ТВАРИННИЦТВО ТА ТЕХНОЛОГІЇ ХАРЧОВИХ ПРОДУКТІВ

Засновник:
Національний університет біоресурсів і природокористування України

Рік заснування: 2010

Рекомендовано до друку та поширення
через мережу Інтернет Вченою радою
Національного університету біоресурсів і природокористування України
(протокол № 4 від 23 листопада 2022 р.)

Свідоцтво про державну реєстрацію
друкованого засобу масової інформації
серії КВ 25125-15065 ПР від 17 лютого 2022 р.

Журнал входить до переліку наукових фахових видань України
Категорія «Б». Галузь знань: Сільськогосподарські науки,
спеціальності – 181 «Харчові технології»,
204 «Технологія виробництва і переробки продукції тваринництва»,
207 «Водні біоресурси та аквакультура»
(Наказ Міністерства освіти і науки України від 17 березня 2020 р. № 409)

Журнал представлено у міжнародних наукометричних базах даних, репозитаріях та пошукових системах: Index Copernicus International,
Google Scholar, Academic Resource Index ResearchBib,
Національна бібліотека України імені В. І. Вернадського, MIAR, BASE, AGRIS

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https://animalscience.com.ua/uk

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Founder:
National University of Life and Environmental Sciences of Ukraine

Year of foundation: 2010

Recommended for printing and distribution
via the Internet by the Academic Council
of National University of Life and Environmental Sciences of Ukraine
(Minutes No. 4 of November 23, 2022)

Certificate of state registration
of the print media
Series KV No. 25125-15065 PR of February 17, 2022

The journal is included in the list of Professional Scientific Publications of Ukraine
Category "B". Field of knowledge: Agricultural Science,
specialties – 181 "Food Technology",
204 "Technology of Production and Processing of Livestock Products",
207 "Aquatic Bioresources and Aquaculture"
(order of the Ministry of Education and Science of Ukraine of March 17, 2020, No. 409)

The journal is presented international scientometric databases, repositories and scientific systems: Index Copernicus International,
Google Scholar, Academic Resource Index ResearchBib,
Vernadsky National Library of Ukraine, MIAR, BASE, AGRIS

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Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 13(4), 7-18
Received: 12.09.2022 Revised: 10.10.2022 Accepted: 26.10.2022

UDC 664.3.032.9
DOI: 10.31548/animal.13(4).2022.7-18

Research on the effectiveness of means for intensifying oil compression in twin-screw extrusion presses

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Abstract. The relevance of the study is due to the problem that is characteristic of various types of screw press structures, which leave up to 16% of the oil in the cake when pressing sunflower oil. In this regard, the purpose of this study is to find rational methods to improve the design of twin-screw extrusion presses to increase the oil yield. The leading methods for solving this problem are empirical research methods that allow comprehensively considering the stages of improving the geometric configuration of the screw shaft based on observation and finding a rational solution to the problem by measuring and experimenting. The paper analyses the features of technical means for grinding oil-containing raw materials in presses and extrusion presses, justifies the need to develop new grinding working bodies. The need to supplement the existing ideas about the interaction of special grinding working bodies with transport and compressive

Suggested Citation:
Hudzenko, M., Vasyliv, V., Zheplinska, M., Burova, Z., & Sarana, V. (2022). Research on the effectiveness of means for intensifying oil compression in twin-screw extrusion presses. *Animal Science and Food Technology*, 13(4), 7-18.

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screw nozzles and the development of grinding units for twin-screw extrusion presses produced in Ukraine are clarified. Experimental studies were conducted with the developed design of new working bodies – cylindrical-conical nozzles. They create an intermediate compression of oil-containing raw materials, aimed at intensifying grinding with simultaneous mixing of the processed oil-containing material. A triple repetition of the increase in the degree of compression was obtained when installing three pairs of cylindrical-conical nozzles along the length of the screw shaft. Their use allowed for intensifying the degree of pressure increase and the additional oil yield of up to 3.1%. With each processed ton of seeds, due to the increase in the amount of pressed oil, the profit of enterprises that will improve twin-screw extrusion presses with these working bodies will also increase accordingly.

Keywords: oil production, oil press, geometric parameters, cylindrical cone nozzles, oil yield

Introduction

Most vegetable oil in the food industry is produced in large industrial plants using solvent extraction, and screw presses are mainly used for pre-pressing seeds with a high oil content (Famurewa *et al.*, 2021). In modern economic conditions, the chosen technology for processing raw materials and the selection of necessary equipment, especially screw presses, is of great importance in the production of oil. In agricultural enterprises specialising in oil production, a mechanical method of squeezing oil in screw presses or extrusion presses is used.

Pressing oil-containing raw materials or pressing oil is the main technological operation in the production of oil, which determines the efficiency of the entire enterprise (Mushtruk *et al.*, 2020). In most cases, the oil is pressed from a mass (pulp) previously prepared from oilseeds. However, when using simplified technological schemes, it is possible to squeeze oil directly from purified oil-containing seeds. The main technological schemes (Alonge & Jackson, 2019) and various types of modern single-screw press designs (Indartono *et al.*, 2019; Fakayode & Ajav, 2019; Pedretti *et al.*, 2019), single-screw and twin-screw extrusion presses (Romero-Guzmán *et al.*, 2020; Evon *et al.*, 2021), which are used in agricultural processing plants and oil pressing enterprises, are described and widely investigated in the processing of various oilseeds. The development of screw presses and extrusion presses in various fields of technology is associated with using continuous production methods and the ability to convert extrusion presses to meet any industry requirements (Choton *et al.*, 2020). Screw presses for oil pressing have a simple design, are easy to maintain, do not require highly qualified technicians to operate, are adapted to different types of oil, can be quickly installed on small farms, and the by-product (cake) can be used as animal feed (Amiolemhen & Eseigbe, 2019).

Most often, the actual task in the design of oil presses is to create universal machines for squeezing oil from almost all types of oil-containing crops (with an oil content of at least 20-15%) (Mushtruk *et al.*, 2020; Lara-Ojeda *et al.*, 2021). The re-equipment of the press by replacing individual parts (screw nozzles, seer slats, yield nozzles in the press matrix, etc.) and setting specific technological parameters of the working process for this raw material is provided to reconfigure the work of the press from one raw material to another. In this regard, there are many different proposals for combining preparatory operations (grinding, water-heat treatment) in one machine – a screw press. These proposals are partially implemented in the form of serial press units, twin-screw extrusion presses, or more often, in the form of described inventions to patents. In addition to design proposals, various types of optimisation methods used for various processes, including modelling and software used in optimisation processes for oil extraction from plant materials, are considered (Bup & Foncha, 2020).

In oil production, screw presses are constantly being improved to increase oil yield, reduce energy consumption during the pressing process, improve the quality of the pressed oil, etc. (Alonge & Jackson, 2019; Chowdhury & Mahmud, 2020). One of the main areas of development of modern presses for pressing oil is the intensification of their technological process by simultaneous action of various physical-mechanical factors on oil-containing raw materials. The design of twin-screw extrusion presses involves combining several processes in one machine: grinding, heating, and compressing oil-containing raw materials with their separation into oil and cake. The type of working elements of the pressing path of the extrusion presses and their design parameters have a substantial impact on these processes. Considering a substantial number of screw and extrusion presses designs (Badmus *et al.*, 2019; Romero-Guzmán *et al.,* 2020; Famurewa *et al.*, 2021), the current expansion of their functionality and, the development of designs of working bodies of these presses for low-production enterprises are becoming relevant.

The purpose of the study is to determine the trend in the development of designs of working bodies of screw presses due to the analysis of modern studies. The main task of the paper was to offer new development with improving the quality characteristics of the twin-screw extrusion presses.

Literature Review

Many studies by foreign researchers are aimed at understanding the mechanism of interaction of the state of preparation of oil-containing raw materials with the design and technological parameters of screw presses. Their work is being conducted to develop effective press designs to increase oil yield. Numerous attempts have been made to improve the efficiency of oil extraction by pressing. In

general, three areas of work were investigated: optimisation of the operating parameters of the oil pressing process; improvement of the geometric configuration of the screw path of the press; preliminary preparation of seeds for pressing. However, many of these studies are the result of criteria based on the experience and intuition of press manufacturers and operators, rather than a thorough theoretical analysis of the physical principles involved in the oil extraction process. Although screw presses have been used for decades in the oil extraction industry, there are no satisfactory mathematical models to describe the pressing process, especially in the case of solid and liquid phase separation processes.

The operating conditions of the screw press also have a substantial impact on the efficiency of oil pressing. These conditions are considered in the papers (Indartono *et al*., 2019; Bogaert *et al*., 2020; Eseoha & Akubuo, 2021): seed properties, temperature, seed feed rate, screw rotation speed, and screw profile, which can determine the degree of filling of the screw shaft, pressure increase and friction. Thus, the effect of pressure on pressing efficiency can be investigated by applying different seed moisture content and operating modes (conditions) during the oil pressing process with a screw press, and different screw configurations. For all the seed moisture values tested, the oil pressed at a pressing temperature of 60 and 75°C was greater than the amount of oil at 45°C, and the oil yield improved with increasing pressing temperature. Similar conclusions that oil pressure is affected by an increase in temperature are confirmed experimentally in the study (Karaj & Muller, 2021) when using various design parameters of a screw press.

The study by Indartono *et al*. (2019) covered the effect of seed moisture content, pressing temperature, and seed feed rate on oil yield and quality. An innovative single-screw press with various screw shafts was developed to determine the optimal performance of squeezing oil from Calophyllum seeds. It was confirmed that with a low moisture content (less than 5%) in oil-containing raw materials, the pressure in the screw press can increase due to increased seed hardness. It is known that the higher the pressure in the screw press, the larger the volume of oil pressed. It was identified that humidity of less than 5.5% does not improve oil yield.

Seed moisture is a parameter that can be measured and changed as needed. An important factor in optimising the process of pressing oilseeds is to determine the most effective humidity indicator, at which the oil yield is the highest (Antoniassi *et al*., 2022). Based on the research of many scientific papers, the study (Bălțatu *et al*., 2022) concluded that there is an optimal humidity level for each type of oilseed.

The pressing time of oil-containing raw materials in a screw press in operation (Bogaert *et al*., 2020) is defined as a key parameter for intensifying oil compression. In the paper, two sets of working bodies were investigated (while the screw nozzles had different profiles of turns, had breaks, or were placed so as to create a reverse flow). The first set, consisting of the original Reinartz AP08 screw model (recommended by the manufacturer), had a constant diameter and reverse trajectories of the compression screw nozzles. The second set, Olexa arrangement, had an increased diameter of compressive screw nozzles and a larger number of compression valves to break the flow of oil-containing raw materials in front of each compressive nozzle. This geometry ensured the alternation of high-pressure (compression) and low-pressure (relaxation-mixing) sections. Oil was pressed exclusively in the compression sections. Intensive mixing of the oil-containing material was detected in the low-pressure sections. In some designs of industrial screw presses, a special ring is added to the set of working bodies of the screw shaft, which is called a ripper. It slows down the flow of raw materials through it and increases intermediate shear forces in the area in front of it. This ripper is recommended for additional grinding of seeds with a high fibre content, such as flax seeds. If this is not done, the seeds stick together to form layers that make it difficult to extract oil.

The design of a twin-screw extrusion press equipped with set screw nozzles, some of which are wound in the opposite area, was proposed to obtain more intensive mechanical processing of seeds. This configuration leads to a longer time of raw materials in their zone and a high shear pressure in the upper engagement of the coils (Vasilachi & Biriș, 2019). The consistent placement of different screw nozzle combinations results in more efficient material compression, dynamic dynamic plug creation, and good oil compression, which is caused by an effective pressure increase. Due to the selection of replaceable elements of screw nozzles, it is possible to provide the necessary geometry of the working channel, and therefore the corresponding shear stresses in different sections along the length of the twin-screw extrusion press, which makes it more versatile.

The advantage of the modular design of a twin-screw extrusion press is described in the paper (Evon *et al*., 2021). It confirms the effective ability to configure the screw using a variety of elements that have different profiles of screw turns, and the ability to swap different working sections in the extrusion presses. The correct choice of screw configuration is an important point to maximise the quality of extruded products.

In the study (Lyng *et al*., 2022), experiments were conducted to examine the effect of a combination of different working bodies on the extrusion process and on the quality characteristics of the product. Various screw configurations were investigated. For example, configuration A consisted of one reverse element section, one mixing section, and five transport sections. Screw configuration B consisted of two reverse element sections and four transport sections, while screw configuration C consisted of three reverse element sections and three transport sections. All three screw configurations had one mixing section. The ability to combine both the working elements of the screw shaft and individual sections is a great advantage of the design of extrusion presses over conventional presses.

