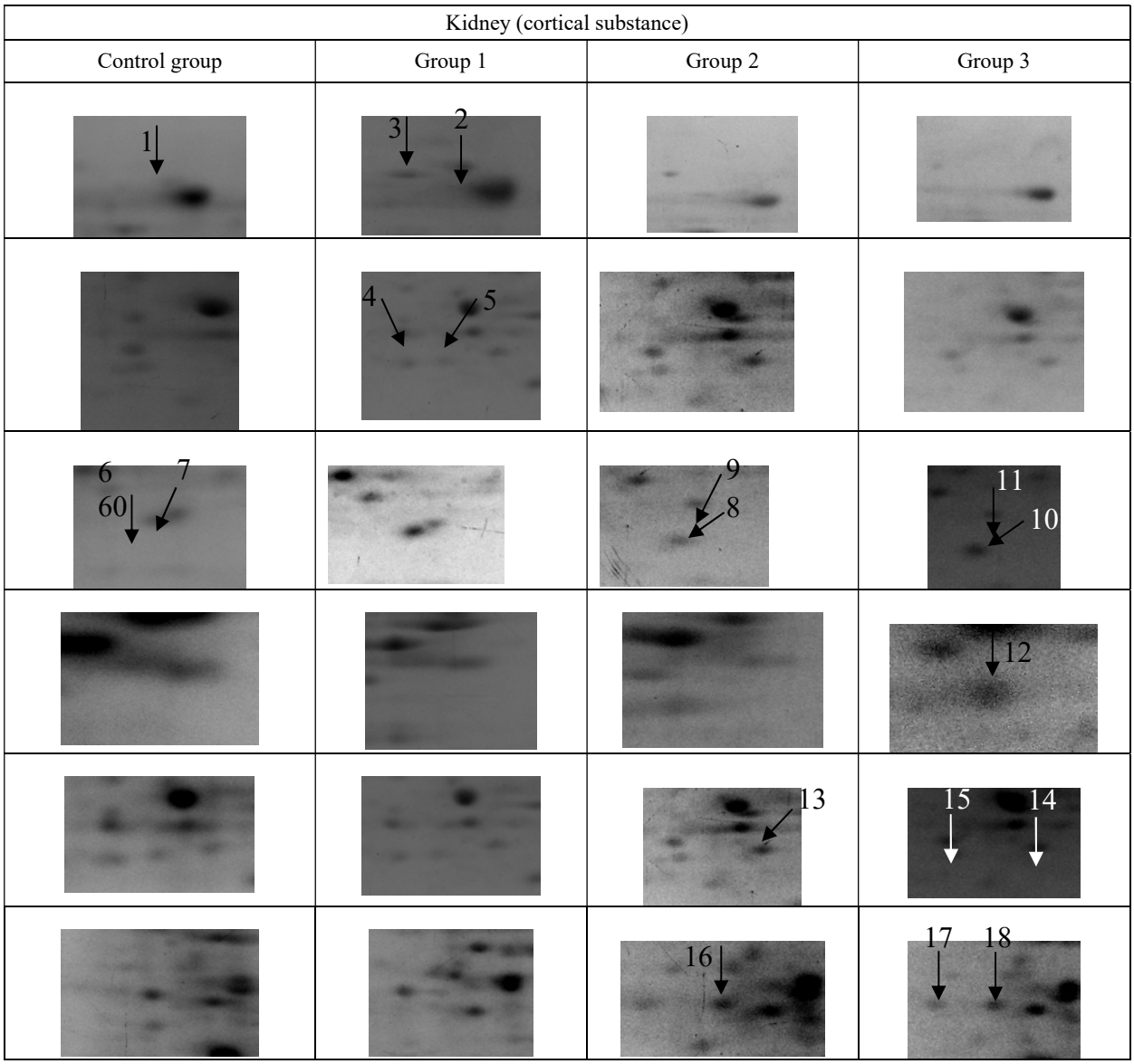

Figure 1. Fragments of DE zones of proteins, showing changes depending on the fattening diet (cortical substance of the kidney). Identified fractions are shown by arrows with numbers in accordance with the Table 1