There are many different proposals for improving the design of oil-pressing screw presses in the scientific

literature (Olaoye *et al.*, 2020; Sheikh & Zakiuddin, 2019; Hudzenko *et al.*, 2020). Conventionally, they can be divided into two directions: creating a screw press for squeezing oil from a specific crop (Ogunlade & Aremu, 2020; Alabi *et al.*, 2022), or a universal screw press that combines not only pressing operations, but also the presence of auxiliary chambers (grinding, water-heat treatment (Fakayode & Ajav, 2019; Didur *et al.*, 2019), or even a complete set of individual new working bodies, leading to an increase in oil yield and press productivity and a simultaneous reduction in production areas due to a reduction in auxiliary equipment. For example, the paper (Evon *et al.*, 2021) describes a developed universal twin-screw extrusion presses to provide efficient thermo-mechanical-chemical pretreatment of lignocellulose biomass before using it as a source of mechanical hardening in fully bio-fibre boards. Various lignocellulose byproducts of crop production have already been successfully pretreated using this process, such as grain straw (especially rice), coriander straw, flax straw pods, and the bark of amaranth and sunflower stalks (Evon *et al.*, 2021). Its mixing ability and the ability to add various components at the end of the screw profile are also advantages of this extrusion press. Such a combined process in one pass of the extrusion press reduces production time and costs and can lead to a reduction in enterprise size since all operations are performed in one step. Such a one-step extrusion operation can be a source of valuable intensification of the industrial process.

From the analysis of various scientific papers, it was identified that the implementation of additional intermediate compression of oil raw materials in the press path attracts great attention from engineers. Among many patented ideas, there are often structurally complex ones to create, which substantially increase the complexity of manufacturing, maintaining the structure, and, accordingly, the final cost of the product.

Materials and Methods

Experimental studies were conducted based on the training, research, and production laboratory on the vegetable oil production line (VOPL) and at the enterprise of PE "Plasma" from 2011 to 2019. The pressing equipment of this line consisted of serial twin-screw extrusion presses of the EK 75/1200 brand produced by NPP "Extruder" and a final pressing machine. The main technical characteristics of the EK-75/1200 serial press extrusion press are shown in Table 1.

Table 1. *Technical characteristics of the EK75/1200 press extrusion press*

Parameter	Value
Productivity (for whole sunflower seeds), kg/h	150-175
Installed capacity, kW	18.3
Power consumption, kW	up to 16
Electric motor power, kW	7.5
Power of electric heaters, kW	up to 12
Heating temperature of cases (depending on raw materials), °C	up to 150
Shaft speed, rpm	30-60

Source: EK 75/1200 extrusion presses operation data sheet

One of the three twin-screw extrusion presses was improved to examine the intensification of oil pressing in screw presses. In production conditions, experimental studies were conducted, which consisted of two stages. The first stage was conducted with the design of the pressing path of the manufacturer and the second – with a new design of the pressing path and sets of working bodies, which included developed cylindrical-conical nozzles (Fig. 1).

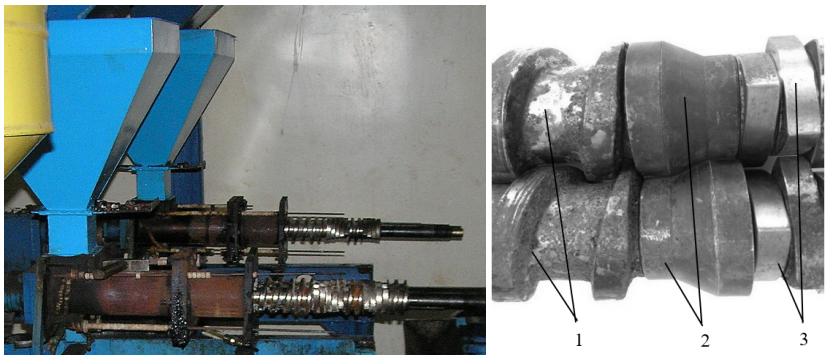

Figure 1. Fragments of working bodies of a twin-screw extrusion presses: a) – the experimental extrusion presses during the replacement of working bodies, b) – a fragment of working bodies, which included the developed cylindrical-cone nozzles: 1 – screw nozzles; 2 – cylindrical-cone nozzles; 3 – triangular cam nozzles

The design-technological parameters of the experimental extrusion press, the geometric parameters of the screw shaft, both factory and developed new nozzles, their ratio, composition, and characteristics of measuring devices are described in the papers (Hudzenko *et al*., 2020; Gudzenko *et al*., 2021).

The shaft speed was measured using a UNI-T UT-372 digital tachometer. The drive and the case heating system were controlled from the control panel, which is made as a separate unit. The control panel scheme is implemented based on the Microchip 16f874 microcontroller, with LCD display indicators that present the values of set and controlled parameters. During the experiment, the temperature values in two heating zones, the current consumed by the electric motor, and the voltage of each of the three supply phases were recorded. Temperature measurement in two heating zones of the extrusion presses case was performed with thermocouples with Philips KTY-81 elements.

A batch of whole sunflower seeds with a humidity of 7.4% was selected as the investigated raw material, which was determined by the Farmcomp WILE-55 moisture meter. Before pressing, the seeds were cleaned on an air-sieve separator. The choice of devices and measuring equipment was conducted on the condition that they provide measurement accuracy regulated by standard methods described more thoroughly in the paper (Hudzenko *et al*., 2020). The methods of mathematical planning and those developed in the course of previous studies (Mushtruk *et al*., 2020; Gudzenko *et al*., 2021) were used, and a second-order central compositional plan was chosen as the basis to implement the experiment. Processing of data obtained during the experiment and their graphic design was conducted using Microsoft Office Excel 2016, Compass-3D v18.1 computer software.

A control batch of seeds in the general flow was processed in VOPL to determine the oil yield, considering the production conditions of operation. During the experiment, seeds entered the extrusion presses by gravity through a hopper. In order not to violate the technological conditions of oil compression and approximate the experiment to production conditions, the flow of seed mass into the extrusion press hopper was not interrupted, and all measurements were made when the extrusion presses reached steady-state operation. All readings from the control panel, temperature sensors, and oil yield measurement were performed in a control unit of time of 10 minutes. During this time, the oil and cake coming out of the extrusion press were collected in special containers, which were then weighed with Domotec Plus DT52 electronic scales. Thus, the oil yield was calculated based on equation (1):

$$Y_o = \frac{w_o}{w_c} \times 100 \quad (1)$$

where Y_o – oil yield, %; w_c – cake weight, kg; w_o – oil weight, kg;

All control measurements in this study were repeated three times, and the data obtained were presented on average.

Results and Discussion

From the conducted literature and patent search for means for intensifying oil pressing, the task of improving the twin-screw extrusion presses was conducted by introducing new working bodies that create intermediate support for oil-containing raw materials along the length of the press path and intensifying crushing with simultaneous mixing of the processed material.

The physical essence of the improvement made for the EK-75/1200 twin-screw extrusion press is confirmed and partially described when optimising the geometry of the screw shaft of the single-screw press reflected in the study (Carré, 2022). Namely, it indicates the generally accepted statements that high pressure is needed for maximum oil production. hus, it is necessary to create a situation where the oil-containing raw material resists the traction of the screw turns, sufficient to create this pressure, without moving to a situation where the screw shaft can no longer overcome this resistance force, jamming the shafts. From the standpoint of screw shaft geometry, it is necessary to consider the importance of the phenomena of backflow and sliding of oil-containing raw materials, which reduce the performance of presses when the counteraction power exceeds the push power. Therefore, these phenomena have a limiting effect on pressure. In addition, increasing the time during which the material is compressed leads to additional heating, which should also be considered.

Special attention should be paid to the plasticity of oil-containing raw materials. It depends on parameters such as the water and oil content of the raw materials before pressing and their temperature and grinding condition. Plasticity, in turn, is explained as the compressibility of oil-containing raw materials, its ability to resist pushing through the turns of screws and, consequently, create pressure or move or flow backwards depending on the geometry of the screw and pressing cage. In order not to change the design of the extrusion presses within the working length of the screw shaft, which is 997 mm, when introducing into its composition the developed working bodies, the length of which is 45 mm, it is proposed to make changes to the set of working bodies of the screw shaft in several variants: reduce the length of the first pair of screw nozzles; apply a reduced number of triangular cam nozzles in groups with cylindrical-conical nozzles; by removing or reducing the length of intermediate rings.

An important point of intensification of oil displacement by new working bodies is the interaction of the working surfaces of a pair of cylindrical-conical nozzles 1 and 2 (Fig. 2). On the shafts 3, 13, screw nozzles 4, 5, 6, 7, cylindrical-conical nozzles 1, 2, groups of triangular cam nozzles 8, 9 are mounted through a keyway connection (not shown in the figure). Screw nozzles 4, 6 and 5, 7 are made with the same outer D1 (75 mm) and inner d1 (55 mm) diameters and differ only in the length of the nozzle, the pitch of the screw crest and its thickness, the values of which are given in Table 2.

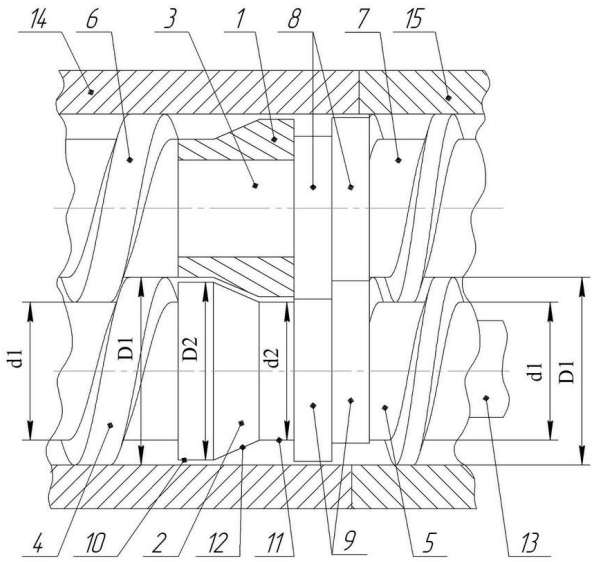

Figure 2. Layout of cylindrical-cone nozzles on the shafts of a twin-screw extrusion press.

Note: 1, 2 – cylindrical-cone nozzles, 3, 13 – shafts, 4, 5, 6, 7 – screw nozzles, 8, 9 – triangular cam nozzles, 10, 11 – cylindrical surfaces of the cylindrical-cone nozzle, 12 – cone surface of the cylindrical-cone nozzle, 14 – section with impermeable walls, 15 – pressing cage section with permeable walls for oil drainage, d1, D1 – inner and outer diameter of the screw nozzles, d2, D2 – inner and outer diameter of the cylindrical cone nozzle

Table 2. *Geometric characteristics of screw nozzles (SN) according to scheme No.53*

	SN1	SN2	SN3	SN4	SN5	SN6	SN7	SN8	SN9
Nozzle length, mm	144	88	96	96	96	48	48	48	48
Screw crest pitch (turn), mm	48	44	37	37	32	24	24	16	12
Screw crest thickness, mm	5	6	7	7	9.9	7.3	7.3	4.7	3.5

Cylindrical-cone nozzle *1* has a simple structure and is made in the form of a rotation part, which has two cylindrical floors on the outside *10*, *11* of larger diameter D2 and smaller diameter d2 and truncated cone surface *12* between them. Cylindrical-conical nozzles are installed on parallel shafts *3, 13* in front of a group of triangular cam nozzles to create an intermediate compression zone for raw materials. Therewith, it is necessary to observe the condition that the cylindrical-conical nozzle *2* is directed to the screw nozzle *4* with the side with a larger diameter D2, and the second cylindrical-cone nozzle *1* is directed to the screw nozzle *6* with the side with a smaller diameter d2. It is recommended to produce the diameter D2 of cylindrical-conical nozzles within the limits given in the ratio (2):

$$D2 = (0.924 \div 0.987) \times D1 \quad (2)$$

where D1 is the outer diameter of the screw nozzles *4*, *5* accordingly;

Reducing diameter D2 of a larger cylindrical surface *10* of the cylindrical-cone nozzle *2* in relation to the outer diameter D1 of the screw nozzles, even by a few millimetres, ensures the penetration of oil-containing raw materials through the formed gaps with simultaneous compression in the area of action of the cylindrical-conical nozzle *1,* and in the area of operation of the cylindrical-conical nozzle *2* a light flow of oil-containing raw materials is formed in the opposite area, which contributes to the renewal of oil-containing layers. Therewith, the live cross-section in the described nozzle area is sufficient to prevent the jamming of the shafts. The value of the diameter d2 of cylindrical-conical nozzles is assumed to be equal to the value of the internal diameter d1 of screw nozzles. As for the group of cam nozzles that are placed behind cylindrical-conical nozzles, their number in each group can consist of at least two or three triangular cam nozzles and an angular displacement of each subsequent one by at least 15°.