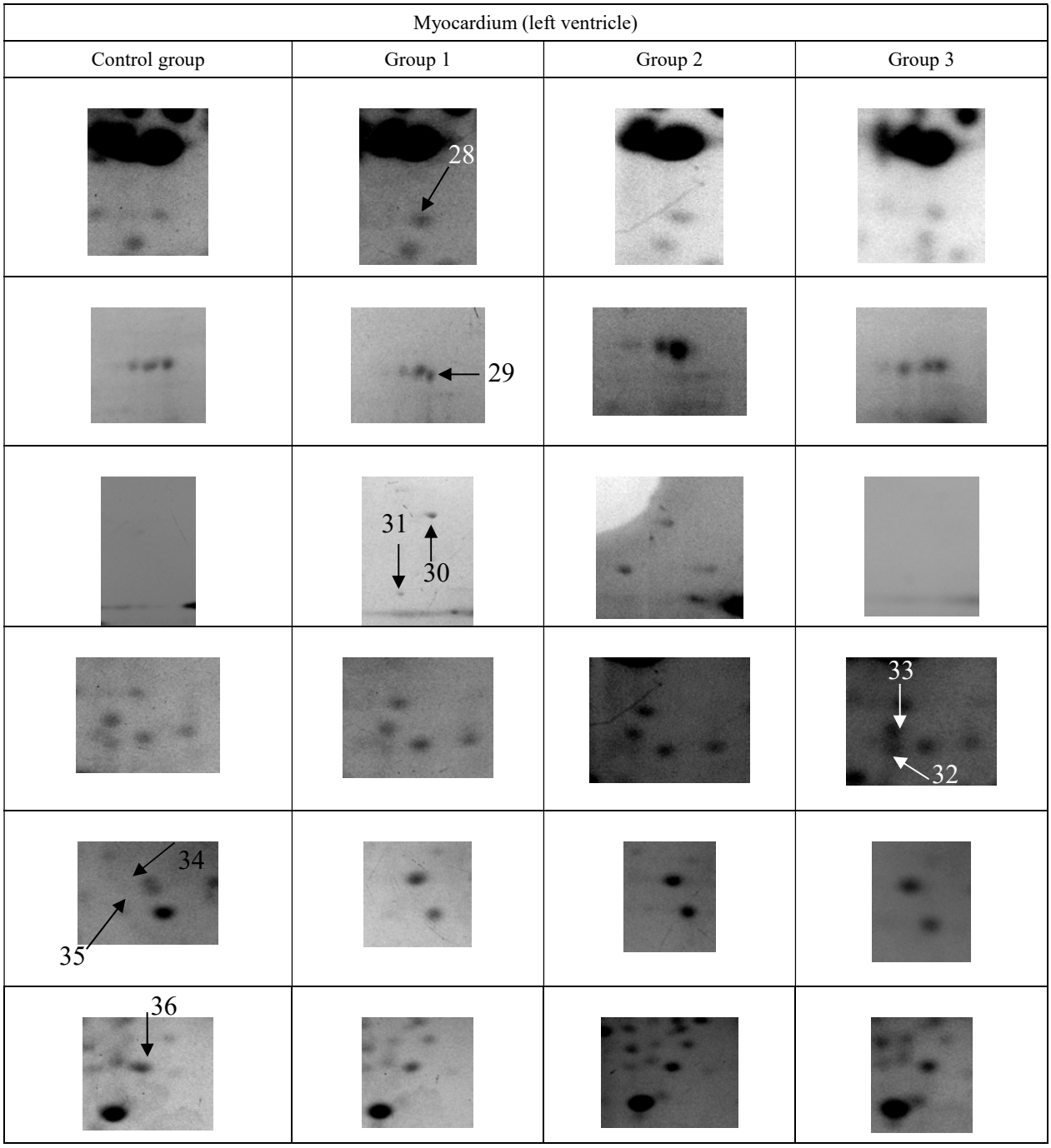

Figure 2. Fragments of DE zones of proteins showing changes depending on the fattening diet (Myocardium (left ventricle). The identified fractions are shown by arrows with numbers in accordance with the Table 1

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.

REFERENCES

1. Giro, T.M., Kulikovsky, A.V., Knyazeva, A.S., Domnitsky, I. Yu., Giro, A.V. (2020). Biochemical and microstructural profile of the thyroid gland from lambs raised on experimental rations. *Food Processing: Techniques and Technology*, 50(4), 670–680. https://doi.org/10.21603/2074–9414–2020–4–670–680 (In Russian)
2. Paredi, G., Raboni, S., Bendixen, E., de Almeida, A.M., Mozzarelli, A. (2012). «Muscle to meat» molecular events and technological transformations: the proteomics insight. *Journal of Proteomics*, 75(14), 4275–4289. https://doi.org/10.1016/j.jprot.2012.04.011
3. Giro, T.M., Ilina, L.A., Kulikovsky, A.V., Ziruk, I.V., Giro, A.V. (2022). Molecular genetic studies of microbiocenosis and microstructure of jejunum wall in young rams grown on biofortified feed additives. *Foods and Raw Materials*, 10(2), 310–317. https://doi.org/10.21603/2308–4057–2022–2–541
4. Shishkin, S.S., Kovalev, L.I., Kovaleva, M.A., Ivanov, A.V., Eremina, L.S., Sadykhov, E.G. (2014). The application of proteomic technologies for the analysis of muscle proteins of farm animals used in the meat industry (Review). *Applied Biochemistry and Microbiology*, 50(5), 421–432. https://doi.org/10.1134/S0003683814050093
5. Zamaratskaia, G., Li, S. (2017). Proteomics in meat science — Current status and future perspective. *Theory and Practice of Meat Processing*, 2(1), 18–26. https://doi.org/10.21323/2414–438X-2017–2–1–18–26 (In Russian)
6. Nair, M. N., Zhai, C. (2020). Application of proteomic tools in meat quality evaluation. Chapter in a book: Meat Quality Analysis: Advanced Evaluation Methods, Techniques, and Technologies. Academic Press, 2020. https://doi.org/10.1016/B978–0–12–819233–7.00019–7
7. Ortea, I., O'Connor, G., Maquet, A. (2016). Review on proteomics for food authentication. *Journal of Proteomics*, 147, 212–225. https://doi.org/10.1016/j.jprot.2016.06.033
8. Yu, T.-Y., Morton, J.D., Clerens, S., Dyer, J.M. (2016). Proteomic investigation of protein profile changes and amino acid residue level modification in cooked lamb longissimus thorais et lumborum: The effect of roasting. *Meat Science*, 119, 80–88. https://doi.org/10.1016/j.meatsci.2016.04.024
9. Li, Z., Li, X., Gao, X., Shen, Q.W., Du, M., Zhang, D. (2017). Phosphorylation prevents in vitro myofibrillar proteins degradation by μ-calpain. *Food Chemistry*, 218, 455–462. http://doi.org/10.1016/j.foodchem.2016.09.048
10. Naveena, B.M., Jagadeesh, D.S., Babu, A.J., Rao, T.M., Kamuni, V., Vaithivanathan, S., Kulkami, V. V., Rapole, S. (2017). OFFGEL electrophoresis and tandem mass spectrometry approach compared with DNA-based PCR method for authentication of meat species from raw and cooked ground meat mixtures containing cattle meat, water buffalo meat and sheep meat. *Food Chemistry*, 233, 311–320. https://doi.org/10.1016/j.foodchem.2017.04.116
11. Ferreira, A.M., Grossmann, J., Fortes, C., Kilminster, T., Scanlon, T., Milton, J. et al. (2017). The sheep (Ovis aries) muscle proteome: Decoding the mechanisms of tolerance to Seasonal Weight Loss using label-free proteomics. *Journal of Proteomics*, 161, 57–67. http://doi.org/ 10.1016/j.jprot.2017.03.020
12. Kiran, M., Maheswarappa, N.B., Banerjee, R., Ch, V., Rapole, S. (2021). Impact of stunning before slaughter on expression of skeletal muscles proteome in sheep. *Animal Biotechnology*, 1–8. http://doi.org/10.1080/10495398.2021.1976198
13. Gao, X., Zhao, D., Wang, L., Cui, Y., Wang, S., Lv, M. et al. (2021). Proteomic changes in sarcoplasmic and myofibrillar

proteins associated with color stability of ovine muscle during post-mortem storage. *Foods,* 10(12), Article 2989. https://doi.org/10.3390/foods10122989
14. Zvereva, E.A., Kovalev, L.I., Ivanov, A.V., Kovaleva, M.A., Zherdev, A.V., Shishkin, S.S. et al. (2015). Enzyme immunoassay and proteomic characterization of troponin I as a marker of mammalian muscle compounds in raw meat and some meat products. *Meat Science*, 105, 46–52. https://doi.org/10.1016/j.meatsci.2015.03.001
15. Capozzi, F., Trimigno, A., Ferranti, P. (2017). Proteomics and metabolomics in relation to meat quality. Chapter in a book: Poultry Quality Evaluation: Quality Attributes and Consumer Values. Woodhead Publishing, 2017. https://doi.org/10.1016/B978–0–08–100763–1.00009-X
16. Della Malva, A., Marino, R., Santillo, A., Annicchiarico, G., Caroprese, M., Sevi, A. et al. (2017). Proteomic approach to investigate the impact of different dietary supplementation on lamb meat tenderness. *Meat Science*, 131, 74–81. http://doi.org/10.1016/j.meatsci.2017.04.235
17. Troshina, E. A., Senyushkina, E. S., Terekhova, M. A. (2018). The role of selenium in the pathogenesis of thyroid disease. *Clinical and Experimental Thyroidology*, 14(4), 192–205. https://doi.org/10.14341/ket10157 (In Russian)
18. Pecoraro, B.M., Leal, D.F, Frias-De-Diego, A., Browning, M., Odle, J., Crisci, E. (2022). The health benefits of selenium in food animals: a review. *Journal of Animal Science and Biotechnology,* 13, Article 58. https: //doi.org/10.1186/s40104–022–00706–2
19. Cao, J., Guo, F., Zhang, L., Dong, B., Gong, L. (2014). Effects of dietary Selenomethionine supplementation on growth performance, antioxidant status, plasma selenium concentration, and immune function in weaning pigs. *Journal of Animal Science and Biotechnology*, 5(1), Article 46. http://doi.org/10.1186/2049–1891–5–46
20. Shishkin, S.S., Kovalyov, L.I., Kovalyova, M.A., Lisitskaya, K.V., Eremina, L.S., Ivanov, A.V., et al. (2010). "Prostate cancer proteomics" database. *Acta Naturae*, 2(4(7)), 95–104. https://doi.org/10.32607/20758251–2010–2–4–95–104
21. Multi-Level Information Database "Muscle organs proteomics" Retrieved from http://mp.inbi.ras.ru. Accessed September 18, 2022

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