The process of intensifying oil compression in the area of action of cylindrical-conical nozzles is conducted as follows. With screw nozzles, oil-containing raw materials (sunflower seeds) are forcibly moved along the shaft to the area of operation of a pair of cylindrical-conical nozzles.

Some of the raw materials find resistance in the wall of the cylindrical-conical nozzle *2* with a diameter of *D2*, partially squeezed into the space between the outer cylindrical surface *10* and the inner surface of the section with impermeable walls *14*. In this space, the oil-containing material undergoes intense compression. Most of the oil-containing material that met resistance in nozzle *2* under the pressure of a new portion of raw materials from the screw nozzles moves to the area of the cylindrical-conical nozzle *1* of the cylindrical body with a smaller diameter *d2*, which contains more free space in cross-section than a cylindrical body with a larger diameter *D2*. In the action of the zone of these cylindrical surfaces, there is a destruction of the stable flow of raw materials created by screw nozzles. In the contact area of cone surfaces *12* of both cylindrical-cone nozzles *1* and *2*, the combined flow of oil-containing material is substantially compacted from the side of nozzle *1* and to a greater extent changes the trajectory of raw materials towards nozzle *2*, although, in the area of cone surfaces, the live cross-section remains constant. In the next contact zone of the body, the part of the cylindrical surface of the larger diameter of the cylindrical-conical nozzle *1* with the inner surface of the section with impermeable walls, oil-containing raw materials are re-compacted. In the adjacent contact zone of the body of the part of the cylindrical surface of the smaller diameter of the cylindrical-conical nozzle *2,* where the live cross-section of the free volume of the part is larger, the next change in the flow layers occurs while simultaneously delayering it. Moving to the zone of two groups of triangular cam nozzles 8 and 9, there is a sharp drop in internal pressure in the oil-containing raw materials, which contributes to loosening with the simultaneous process of crushing and grinding the seed petals compressed in the previous zone of cylindrical-cone nozzles, and therefore renewing the surfaces of oil-containing capillaries. Raw materials are intensively deformed, as a result of which oil flows out not only from the surface but also from the internal capillaries of those particles that have been deformed. In the inter-turn space of pairs of subsequent screw nozzles towards the movement of the oil-containing mass *5* and *7*, which have an even smaller free volume than the previous screw nozzles, raw materials are compacted while oil is squeezed out through special holes in the pressing cage section *15*. Thus, the described interaction of the working surfaces of a pair of cylindrical-cone nozzles together with screw and triangular nozzles provides intermediate support for oil-containing raw materials and the intensification of destruction with simultaneous mixing. And the final support for oil-containing raw materials is created by cone nozzles located on the shafts after the last pair of screw nozzles in the matrix zone.

The main technological and design parameters that affect the efficient operation of the extrusion presses are: the heating temperature of the working area, the speed of rotation of the shafts, the gap in the matrix. Step-by-step studies were conducted at different values with the factory and a new set of working bodies of the pressing path, which included the developed cylindrical-conical nozzles. The results of the first stage of research are presented in the paper (Gudzenko *et al.*, 2021). The initial variable parameters were made the heating temperature of the buildings to obtain a more reliable assessment of the results. Regarding the size of the gap in the matrix (5.0 mm) and the speed of rotation of the shafts (56 rpm), they were left with the values obtained in previous studies. All other technological modes and conditions remained unchanged for the same batch of seeds.

First of all, the difference in geometric parameters and the nature of changes in the free volume along the length of the working path of the press for set No.1 (Fig. 3), which are recommended by the manufacturer and the updated set of working bodies No.2 (Fig. 4), which includes cylindrical-cone nozzles in each conjugate pair of nozzles will be analysed ("SN" – screw nozzles; "CN" – triangular cam nozzles; "CCN" – cylindrical-cone nozzles).

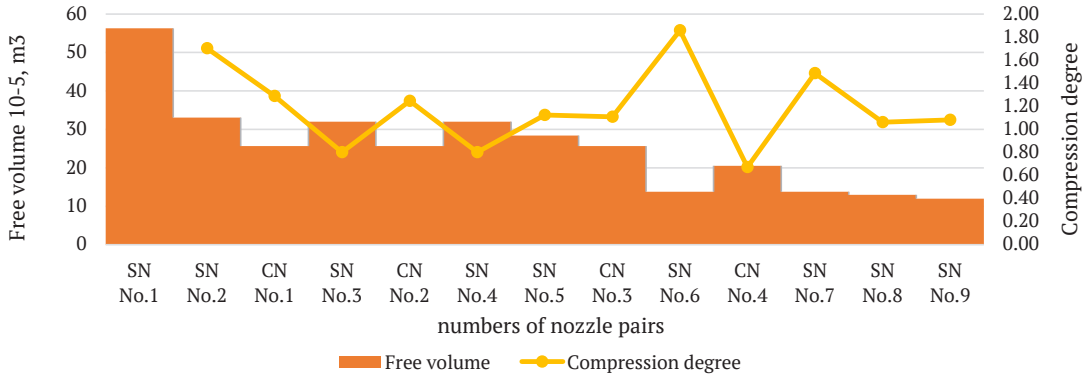

Figure 3. Change in the free volume of working bodies and the degree of compression of raw materials along the pressing path of the screw shaft (set No. 1 of working bodies for sunflower seeds with screw and cam nozzles)

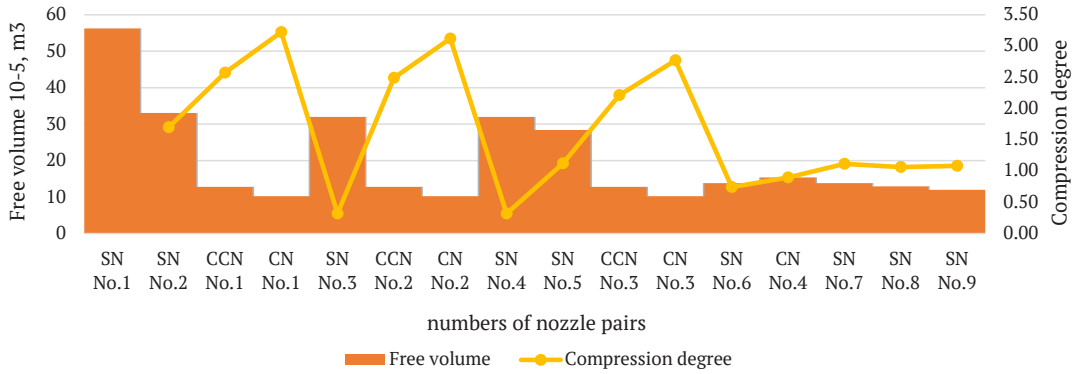

Figure 4. Change in the free volume of working bodies and the degree of compression of raw materials along the pressing path of the screw shaft (set No. 2 of working bodies for sunflower seeds with screw, cam, and cylindrical-cone nozzles)

When comparing charts (Fig. 3 and 4) it is noticeable that when using cylindrical-cone nozzles between screw and cam nozzles on shafts, the value of the compression ratio increases with the increase in the zone of grinding nozzles, and decreases in the screw nozzle following the movement of raw materials. When installing three pairs of cylindrical-conical nozzles along the length of the shaft, a triple repetition of a more rapid increase in the degree of compression is obtained. This ensures intermediate compaction of oil-containing raw materials and intensification of destruction with simultaneous mixing of the processed material.

Based on the obtained experimental results, and after performing their computer processing, graphical dependences of oil yield depending on the set of working bodies (Fig. 5) at variable heating temperatures of enclosures are created. When comparing the obtained oil yield values, the effectiveness of using the developed cylindrical-cone nozzles is evident. Ultimately, with set No. 2, the yield of sunflower oil at different heating temperatures had an increase of up to 3%.

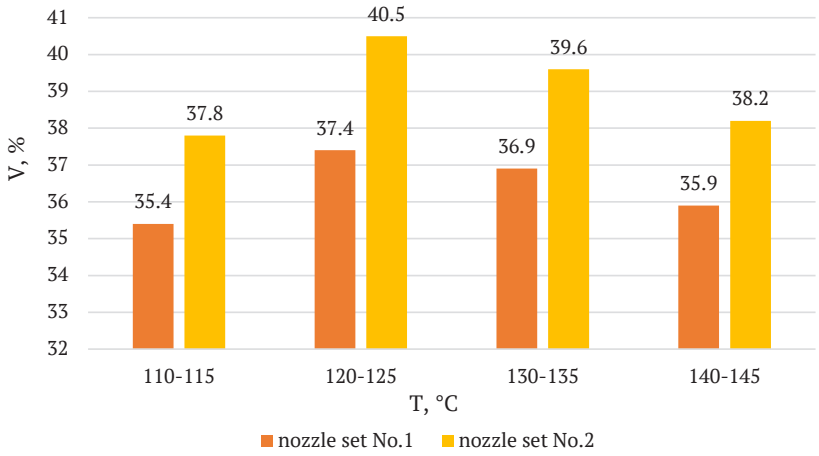

Figure 5. Dependence of the sunflower oil yield on the set of installed nozzles in the extrusion presses pressing path at different heating temperatures of the cases.

Note: 1 – with nozzles from manufacturer, 2 – with cylindrical-cone nozzles

As noted in the previous paper, (Gudzenko *et al.*, 2021) studies conducted with different sets of working bodies, and accordingly with changed geometric parameters of the extrusion presses path, confirmed their effect on the oil yield. With the same operating modes and technological adjustments of the extrusion presses, the presence of additional intermediate compression of raw materials by cylindrical-cone nozzles affects the throughput, namely the productivity of the extrusion presses. However, such changes in the press path caused an increase in pressure at their limits, followed by a decrease in it in the groups of cam nozzles, which was accompanied by a slightly higher load on the engine. From the studies of the first stage of experiments with set No. 1, it is known that with an increase in the throughput from the increased gap in the matrix and with an increase in the speed of the screw shafts, the oil yield decreases.

In the research on single-screw presses (Indartono *et al.*, 2019) it is noted that the improvement in oil yield

was due to a forced increase in the degree of filling inside the screw press, which increased the pressure to 1400-1500 kgf*cm^{-2} inside the screw press. In addition, this was due to the longer residence time of the material inside the screw press, so the pressing time was increased. However, if the seed feed rate is too high, it will block the filtration module of the pressing cage chamber and matrix with solid particles, which will lead to clogging of the oil outlet holes of the screw press. However, this disadvantage of single-screw presses was eliminated when using twin-screw extrusion presses with shafts that rotate in the same area and engage with each other. This ensures the forced movement of oil-containing raw materials along the press path to the cake outlet. Feeding the cavity between the turns of the twin-screw extrusion presses EK-75/1200 in the loading neck, when the extrusion presses enter a steady-state mode of operation,]is provided by gravity seed flow from the hopper, without forced feeding seeds into it. There is a forced feeding device for feeding seeds to the working chamber In the design of twin-screw extrusion presses that have been investigated in the paper (Dufaure *et al.,* 1999). For its use, the regulatory limits are specified. When the input seed flow rate was too low, the free volume of the working bodies was not fully filled, a dynamic plug could not form around the return screw, and therefore the pressure exerted on the raw material was insufficient to remove the oil through the filter module (pressing cage chamber), the raw material (crushed seeds) was simply transported to the yield of the extrusion presses matrix. If the input seed flow rate was too high, the working chamber of the extrusion press was clogged and its raw material contents were pushed back through the feed device on the first module. An increase in the maximum threshold value of the input seed flow rate could be obtained by increasing the speed of rotation of the screws, but this affected the reduced oil yield since the oil-containing raw materials were forced out of the extrusion presses faster. Notably, the speed of rotation of the shafts under study in operation (Dufaure *et al.,* 1999) of the BC21 twin-screw extrusion presses was 125-200 rpm, and the seed feed rate was in the range of 5-20 kg*h. Overall, the authors argue that the efficiency of pressing in the twin-screw extrusion presses was achieved by selecting the optimal configuration of the screw shaft profiles, controlling the internal parameters of the oil pressing operation (seed inlet flow rate, screw shaft rotation speed, pressing temperature) and controlling the external parameters (seed humidity). The paper notes that increasing the length of the reverse screw and reducing its pitch helped to seal the dynamic plug and increase the back pressure effect under the filtration module, which also led to a higher oil yield. It is also indicated that a twin-screw extrusion press can be used with greater efficiency than a single-screw press.

Since the screw press is a closed system, for a deeper understanding of the processes that occur in its working chamber, namely, the study of the flow areas of oil-containing raw materials from the influence of the configuration of the screw shaft geometry in operation (Azizi *et al*., 2015) it is proposed to apply simulation modelling of hydrodynamic flows of raw materials before implementing scientific findings in metal. The authors claim that computational hydrodynamics allows for a deeper analysis of aspects of flow dynamics due to local effects of the geometry of working bodies, modelling will provide information about the mixing behaviour of an inhomogeneous mixture of palm fruits and the degree of its compaction due to the applied hydraulic force. A successful simulation model is one of the available process tools that can replace expensive manufacturing and test runs of equipment to predict the performance of a screw press unit without compromising valuable metalworking resources, working time, and finances. However, the paper itself lacks detailed information about how this software papers in action. Only described are the expectations from its implementation that computational hydrodynamics will provide a solution to the problems associated with the operation of the screw press for palm oil, which will ultimately improve the speed of oil extraction. But the idea itself can be borrowed for implementation in future research

The results of investigating the dependence of oil yield at different heating temperatures of housings obtained in Figure 5 are confirmed by the trend of the obtained results of oil pressing research in other papers of researchers. For example, in operation (Fakayode & Ajav, 2019), when using a single-screw press with a roaster for squeezing moringa fruit oil, at a temperature of 80°C and a heating time of 30 minutes of raw materials in the roaster and an applied pressure of 20 MPa, it leads to an increase in oil yield – 81.66%. However, at higher temperatures and heating times, the oil yield decreased. This is due to the fact that at higher temperatures, protein coagulation and reduced viscosity occur faster, which leads to an increase in yield in a short period of time; while an increase in the heating duration at higher temperatures caused a substantial loss of moisture, which led to the solidification of the raw material, which, as a result, leads to a decrease in the efficiency of oil extrusion. Softening of tissues weakens the cellular structure, which makes it very susceptible to destruction under the influence of pressure. The interaction of temperature and pressure at higher levels tends to become counteracting, as increasing the temperature reduces the viscosity of the oil, thereby increasing its fluidity through the compressed medium, while increasing the pressure makes the oil harder, which limits the flow of oil. These results are similar to those of other researchers cited in the paper, who observed a similar trend when extracting jatropha kernels, peanuts, and walnuts.

Considering new factors affecting the energy consumption and productivity of the extrusion presses, it was concluded that additional experiments should be conducted with changes in the size of the outlet hole in the matrix with different numbers of cylindrical-conical nozzles on the shaft. And given the change in electricity and oil tariffs, it is necessary to further investigate the rationality of the cost of increasing the oil yield at different energy

costs. Thus, in modern realities, these statements are the actual subject of subsequent research on the improvement of twin-screw extrusion presses.

Conclusions

Based on the analysis and systematisation of oil extrusion processes, principles of operation and designs of Ukrainian and foreign machines and assessment of their interaction with oil-containing raw materials, this study obtained new scientific results that expand the understanding of the patterns of grinding processes in different functional areas of twin-screw extrusion presses. Experimental studies have confirmed that the oil yield depends on the degree of compression, which is due to the geometric parameters of the screw shaft. The general nature of the change in free volume in the zones of conjugated cylindrical-cone nozzles leads to a more rapid increase in the degree of compression, but sufficient so that raw materials do not clog in their flow section. Experimentally confirmed the theoretical statement that the step-by-step intermediate compaction of oil-containing raw materials in the interface zone of cylindrical-cone nozzles and screw nozzles in front of them with simultaneous destruction and mixing of the upper and lower layers of the processed material relative to the inner shaft in the interaction zone of the surfaces of cylindrical-cone nozzles and subsequent pressure relief in the zone of triangular cam nozzles destroys the layer gaps of encapsulated oil and leads to its more efficient extrusion from the extrusion presses. Comparing the obtained values of sunflower oil yield on a standard set of working bodies of the manufacturer (set No. 1) with an improved set of working bodies with cylindrical-cone nozzles in their composition (set No. 2), the effectiveness of set No. 2 was recorded. Namely, their use gave an increase in oil yield from 2.3% to 3.1% in all four cases with a change in the heating temperature of the cases. The practical importance of this study is relevant for owners of oil production enterprises that are equipped with twin-screw extrusion presses. After retrofitting the screw shafts of the extrusion presses with cylindrical-cone nozzles, up to 3% of the oil can be obtained additionally. When converted to the production capacity of the enterprise in hundreds of tonnes, the increase in profit due to additional litres of oil yield is evident. Based on the results of this study, the prospects for further research are outlined. They consist in clarifying the rational geometric parameters of the developed cylindrical-cone nozzles, from the standpoint of energy efficiency. That is, they should be considered in conjunction with other design parameters of the extrusion presses, which will reduce energy costs for the oil extrusion process.

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Дослідження ефективності засобів для інтенсифікації відтискання олії в двогвинтових прес-екструдерах

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Анотація. Актуальність дослідження зумовлена проблемою, яка характерна для різних видів конструкцій шнекових пресів, які при відтисканні соняшникової олії залишають в макусі до 16 % олії. У зв'язку з цим дане дослідження спрямоване на знаходження раціональних методів по вдосконаленню конструкції двогвинтових прес-екструдерів з метою збільшення виходу олії. Провідними методами для вирішення цієї проблеми є емпіричні методи дослідження, що дозволяють на основі спостереження комплексно розглянути етапи удосконалення геометричної конфігурації шнекового валу та шляхом вимірювань і експерименту знайти раціональний варіант розв'язку поставленої задачі. В роботі проаналізовано особливості технічних засобів для подрібнення олієвмісної сировини в пресах та екструдерах, обґрунтовано необхідність розроблення нових подрібнювальних робочих органів. З'ясовано необхідність доповнення існуючих уявлень про взаємодію спеціальних подрібнювальних робочих органів з транспортувальними і стискаючими гвинтовими насадками та розроблення подрібнювальних вузлів для двогвинтових прес-екструдерів вироблених в Україні. Проведено експериментальні дослідження з розробленою конструкцією нових робочих органів – циліндрично-конусних насадок. Вони створюють проміжне стискання олієвмісної сировини, спрямоване на інтенсифікацію подрібнення з одночасним перемішуванням перероблюваного олієвмісного матеріалу. Отримано потрійне повторення збільшення ступеню стискання при встановленні трьох пар циліндрично-конусних насадок по довжині шнекового валу. Їх застосування дозволило інтенсифікувати ступінь наростання тиску і збільшити вихід олії до 3,1 %. З кожної переробленої тони насіння, за рахунок збільшення кількості відтиснутої олії, відповідно, зросте і прибуток підприємств, які вдосконалять двогвинтові екструдери цими робочими органами

Ключові слова: виробництво олії, олієвіджимний прес, геометричні параметри, циліндрично-конусні насадки, вихід олії

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 13(4), 19-29
Received: 15.09.2022 Revised: 13.10.2022 Accepted: 27.10.2022

UDC 006:637.52.04
DOI: 10.31548/animal.13(4).2022.19-29

Analysis of the use of plant components in the production of meat products

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Abstract. The inclusion of plant components in meat products plays a substantial role in human nutrition, since such inclusions are a source of biologically active substances, promote digestion, and increase the nutritional composition of food products. The purpose of this paper is to review the latest studies on the use of plant components in the production of meat products and search for new ones that can be used as substitutes for artificial additives and meat raw materials. During the study, methods of analysis, comparison, and synthesis of papers of Ukrainian and foreign researchers located in scientometric databases, such as Web of Science, Scopus, and internet resources, are used. During the study of the literature, the main problems of meat products production are considered: spoilage of products during storage, increased content of nitrates and phosphates, development of pathogenic microflora. It is identified that the main centre of research is oxidative processes during the production and storage of finished meat products. It is identified that due to the high content of vitamins, phenolic compounds, and micro- and microelements, plant inclusions can act as inhibitors in such reactions. In the course of the study, it is determined that the most commonly used: peel, pulp, juices, and extracts of fruits and vegetables as plant inclusions. The main task of such inclusions is to replace synthetic inclusions with natural ones to increase the nutritional value and composition of food products. A comparative analysis of the nutritional value and chemical composition of fruits, vegetables, and their juices is conducted to select the most fitting plant inclusions that are common on the Ukrainian market. The indicators are summarised in the table. Replacing artificial antioxidants, preservatives, and other compounds with their natural counterparts is essential for modern manufacturing, as such technological solutions will help make food more functional, healthy, and increase the level of beneficial compounds in food

Keywords: chemical composition, natural antioxidants, phenolic substances, natural substitutes, plant inclusions

Suggested Citation:
Zinchenko, R., & Slyva, Yu. (2022). Analysis of the use of plant components in the production of meat products. *Animal Science and Food Technology*, 13(4), 19-29.

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Introduction

The meat processing industry is one of the most popular industries in Ukraine, which is why the largest number of producers are focused on creating innovative approaches in the processing of meat products. In recent years, when production, formulations, and processing technologies have reached a stable position, manufacturers focused on creating safe products and improving their range (Koval *et al.*, 2021).

Meat and meat products are the main source of high-quality protein, fat, minerals, and vitamins. Due to the growth of the population, and the level of consciousness of consumers, meat consumption per unit of the population increased by 3 times compared to the 1960s (Yarmolenko, 2020), which led to the need for quantity, quality, and safety of these food products.

The segment of meat processing industry occupies a fairly large volume in the food industry of Ukraine, approximately 18% (Ishchuk, 2020), second only to the dairy and grain sectors in terms of output. The high level of competition and an insufficient number of suppliers of raw materials led to an increase in the cost of products, but this did not reduce the demand for them. That is why modern research and production have focused on adding plant inclusions to the production of meat products.

According to classical technologies, the production of meat products provides for the addition of synthetic additives at certain stages of production processes. In particular, phosphates are added at the stage of preparation of minced meat, to increase the emulsifying and moisture-binding properties of meat products, inhibit oxidative processes and colour formation, and stabilise the pH of the product. Along with phosphates, sodium nitrite is added, which is most often used in meat products, as a dye. Antioxidants are added during the preparation of minced meat to inhibit oxidative processes (Klymenko *et al.*, 2021).

Oxidative reactions in meat products are one of the centres of attention of researchers in the field of production and storage of meat products because of their role in the deterioration of safety and quality and the formation of toxic compounds that are directly related to changes in aroma and taste. In addition to causing a change in the colour of meat, which, as a result, leads to rejection of the product by consumers, oxidative reactions lead to the following losses: loss of vitamins, essential amino and fatty acids, which are caused by undesirable lipid oxidation in meat products (Turgut *et al.*, 2017; Zahid *et al.*, 2019). In addition, processed meat products are very susceptible to loss of colour, loss of functionality of lipids, proteins, and the appearance of rancid taste due to highly reactive nitrogen intermediates formed during oxidation. (Reshi *et al.*, 2017; Klymenko *et al.*, 2021). Synthetic food preservatives such as butylated hydroxyanisole and butylated hydroxytoluene, which are used as inhibitors of the fat oxidation process, are effective in stopping spoilage processes in meat and meat products, but simultaneously, these preservatives cause serious health concerns and allergic reactions (Zahid *et al.*, 2019). The use of antioxidants from plant components for storing meat products is an alternative solution to prevent the occurrence of such problems. Organoleptic characteristics of meat products are also improved by introducing plant extracts into meat products (Golub & Horbach, 2018).

The purpose of the study was to analyse the latest studies on the use of juices, zest, and other plant components in the production of meat products.

Analysis of the Use of Vegetable Components in the Production of Meat Products

Given the level of consumer awareness, the trend of healthy eating, and the growing demand for organic products, over the past 20 years, a large number of researchers have been trying to investigate the use of natural substitutes in the production of meat products. Thus, trying to replace artificial or chemical substances in the composition of meat products, while not losing, or even increasing organoleptic, physico-chemical, and microbiological indicators (Krivosheeva *et al.*, 2020). It was due to such studies that the qualitative composition of nutrients and the shelf life of the product increased. The peel, zest, pulp, juices, and seeds of fruits and vegetables are most often used as plant components (Rodriguez-Amava, 2015; Turgut *et al.*, 2017; Mohammed *et al.*, 2022).

In the study (Turgut *et al.*, 2017), 0.5 and 1% pomegranate peel extract was added to beef meatballs and stored at -18±1°C. The results showed that the oxidation of lipids and proteins was suspended, and organoleptic parameters remained constant. This indicates a high antioxidant property of pomegranate. In addition, other parts of the fruit (juice, seeds) have antimicrobial properties. Due to the high content of punicalagin and ellagic acid, pomegranate juice can be used as a natural antibiotic to destroy or suppress pathogenic microorganisms. Antioxidant activity is explained by the high content of phenolic compounds, including anthocyanins (3-glucosides and 3.5-diglucosides delphinidin, cyanidin and pelargonidine), ellagic acid, punicalin, punicalagin, pedunculagin, and various flavonoids (Turgut *et al.*, 2017; Saleh *et al.*, 2017). Ukrainian research focused on the use of pomegranate juice as a vegetable dye that can replace the action of sodium nitrite, according to the results, improved organoleptic parameters with increased content of vitamins in the product were achieved (Verchenko *et al.*, 2019).

In another study, ginger root extract was added to the production of fried sausages from poultry meat. Ginger is one of the traditional medicinal plants. Ginger contains active ingredients that have antibacterial, anti-flatulence, antimicrobial, anti-inflammatory, antidiabetic, antispasmodic, anti-cancer, and antioxidant properties. A 5% solution of ginger root extract was added to the sausage recipe, which led to an increase in the shelf life of sausages, while the pH value remained within the normal range. The action is explained by the high content of phenolic compounds

gingerol, which can inhibit chain reactions during lipid oxidation. In addition, the introduction of ginger extract leads to the saturation of the product with a large amount of vitamins and minerals, which can increase the resistance to infectious diseases and increase the immunity of the human body (Reshi *et al.*, 2017; Rubanka *et al.*, 2017).

An effective antioxidant can be mango juice and its peel. Studies results of a group of authors (Manzoor *et al.*, 2022), showed that when adding mango peel extract to chicken sausages, in concentrations of 2%; 4%; 6%, it will allow not making substantial changes to recipes. In organoleptic parameters, when using an extract concentration of exactly 4%, it had a negative effect on the colour of the product. The study showed that sausages with the addition of the extract show a higher content of thiol and a lower content of carbonyl, which prevents protein oxidation.

From previous studies, it can be stated that the main factor in the replacement of antioxidants is a decrease or complete inhibition of the oxidation of proteins and fats during the life cycle of a meat product. Therefore, (Bozhko, 2015) in his research focused on the search for natural inhibitors and replacing nitrite and its derivatives with natural antioxidants. In the course of his studies, he identified the fact that in case of improper storage of the product, or during violations of technological processing conditions, nitrosamines can form, a substantial part of which has a carcinogenic effect or is toxic to the human body. A drug was used that included β-carotene and vitamin E, which are natural antioxidants to neutralise them. These two vitamins are present in large quantities in juices and vegetables.

A separate issue in the production of meat products is the large amount of phosphates. Excess phosphates in the body cause a violation of the internal intestinal microflora, can lead to the development of allergies. In addition, excess phosphates negatively affect the functioning of the kidneys and liver (Volkova *et al.*, 2021). According to the study by Yu. Krizhova and I. Moskalenko (2021), phosphates were replaced with amylopectin starch, and sodium nitrite – with beet juice during the production of diet cooked sausages. The results of the study showed that organoleptic parameters reached the planned values. Special attention should be paid to the recommendations for a complete replacement of sodium nitrite with beet juice. In addition, beetroot contains substantial amounts of tocopherol, carotene, folic and ascorbic acids, which lead to an antioxidant effect, due to their action as an inhibitor of oxidative reactions. Therefore, beetroot juice can function as a natural substitute for artificial antioxidants (Rodriguez-Amava, 2015; Krizhova & Tkachenko, 2021).

Another group of researchers (Thangavelu *et al.*, 2022) investigated that consumption of processed meat products declined substantially for many reasons. One of the reasons is the likelihood of a negative impact on consumer health, which is associated with the composition of processed meat products, namely high salt and fat content, the presence of synthetic additives, etc. The use of these ingredients can lead to: cardiovascular problems, obesity, kidney problems, and even cancer. Recently, consumers have been demanding the absence of synthetic additives and non-dairy components in meat products. This requirement causes enterprises for the production of meat products to change the recipe, reduce the amount of phosphates, salt, etc. In particular, to reduce the proportion of phosphate use, researchers (Thangavelu *et al.*, 2022) in the production of fresh sausages, added apple pomace as a byproduct in the production of juice and coffee bean husks. In the future, the raw product was subjected to processing by ultrasound and preparation by ultrahigh-frequency radiation. As a result, it was identified that due to such a technological solution and the addition of plant components, it is possible to reduce the use of phosphates by up to 80% without a negative impact on the physico-chemical and technological properties of sausages. In turn, the complete removal of phosphates from the technological stages negatively affected the quality of sausages.

In search of functional food products, some researchers turn to the use of seaweed in their production technologies. So a group of researchers (Mohammed *et al.*, 2022), suggested adding seaweed to pork sausages *Porphyra umbilicalis* – Nori. Seaweed contains biologically active compounds such as: proteins, minerals, vitamins, dietary fibre, polyphenols, carotenoids, and tocopherols. In addition, they are low in lipids, and some types of algae contain high levels of polyunsaturated fatty acids. In addition, studies were conducted on the addition of other types of algae to the recipe of fresh sausages: *Himanthalia elongata* – sea spaghetti, *Alaria esculenta* – Irish wakame and red seaweed – *Palmaria palmata* – dulse. As a result, a disadvantage of the use of seaweed was identified – a change in colour and taste (López-López *et al.*, 2009; Mohammed *et al.*, 2022).

Another main object of research was natural essential oils. They are among the best alternatives to synthetic antioxidants due to their strong antimicrobial action. Essential oils have been widely used in food production due to their antibacterial, antifungal, and antioxidant properties, and as food flavourings. The main advantage of essential oils is that they can be used for any food. Essential oils contain many phytochemicals, including phenol and compounds such as flavonoids, which are sources of natural antioxidants. In studies (Gerges & Asuoty, 2022), basil essential oil and acetic acid were added in various concentrations to chicken sausages. The finished products were stored in the refrigerator at 4°C and freezer at -18°C for 15 days. As a result of the conducted studies, inhibition of pathogenic microflora and suspension of oxidative processes were detected (Viuda-Martos *et al.*, 2010; Gerges & Asuoty, 2022).

In the study (Kotecka-Majchrzak *et al.*, 2021), hemp pulp was used as a byproduct of cold-pressed oil, of various concentrations, in the production of meatballs. According to the technology, meatballs were baked, cooled, and packed in a vacuum. The results showed that such a technological solution allowed to increase the shelf life of the product to 12 days by inhibiting the processes of lipid oxidation.

Materials and Methods

The search used papers placed in scientometric databases in three electronic bibliographic databases: Web of Science, Scopus, and Science Direct, and on the Google Scholar platform published in English, Ukrainian, and Polish.

During the analysis and review of the latest scientific achievements, 323 scientific sources of various types were processed: abstracts and materials of scientific conferences; studies; textbooks; monographs; dissertations; thematic internet sources; technologies for the production of meat products by Ukrainian and foreign authors.

The search was conducted in the following key words and phrases: the meat processing industry; the development trend of the meat industry; plant use; antioxidants; natural antioxidants; meat products; the use of fruits in the production of meat products; the use of fruit juices in meat during production; lipid oxidation in meat products; flavonoids; the use of pomegranate in the production of meat products; the use of ginger in the production of meat products; the use of sea buckthorn in the production of meat products; the use of pumpkin juice in the manufacture of meat products; the use of carrot juice in the manufacture of meat products; the use of mango in the production of meat products; the use of black currant in the production of meat products; the use of sea buckthorn in the production of meat products; the use of elderberry; the use of oil from cannabis; oil from industrial hemp; chemical composition: elderberry, currant, raspberry, carrot, pumpkin, ginger, carrots, beets, pomegranate, hemp oil; chemical composition of juice from elderberries, currants, raspberries, carrots, pumpkins, carrots, beets, pomegranate; study: elderberries, currants, raspberries, carrots, pumpkin, ginger, carrots, beets, pomegranate, hemp oil; nutritional value: elderberry, currant, raspberry, carrot, pumpkin, ginger, carrots, beets, pomegranate, hemp oil; vitamins; trace elements contained in apples, currants, raspberries, carrots, pumpkin, ginger, carrots, beets, pomegranate, hemp oil; macronutrients contained in apples, currants, raspberries, carrots, pumpkin, ginger, carrots, beets, pomegranate, hemp oil; the nutrient composition of food products; bioactive substances in food products; carotenoids; β-carotene; manufacturing technology of chopped semi-finished products; technology for production of natural sausage, and their Ukrainian or Polish translation. All studies and internet sources were summarised in an Excel spreadsheet and checked for duplication. Duplicate studies were deleted (n=86). Studies that passed the first stage of verification (n=237) were reread in detail and those that were clearly irrelevant for information (n=181) were also deleted. Of the remaining 56, internet sources that did not have links to scientific literature (n=13) were removed.

In the further study of the papers, the following methods were used: analysis – when analysing the latest findings on the use of plant substitutes in meat products; comparison – when comparing the effects of the use of plant-based antioxidants with the effects of standard food additives used in the production of meat products; synthesis – to deduce the probability of using natural substitutes or Ukrainian analogues of the investigated ones; generalisation – when searching for common signs of plant inclusions and their effects when used in the production of food products.

After analysing the latest studies of Ukrainian and foreign experts, it is necessary to identify several main areas of research on the use of plant components for partial or complete replacement of synthetic additives in the production of meat products, as natural additives that have antiseptic and antioxidant properties, to increase the level of nutritional value by adding plant inclusions or combinations thereof.

The results of the analysis show that carotenoids, vitamin C, vitamin E, and phenolic compounds are plant-based antioxidants that are used to stabilise and can extend the shelf life of meat products.

Description of Nutrients that Can be Used as Antioxidants

Carotenoids are the most common, numerous, and important group of natural pigments. According to their chemical structure, carotenoids belong to the class of terpenes and are widely distributed in both photosynthetic and non-photosynthetic organisms (Simonova, 2010). Of the carotenoids in the production of meat products, β-carotene (provitamin A) is the most applicable. In nature, β-carotene is a precursor to the fat-soluble vitamin A-retinol. It is used as an antioxidant and food colouring agent E160a. Carotenoids are identified in large quantities in carrots, oranges, persimmons, saffron, tomatoes, spinach, pumpkin, papaya, grapefruit, apricots, pomegranates, mango, watermelon, ginger, melon, avocado, nectarine, and passion fruit (Milne, 2005).

Vitamin E (tocopherol) is a fat-soluble vitamin that is used to prevent the oxidation of fatty acids. It is a group of leaded phenols, labelled as a food additive E306 (a mixture of tocopherols), E307 (α-tocopherol), E308 (γ-tocopherol), and E309 (δ-tocopherol). The antioxidant properties of tocopherols are explained by the ability of the mobile hydroxyl of the chromane core to interact with oxygen free radicals (HO_2, O_2, HO) (Mokrosnop & Zolotareva, 2021). Tocopherols are identified in large quantities in: bananas, almonds, soybeans, carrots, avocado, broccoli, radishes, pomegranates, oatmeal, onions, pumpkin, green leafy vegetables, sea buckthorn, rosehip, black currant and its essential oil (Afanasyeva & Stoychyk, 2015; Kruchanitsya *et al.*, 2019).

Vitamin C (Ascorbic acid) is also a well-known antioxidant, it is identified in many fruits and vegetables and is labelled as a food additive E300. The antioxidant properties of ascorbic acid are due to the reaction with free oxyl and hydroxyl radicals, and it can also reduce α-tocopherol, which enhances the antioxidant effect (Artiushenko, 2021). Most vitamin C in large quantities is identified in: rosehip, sea buckthorn, black currant, kiwi, papaya, grapefruit, lemon, oranges, mango, ginger, celery, raspberry, beetroot,

elderberry, onion, sorrel, tangerine, pumpkin, and apples (Afanasyeva & Stoychyk, 2015; Kruchanitsya *et al.*, 2019).

Phenolic compounds are a group of chemicals based on derivatives of aromatic alcohol – phenol (C6H5-OH), the physiological role of which is associated with antiradical and antioxidant properties. In plant inclusions, the most common are bioflavonoids (vitamin P) – a group of vitamin-like substances that are similar in biological activity to vitamin C. (Afanasyeva & Stoychyk, 2015; Kruchanitsya *et al.*, 2019). Phenolic compounds are identified in large quantities in: raspberries, strawberries, cherries, plums, black currants, elderberries, blackberries, pomegranates, red grapes, and blueberries.

These substances are of natural origin and can be used as a partial or complete substitute for synthetic antioxidants. As a result of the analysis, it can be noted that plants that contain the greatest amount of antioxidants and can be used as additives for partial or complete replacement of synthetic components are: mango, pomegranate, ginger, beetroot, brown and green algae, apples, essential oils. However, a substantial part of these fruits and vegetables are not very common in the Ukrainian market (Rubanka *et al.*, 2017; Simakhina & Naumenko, 2021; Mohammed *et al.*, 2022). The best solution is to select a combination of several fruits, vegetables, and products made from them that could achieve the desired effect during production. Considering the demand on the national market, the following plants, their components, and combinations were selected: pomegranate, ginger, beetroot, carrot, raspberry, pumpkin, elderberry, and sea buckthorn.

Description of Foods Containing Natural Antioxidants

Pomegranate is grown in countries with subtropical climates, present on the market in large quantities. Pomegranate peel and juice contain vitamins B_6, B_{12}, C, H, P, E, fibre, minerals, and trace elements – phosphorus, calcium, manganese, iodine, magnesium, iron, potassium, and sodium. In addition, organic acids – tartaric, succinic, citric, etc. Pomegranate peel contains a lot of tannins, ursolic acid, alkaloids, and it also has good antiseptic properties. In the production of meat products, it can be used as a substitute for meat raw materials, artificial antioxidants, and a partial substitute for antiseptics. (Turgut *et al.*, 2017; Burak, 2012; Kruchanitsya *et al.*, 2019).

Ginger is grown in tropical areas and is available on the market in large volumes. Ginger root juice contains vitamins: B_6, B_1, B_9, B_5, B_2, A, C, E, and K; minerals and trace elements: potassium, calcium, magnesium, sodium, phosphorus, manganese, copper, iron, selenium, zinc, lysine, phenylalanine, threonine, methionine, curcumin, etc.; antiseptic and mucolytic substances: camphene, cineol, bisabolene, resinous substance gingerol forms an astringent and sharp taste; essential acids – oleic, linoleic, and caprylic (Reshi *et al.*, 2017; Rubanka, 2017). In the production of meat products, it can be used as a substitute for meat raw materials, artificial antioxidants, a partial substitute for antiseptics, flavourings, and spices.

Beet is a root vegetable grown in large volumes in Ukraine and is a component of a substantial number of Ukrainian dishes. This root vegetable is rich in vitamins: C, PP, B; amino acids: lysine and arginine; organic acids: pantothenic, folic, sorrel, and malic; phenolic compounds; minerals and trace elements: iron, calcium, magnesium, iodine, zinc, copper, and various carotenoids (Afanasyeva & Stoychyk, 2015). In the production of meat products, it can be used as a substitute for meat raw materials, artificial antioxidants, artificial phosphates, and synthetic food dyes.

Raspberry is a berry that is grown on the territory of Ukraine in large volumes. Berries and their juice are actively used in the food industry. Raspberry berries contain vitamins: C, B_1, B_2, PP, A, and B_9; minerals and trace elements: zinc, cobalt, potassium, iron, and manganese; phenolic compounds (Afanasyeva & Stoychyk, 2015). In the production of meat products, it can be used as a substitute for meat raw materials, artificial antioxidants, and synthetic food dyes.

Carrot is a biennial herbaceous plant that is grown in Ukraine in large volumes. Carrots contain vitamins: B_1, B_2, B_5, B_6, B_9, PP, A, C, E, H, and K; minerals and trace elements: magnesium, iron, zinc, potassium, sodium, phosphorus, copper, calcium, manganese, fluorine, iodine, molybdenum, boron, aluminium, sulfur, chlorine, and nickel. Carrots are rich in fibre and carotenoids (Afanasyeva & Stoychyk, 2015). In the production of meat products, it can be used as a substitute for meat raw materials and artificial antioxidants.

Pumpkin is a melon crop, which is grown on the territory of Ukraine in large volumes. It contains large amounts of carotenoids, pectin, and vitamins: A, E, K, B, PP. The main advantage of pumpkin is the presence of vitamin T and L-carnitine, which are used as substances to control weight and promote digestion in the human body (Afanasyeva and Stoychyk, 2015; Muzychuk *et al.*, 2020). In the production of meat products, it can be used as a substitute for meat raw materials and artificial antioxidants.

Elderberry is a shrub or small tree that grows in many parts of Ukraine, the fruits, flowers, and roots of which are used in the food and pharmaceutical industries. Elderberry berries contain vitamins: A, B, and PP; minerals and trace elements: calcium, potassium, iron, magnesium, phosphorus, sodium, zinc, copper, and selenium; phenolic substances; rutin and β-carotene (Khomich *et al.*, 2012; Burak, 2012). In the production of meat products, it can be used as a substitute for meat raw materials, artificial antioxidants, artificial phosphates, and synthetic food dyes.

Sea buckthorn is a bushy tree that grows in Ukraine and has yellow, orange, or orange-red berries. Sea buckthorn berries contain large amounts of vitamins C, E, B, K1, and carotenoids; minerals and trace elements: potassium, calcium, magnesium, iron, zinc, copper, manganese, bromine, and iodine; organic acids: malic, oxalic, and tartaric; tannins; higher fatty acids; high content of phenolic compounds (Bondarchuk & Kurylenko, 2022; Muzychuk *et al.*,

2020). In the production of meat products, it can be used as a substitute for meat raw materials, artificial antioxidants, and animal fat substitutes.

Black currant is a perennial shrub plant that grows in Ukraine in large volumes. The main active ingredients in black currant fruits are carbohydrates: glucose, fructose, rhamnose, and sucrose; vitamins: C, B_1, B_2, B_6, BC, E, K, minerals and trace elements: potassium, calcium, magnesium, silicium, iron, sodium, manganese, copper, zinc, phosphorus, cobalt, molybdenum, chromium, selenium, boron; carotenoids; pectin substances, organic acids; phenolic compounds; coumarins and flavonols (Afanasyeva & Stoychyk, 2015; Shtonda & Pasichnyi, 2019). In the production of meat products, it can be used as a substitute for meat raw materials, artificial antioxidants, artificial phosphates, and synthetic food dyes.

Hemp oil is made from hemp that does not contain cannabinoids, is made by cold or hot pressing, and is widely used in the pharmaceutical and culinary industries. The main active ingredients in it are fats (omega-3, 6, and 9; oleic, linoleic, palmitic, organic acids.), minerals: zinc, magnesium, iron, calcium, manganese; vitamins: A, E, B, and K; tannins (Oniskiv & Pokotilo, 2014; Kotecka-Majchrzak *et al.*, 2021). In the production of meat products, it can be used as a substitute for meat raw materials, artificial antioxidants, and animal fat substitutes.

Over the past 10 years, the number of papers on the use of plant inclusions in the production of meat products has grown rapidly, demonstrating the interest of researchers and enterprises in developing the production of healthy and safe products. The main task of this study is to identify plant inclusions or combinations of them that can be used in the production of meat products.

During the literature review, it was determined that plant inclusions containing phenolic substances and vitamins can be used as natural substitutes for synthetic anti-oxidants to extend the shelf life of meat products and inhibit lipid oxidation, which is confirmed by studies (Turgut *et al.*, 2017; Burak, 2012; Rubanka, 2017).

Another advantage of using plant inclusions is the achievement of improved organoleptic parameters of the product (Gerges & Asuoty, 2022). However, there are papers that raise doubts about whether they are actually improving. According to the studies (López-López *et al.*, 2009; Mohammed *et al.*, 2022), taste and colour indicators were changed, which did not satisfy all consumers of the product. Also in the study (Manzoor *et al.*, 2022), the colour of the product has changed, which may negatively affect the overall attractiveness for the buyer, but in the other one (Krizhova & Moskalenko, 2021), the colour indicator was achieved as planned. Also in this study, the amount of synthetic phosphates was reduced, which did not affect the quality indicators of the final product. Therewith, in the study (Thangavelu *et al.*, 2022), a decrease in phosphate levels led to a negative impact on the quality of sausages.

A decrease in the level of pathogenic microflora is also accompanied by the replacement of synthetic additives with plant inclusions, which is confirmed by studies (Kotecka-Majchrzak *et al.*, 2021; Gerges & Asuoty, 2022).

In a larger number of papers, there were recommendations for further research on the behaviour of plant inclusions in meat products. Therefore, it is proposed to consider the best plants and their components that can be used in the production of meat products, according to the authors, by their nutritional value and chemical composition, which are presented in Tables 1 and 2.

Table 1. *Comparative characteristics of the chemical composition and nutritional value of fruits and vegetables*

Chemical composition	Carrots	Beetroot	Pumpkin	Pomegranate	Ginger	Elderberry	Sea buckthorn	Currant	Raspberry
Nutritional value per 100g (g)									
Protein	1.1	1	-	2	2	0.66	2	0.5	1
Fats	0.1	-	-	1	0.5	0.5	6	0.27	0.58
Carbohydrates	12.6	14.1	9	19	17	8.4	8	17	13
Water	84.6	83.4	90.8	78	-	79.8	83	81	84
Dietary fibre	1	1	2	4	2	7	3	7	6
Energy value, kcal	56	61	37.5	100	80	74	93	39	73
Macronutrients (mg)									
Calcium	19	19	25	8	16	38	42	40	41
Magnesium	7	17	14	12	43	5	30	35	30
Sodium	26	45	4	3	13	6	4	16	1
Potassium	130	288	204	236	415	280	193	133	200

Chemical composition	Carrots	Beetroot	Pumpkin	Pomegranate	Ginger	Elderberry	Sea buckthorn	Currant	Raspberry
Trace elements (mg)									
Iron	0.6	0.6	0.4	0.3	0.6	1.6	1.4 mg	0.4	1
Zinc	0.18	-	0.24	0.4	0.34	0.11	-	0.3	0.4
Copper	0.046	0.1	0.18	0.2	0.2	0.061	-	0.1	0.1
Vitamins (mcg)									
Vitamin A (provitamin A)	350		250	1	18	30	25	-	30
Vitamin C	3000	3000	8000	10200	5000	36000	200000	85000	45
Vitamin E	1000	1100	-	600	300	-	5000	1000	30
Phenolic compounds	75	-	50	1130	300	80800	200000	200000	400000

Sources: Zubar, 2010; Afanasyeva & Stoychyk, 2015; Simakhina *et al.*, 2016

Table 2. *Comparative characteristics of the nutritional value and chemical composition of fruit and vegetable juices*

Chemical composition	Carrot juice	Beetroot juice	Pumpkin juice	Pomegranate juice	Ginger juice (extract)	Elderberry juice	Sea buckthorn juice	Currant juice	Raspberry juice
Nutritional value per 100 ml (g)									
Protein	0.95	1	1	0.3	1.82	1.1	1.2	0.5	0.75
Carbohydrates	9	9.9	9	14.2	15.5	5.1	5.7	7.3	24.7
Fats	0.15	0	0.09	0.1	0.75	0.2	2.4	-	-
Dietary fibre	1	1	2	0.2	2	7	1.8	1	3.7
Energy value, kcal	40	42	38	56	80	27	82	41	100
Macronutrients (mg)									
Calcium	24	19	25	12	16	30	22	40	40
Magnesium	14	17	14	5	43	3	30	35	22
Sodium	29	45	4	4	13	5	4	16	10
Potassium	292	148	204	102	415	220	193	133	224
Trace elements (mg)									
Iron	0.46	0.6	0.4	1	0.6	1.44	1.4	0.4	1.2
Zinc	0.18	-	0.24	0.1	0.34	0.1	-	0.3	0.2
Copper	0.046	0.1	0.18	0.1	0.2	0.050	-	0.1	0.17
Vitamins (mcg)									
Vitamin A (provitamin A)	350	-	1500	20	18	21	1500	50	30
Vitamin C	3000	3000	8000	4000	5000	27000	200000	85000	45
Vitamin E	1000	100	400	300	300	-	5000	400	30
Phenolic compounds	75	-	10	1000	300	72000	160000	190000	32000

Sources: Zubar, 2010; Afanasyeva & Stoychyk, 2015; Simakhina *et al.*, 2016

According to the latest sources, the loss of biologically active substances (vitamins, micro-, and macro-elements) in the production of juices is on average: for pome fruits is 21%; for light-coloured stone fruits – 17%. Phenolic substances change by 24% when grinding pome fruits, light-coloured stone fruits – 20%, while intensely coloured fruits change by only about 9.5%.

Notably, only a small part of scientific papers considered inclusions in the form of various components of a particular plant, and none considered combinations of inclusions of the plants described above. Therefore, this area of research remains relevant in the future.

Conclusions

The meat processing industry is one of the largest and most promising branches of the food industry in the Ukrainian market, as evidenced by its constant growth over the past decades. When reviewing the latest published studies, it was identified that plant inclusions are actively used in the production of meat products. The most common use of plant components is identified in the production of semi-finished products. Vegetable components in meat products are used as natural antioxidants and antiseptics, nitrosamines, emulsifiers, dyes, substitutes for animal proteins and fats, etc.

Most often, in the Ukrainian production of meat products, the components that are available in large quantities on the Ukrainian market are used, so the most applicable plant inclusions can be: carrots, beets, pomegranate, ginger, elderberry, sea buckthorn, currant, raspberry, and derived products of their processing: juice, peel, zest, pulp, seeds, pomace, extracts. The use of plant components not only allows replacing the technological component but also helps to: increase the level of the biological and nutritional value of finished food products; increase the level of mineral composition; increase the level of vitamin composition; increase the level of dietary content of the product; reduce the level of consumption of artificial nitrates and phosphates. The use of plant inclusions can reduce the level of diseases in the human body due to the additional consumption of biologically active substances, which are included in them, and by changing synthetic analogues that can cause various diseases when used in excessive concentrations. The addition of plant inclusions allows expanding the range of food products, increasing the level of consumer satisfaction, and creating new products with increased standards and functionality.

Negative effects of using plant inclusions can be: a change in the pH level; a change in smell; a change in taste parameters; a change in colour.

It is recommended to examine the possibility of using plant components that are most common on the Ukrainian market for their use in the production of meat products; the possibility of combining various plant inclusions by combining them with each other with subsequent use.

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Аналіз використання рослинних компонентів при виробництві м'ясних продуктів

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Анотація. Включення рослинних компонентів в м'ясні продукти відіграють значну роль у харчуванні людей, оскільки такі включення є джерелом біологічно активних речовин, сприяють травленню та підвищують нутрієнтний склад харчових продуктів. Метою цієї статті є огляд останніх досліджень з використання рослинних компонентів при виробництві м'ясних продуктів та пошук нових, що можуть бути використанні в якості замінників штучних добавок та м'ясної сировини. Під час дослідження використовували методи аналізу, порівняння та синтезу наукових праць українських та закордонних вчених, розташованих в наукометричних базах даних, таких як, Web of Science та Scopus; інтернет-ресурсів. Під час опрацювання літератури, були розглянуті основні проблематики виробництва м'ясних продуктів: псування продуктів під час зберігання, підвищений вміст нітратів та фосфатів, розвиток патогенної мікрофлори. Було з'ясовано, що головним центром досліджень є окислювальні процеси під час виробництва та зберігання готових м'ясних продуктів. Встановлено, що через високий вміст вітамінів, фенольних сполук, мікро- та мікроелементів рослинні включення можуть виступати інгібіторами в таких реакціях. В ході досліджень було визначено, що найчастіше використовують: шкірку, м'якоть, соки та екстракти фруктів та овочів у якості рослинних включень. Основним завданням таких включень виступає заміна синтетичних включень на природні з метою збільшення харчової цінності та нутрієнтного складу харчових продуктів. Для підбору найбільш вдалих рослинних включень, що поширені на українському ринку, проведено порівняльний аналіз харчової цінності та хімічного складу, фруктів, овочів та їх соків. Показники зведені в таблиці. Заміна штучних антиоксидантів, консервантів та інших сполук на їх природні аналоги, має важливе значення для сучасних виробництв, оскільки такі технологічні рішення допоможуть зробити їжу більш функціональною, здоровою та підвищити рівень корисних сполук в харчових продуктах

Ключові слова: хімічний склад, природні антиоксиданти, фенольні речовини, природні замінники, рослинні включення

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 13(4), 30-38
Received: 20.09.2022 Revised: 21.10.2022 Accepted: 10.11.2022

UDC: 664.95:665.356
DOI: 10.31548/animal.13(4).2022.30-38

Influence of the method of preparation of clove oil emulsion on the anaesthetic effect in fish (on the example of *Clarias gariepinus*)

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Abstract. In aquaculture, the use of clove oil as a natural anaesthetic for hydrobiontes is gaining popularity. There are several ways to prepare an aqueous emulsion for anaesthesia, which creates a problem for technologists in choosing the most effective one. The purpose of the study was to compare the anaesthetic effect of clove oil in three methods of preparation of the mixture on the youth of the African clary catfish. The study was conducted at the Centre for Aquatic Bioresources and Aquaculture of the National University of Life and Environmental Sciences of Ukraine. Research methods – Ichthyological and statistical. The activity of a water-based clove oil preparation was evaluated using three

Suggested Citation:
Kovalenko, B., Kovalenko, V., Kononenko, R., Klymkovetskyi, A., & Matvieiev, M. (2022). Influence of the method of preparation of clove oil emulsion on the anaesthetic effect in fish (on the example of *Clarias gariepinus*). *Animal Science and Food Technology*, 13(4), 30-38.

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methods: hot, cold, and alcohol. Experimental material – juvenile fish of the clary catfish with an individual body weight from 22 to 285 g. Experimental conditions: the experimental material was kept without feeding for a day before the start of the study; fish anaesthesia was performed in the water at the temperature of +26°C; the duration of manipulations with each fish was minimal. The effectiveness of various methods of preparation of the emulsion was evaluated by the duration of fish entry into the state of anaesthesia and withdrawal from this state. The advantage of the hot method of preparing an aqueous emulsion of clove oil over the other two is established: the duration of fish entry into anaesthesia was 22% shorter compared with the cold method, and 12.3% – with the alcohol method ($p \leqslant 0.05$). The duration of fish recovery from anaesthesia with the hot method of preparation of the mixture was also shorter: compared to the cold method – 18% ($p \leqslant 0.05$), with the alcohol method – 43.2% ($p \leqslant 0.001$). Using correlation analysis, a relationship was established between the duration of fish entry into the state of anaesthesia and the individual body weight of the fish. Degree of binding: high for hot ($r=0.606$) and alcohol methods ($r=0.529$) and medium for a cold method of preparation of an anaesthetic mixture ($r=0.385$). The results obtained are of practical value for improving the method of anaesthesia of fish using clove oil

Keywords: aquaculture, anaesthesia, water emulsion, clary catfish

Introduction

Recent studies have shown that fish experience pain and suffering just like other phylogenetically superior animals (Sneddon, 2015). The use of various methods of calming fish during caviar harvesting, during bonitation, and surgical interventions helps to reduce injury of fish, prevents the manifestation of stress reactions or reduces negative consequences from them, and facilitates the work of the personnel of aquaculture enterprises. Managing stress is one of the main challenges in achieving animal welfare today (Herskin & Nielsen, 2018).

The use of anaesthetic substances in fish handling complies with the requirements of European legislation (Directive 2010/63/EU, 2010), the norms of which should be implemented in the legislation of Ukraine on the way to accession to the EU.

The state of anaesthesia is achieved in various ways, in particular, the effect of low temperatures on the fish body (Olifirenko *et al.*, 2020), or the use of carbon dioxide (Mitsuda & Yoshizawa, 1991). Sometimes these two methods are combined (Tan *et al.*, 2022). However, the most common method of anaesthesia in fish farming is chemical, using anaesthetic drugs, divided into synthetic (MS-222, novocaine, phenasepam, etc.) and natural, such as clove oil and chamomile oil (Ak *et al.*, 2022).

When choosing a drug for anaesthesia, researchers (Park *et al.*, 2017; Bolasina *et al.*, 2017) recommend focusing on the generally accepted requirements for an ideal anaesthetic for fish: rapid anaesthesia (<3 min) and rapid recovery (<5 min) of fish after anaesthesia; solubility in water, affordability and practicality in use, no toxic aftereffect for the fish body and harmful residues in food fish products, no harm to the environment.

The most common anaesthetics in Ukraine are phenasepam, quinaldine, and clove oil. The use of the first two drugs has certain limitations: phenasepam belongs to psychotropic substances and is sold only by prescription; quinaldine has a toxic aftereffect (Zinkovskij *et al.*, 2000).

Clove oil is a representative of essential oils: it has a pronounced pungent smell and bitter taste. This oil is extracted from the flower buds, leaves, and stems of the clove tree (*Syzygium aromaticum*), which grows on the islands of Java, Zanzibar, Madagascar, Sri Lanka, China, and some other countries. The quality of clove oil depends not only on the part of the tree from which it was extracted but also on the time of year and the climate zone in which the plant grew. Clove oil is also used in the food industry (as a food additive and as a means of extending the shelf life of products), in cosmetology (as a flavouring agent) and in medicine (as an analgesic in dentistry) (Hu *et al.*, 2018; Singh *et al.*, 2022).

In aquaculture, clove oil as an anaesthetic is used in working with a large number of hydrobiontes, both marine and freshwater (Teles *et al.*, 2019; da Silva *et al.*, 2021). Clove oil is also used in amphibian breeding (Sirimanapong, 2020). The anaesthetic effect of clove oil can also be used to reduce fish loss during transportation and to facilitate fish slaughter (Teles *et al.*, 2019; Kovalenko & Kovalenko, 2022).

An important property of clove oil is its safety: the drug does not accumulate in the body of animals and does not affect haematological and biochemical parameters, reproductive function of the body, further behaviour, and growth rates of animals (Aydın & Barbas, 2020). This substance has long been a food additive for various products, so it is considered safe for humans. Therewith, there is a lack of data in the scientific literature on the study of the possible effect of this anaesthetic in the long term on the degree of physiological changes in the fish body, especially if this procedure is repeatedly used on one fish, as is the case in working with the breeding stock of sturgeon with the factory method of reproduction of these fish.

The composition of clove oil includes eugenol (active substance, more than 70%), eugenol acetate (up to 13%), caryophyllene, β-myrcene, α- and β-pinenes, ylangen, γ-selinene, β-elemene, heptanol, nonanol, benzyl alcohol, havicol, benzaldehyde, furfural and furfural alcohol, methyl benzoate, etc. (Nowak *et al.*, 2012). The registration number of the drug is CAS 8000-34-8.

Eugenol can be extracted from clove basil (*Ocimum gratíssimum*) and holy basil (*Ocimum sanctum*). According

to the effect on fish, eugenol from these plants does not differ from eugenol obtained from the clove tree. Eugenol is also part of the AQUI-S anaesthetic (Putland *et al.*, 2020; Sharma *et al.*, 2022).

Clove oil does not dissolve in water and forms large droplets on the surface of the water, which makes it difficult for the active substance to enter the body of fish. This property of clove oil requires careful mixing with water until a fine emulsion is formed (Yostawonkul *et al.*, 2019). Therefore, the search for the optimal method of preparing an emulsion of clove oil is relevant.

There are three main ways to prepare an aqueous emulsion of clove oil: hot, cold, and alcohol. When using the hot method, the required amount of clove oil is collected from the bottle using a syringe and poured into a glass of water heated to +50°C, and then mixed until a fine emulsion is formed. The resulting emulsion is poured into a container for fish anaesthesia (Ross & Ross, 2008).

For the cold method, clove oil is collected in a syringe and poured into a container with water, in which the fish will be anaesthetised. Intensive mixing of oil with water manually or using a technical device, for example, a kitchen mixer, is recommended to form an emulsion (Kovalenko *et al.*, 2022). This method is simpler but requires a careful mixing of water with clove oil because in cold water the process of emulsion formation takes longer than in hot water.

When using the alcohol method, clove oil is dosed using a syringe, poured into a glass with ethyl alcohol (95%), in which the oil is dissolved by careful mixing. The prepared mother liquor is poured into a container for anaesthesia of fish. The recommended ratio of clove oil to alcohol is 1:10 (Öğretmen & Gökçek, 2013; Aydın *et al.*, 2015). The solution is prepared immediately before use. Its long-term storage is unacceptable.

In the scientific literature, there is no information on conducting experiments to compare the above-mentioned methods of preparing clove oil for the effectiveness of anaesthetic effects on fish, which was the determining factor for choosing the research subject.

The purpose of this study is a search for the most effective way to prepare an aqueous emulsion of clove oil for the anaesthesia of fish in aquaculture. The task was set to achieve the goal – conduct an experiment to assess the degree of anaesthetic effect of clove oil on juvenile fish of African clary catfish using hot, cold, and alcoholic methods of preparation of the mixture, analyse the results of the experiment, and draw conclusions.

Materials and Methods

An experiment to compare the effectiveness of the anaesthetic effect on fish of clove oil preparation for fish, prepared by three different methods, hot, alcohol, and cold, was conducted in 2022 at the Centre for Aquatic Bioresources and Aquaculture of the National University of Life and Environmental Sciences of Ukraine. The hot method of preparation of the emulsion, the most common in the modern practice of using clove oil for fish anaesthesia (Poplavskaya *et al.,* 2017), was chosen as a control option, cold and alcohol methods – as experimental options.

The object of research is juvenile fish of the African clary catfish (*Clarias gariepinus*), 1.5 months of age, with an individual body weight ranging from 22 to 285 g. The experimental material was kept in an aquarium with a capacity of 400 litres without feeding for one day before the experiment began.

The following laboratory equipment and fish farming equipment were used to conduct the experiment:

- liquid container with lid, working volume 100 ml;
- 1 cm^3 syringe;
- 10 cm^3 syringe
- thermal oximeter;
- stopwatch;
- aquarium aerator;
- aquarium with a capacity of 400 litres – 1 unit;
- aquarium with a capacity of 50 litres – 2 units;
- laboratory glass stick;
- net for catching fish;
- electronic scales;
- bowl for weighing fish.

Clove oil of TM Aromatika (Ukraine) in bottles with a capacity of 5 and 10 ml, purchased in a pharmacy chain in Kyiv was used to prepare an aqueous emulsion of the anaesthetic preparation.

The process of preparing an emulsion of clove oil in three ways included the following steps.

Hot method:

- 1 ml of the drug was collected from a bottle of clove oil with a syringe and poured into a liquid container;
- using a syringe, 10 ml of water heated to a temperature of +50°C was added to the container;
- the container was closed with a lid and shaken manually for 30 seconds until a fine emulsion was formed.

Cold method:

- 1 ml of the drug was collected from a bottle of clove oil with a syringe and poured into a liquid container;
- using a syringe, 10 ml of water at room temperature (+26°C) was added to the container;
- the container was closed with a lid and shaken manually for 30 seconds until a fine emulsion was formed.

Alcohol method:

- 1 ml of the drug was collected from a bottle of clove oil with a syringe and poured into a liquid container;
- using a syringe, 10 ml of 95% ethyl alcohol was added to the container;
- the container was closed with a lid and shaken manually for 30 seconds to better dissolve the oil in alcohol.

Two aquariums with a volume of 50 litres each were prepared for the experiment. Each aquarium was filled with 10 litres of tap water work prepared according to the settling method. One aquarium was used to put fish in a state of anaesthesia, the second – to withdraw fish from a state of anaesthesia. An emulsion of clove oil prepared in one of three ways was poured into an aquarium of water intended for fish anaesthesia and mixed with a glass stick to evenly distribute the emulsion in the water.

The concentration of the drug for the anaesthesia of fish in all variants of the experiment was the same – 0.1 ml of clove oil in 1 litre of water. The choice of such a concentration of the drug for the anaesthesia of clary catfish was made based on the results of a previous study (Kovalenko *et al.*., 2022).

The aquarium for withdrawing fish from the state of anaesthesia was equipped with an aerator to enrich the water with oxygen to speed up the release of fish from anaesthesia in the absence of water flow.

After the above preparation, the experiment began:

1) using a net, one by one, specimens of fish was caught from a 400-litre Aquarium and placed in an aquarium with an aqueous emulsion of an anaesthetic;

2) the changes were observed in the state of fish;

3) a stopwatch was used to record the time when the fish entered a state of anaesthesia, which, in accordance with the protocol of induction of anaesthesia for fish (Zahl *et al.*, 2012), has the following signs: loss of reflex activity, position with the abdomen up, movement of the gill covers – barely noticeable, lack of response to strong external stimuli;

4) the immobilised fish was taken out of the aquarium and weighed on a laboratory scale with an accuracy of ±0.1 g;

5) immediately after weighing, the fish was placed in an aquarium with clean aerated water;

6) changes in the condition of fish were observed and the time of recovery from anaesthesia was recorded for signs of respiratory activation, complete recovery of motor activity and normal body position – with back up, and the presence of a reaction to external stimuli.

Thus, an experiment was conducted on three variants, with the corresponding use of a clove oil preparation prepared in 3 different ways. The total number of clary catfish used in the experiment was 90 fishes. 30 of them were used in each version of the experiment.

The results of the experiment were processed using generally accepted methods of variation statistics. Univariate dispersion analysis was used to assess the effect of the method of preparation of an aqueous emulsion on the time of entry into anaesthesia and the time of recovery from this state.

A correlation analysis of experimental data was performed to assess the relationship between the duration of individual stages of fish anaesthesia (the processes of fish entering and withdrawaling the state of anaesthesia) and individual fish body weight.

The mathematical processing of the digital data of the experiment was conducted using methods generally accepted in animal husbandry (Ruban *et al.*., 2020).

Results and Discussion

The effectiveness of the anaesthetic effect on clary catfish juvenile fish of clove oil prepared by three methods was investigated: hot, cold, and alcohol. The results of the experiment on the comparative evaluation of these three methods of preparation of the mixture for the achieved effect of fish anaesthesia are presented in Table 1.

Table 1. *Influence of the method of preparation of clove oil emulsion on achieving the effect of anaesthesia in clary catfish juvenile fish*

Method of preparation of water emulsion of clove oil	Duration of changes in the condition of fish, seconds	
	Entering a state of anaesthesia	Recovery from anaesthesia
Hot	94.1±7.49	111.7±3.86
Alcohol	116.5±8.81*	196.5±21.10***
Cold	120.2±8.09*	136.1±8.83*

Note: * – $p \leq 0.05$, *** – $p \leq 0.001$ (compared to the hot method)

As can be seen from Table 1, for all variants of preparation of clove oil mixture, the effect of an anaesthetic effect on the condition of experimental biological material of fish was obtained. Therewith, the time interval during which clary catfish juvenile fish were first introduced into a state of anaesthesia, and then, after recovery from the drug and weighing, withdrawed from this state, varied substantially: from 94.1±7.49 to 120.2±8.09 and from 111.7±3.86 to 196.5±21.10 seconds, respectively. The difference between the extreme values of these indicators was 27.7% and 75.9%, respectively, which is objective evidence of the influence of the method of preparing clove oil on the effectiveness of the anaesthetic effect of this drug on fish. Univariate analysis of variance showed that the strength of the influence of the method of preparation of clove oil for anaesthesia factor on the duration of entry of clary catfish juvenile fish into the state of anaesthesia was 6.4% ($p \leq 0.05$), and for the time to withdrawal this state – 19.6% ($p \leq 0.001$).

Evaluation of the results of the experiment based on compliance with existing recommendations for choosing the ideal anaesthetic for fish (Park, *et al.*, 2017; Bolasina, *et al.*, 2017) on the duration of the processes of entering the object of influence into the state of anaesthesia (<3 min) and recovery after this state (<5 min) showed that for all three variants of preparation, the mixture of clove oil met the established criteria for effectiveness.

In the control version of the experiment (hot method of preparation of clove oil mixture), the highest effect of anaesthetic effects on clary catfish juvenile fish was obtained, both in terms of the time of introduction of young catfish into the state of anaesthesia (94.1±7.49) and the duration of the fish recovery process after this state (111.7±3.86). Comparison of the results obtained

in two experimental versions of the experiment with the control showed that the time of the entry of fish into the state of anaesthesia for both methods substantially increased (p≤0.05): for the alcohol method – by 22.4 seconds (12.3%), for the cold method – by 26.1 seconds (22%). The duration of fish recovery from the state of anaesthesia, in comparison with the control option, also increased: with the alcohol method of preparation of the anaesthetic emulsion – by 84.8 seconds (43.2%) (p≤0.001), in the cold method – by 24.4 seconds (18%) (p≤0.05).

The results of the search for a correlation between the live weight of clary catfish juvenile fish and the duration of fish entry into the state of anaesthesia and calculations of the regression coefficient are shown in Table 2.

Table 2. *Correlation and regression coefficients between the live weight of fish and the duration of entry of clary catfish juvenile fish into the state of anaesthesia for various methods of preparation of the anaesthetic mixture*

Method of preparation of clove oil mixture	Correlation coefficient (r)	Regression coefficient, s/10 grams
Hot	0.606***	3.91***
Alcohol solution	0.529***	5.01**
Cold	0.385*	2.96*

Note: *– p≤0.05; **– p≤0.01; ***–p≤0.001

As can be seen from Table 2, the degree of correlation between the live weight of clary catfish juvenile fish and the duration of entry of these fish into the state of anaesthesia was generally positive, but substantially varied depending on the method of preparation of the mixture for anaesthesia: from high, using hot (*r*=0.606) and alcohol methods (*r*=0.529) to medium – using the cold method of preparing an aqueous emulsion of clove oil (*r*=0.385). It should also be noted that there is a substantial correlation between the live mass of clary catfish and the duration of induction of the state of anaesthesia in fish for all variants of the experiment, the value of which was: for hot and alcoholic methods of preparation of the anaesthetic emulsion – p≤0.001, for the cold method – p≤0.05.

Analysis of the degree of dependence between the live mass of clary catfish and the duration of the induction process of anaesthesia in fish showed that an increase in the mass of clary catfish by 10 g leads to an increase in the duration of fish entry into the state of anaesthesia by 3.91 seconds for hot, 5.01 for alcohol, and 2.96 for cold methods of preparation of clove oil. The substantiality of the reliability of the regression coefficients for the experimental variants was: for the hot method of preparation of the anaesthetic mixture – p≤0.001, for the alcohol method – p≤0.01, for the cold method – p≤0.05.

The results of determining the correlation coefficients between the live weight of clary catfish and the time of recovery of these fish from the state of anaesthesia and the degree of regression changes in these indicators for each method of preparation of the anaesthetic mixture are shown in Table 3.

Table 3. *Correlation and regression coefficients between the live weight of clary catfish juvenile fish and the time of recovery from anaesthesia for different methods of preparation of the anaesthetic emulsion*

Method of preparation of clove oil mixture	Correlation coefficient (r)	Regression coefficient, s/10 grams
Hot	0.111	0.37
Alcohol solution	-0.368*	-8.36*
Cold	0.306	2.57

Note: *– p ≤ 0.05

As can be seen from Table 3, the search for a correlation between the live weight of fish and the duration of their recovery from anaesthesia showed radically different results, depending on the experiment option: the practical absence of such a relationship (*r*=0.111) for the hot method of preparing an aqueous emulsion of clove oil; a weak direct connection (*r*=0.306) – for the cold method; inversely proportional relationship, with a degree below average (*r*=-0.368) – for the alcohol method of preparing a solution of an anaesthetic preparation. The substantiality of correlation ties was established only for the alcohol method of preparation of the anaesthetic mixture (p≤0.05).

Only one substantial indicator was considered to analyse the degree of dependence between the weight of fish and the time of its release from the state of anaesthesia – from the option of alcohol method for preparing a mother liquor of clove oil for fish anaesthesia, with a regression coefficient of -8.36 at the substantiality level at p≤0.05. This indicator shows that with an increase in the body weight of the clary catfish by 10 grams, the time when the fish comes out of the state of anaesthesia decreases by 8.36 seconds.

Based on the results obtained, it can be concluded that there is a moderate tendency to increase the time of the recovery of the African clary catfish from the state of anaesthesia with a decrease in the live weight of the object of anaesthetic action only when using an alcoholic method of preparing the mother liquor of clove oil for anaesthesia of this type of fish.

The establishment of a positive correlation between the individual weight of clary catfish juvenile fish and the duration of fish introduction into anaesthesia confirmed the results of the first study of the authors conducted on this fish species earlier (Kovalenko *et al.*, 2022). Thus, in the previous experiment conducted on fish with an average body weight of 928 g, against 575 g in the second experiment, under similar conditions (cold method of preparation of clove oil emulsion for anaesthesia, option with a water temperature of +26°C), the time of entry of fish into the state of anaesthesia was recorded, almost one and a half minutes longer than in this study, confirming the identified dependence. However, the conclusion made in the previous study about the presence of an inverse relationship between the individual weight of fish and the time of its release from the state of anaesthesia was confirmed only in the version with the alcohol method of preparing the mother liquor of clove oil. In the version with the cold method of preparing the emulsion for anaesthesia, the effect of body weight on the duration of fish recovery from the state of anaesthesia was the opposite – a direct weak relationship between these indicators was established. Such different results of assessing the degree of correlation between the live mass of fish and the duration of their withdrawal from the state of anaesthesia, depending on the method of preparation of clove oil mixture, dissonant with the indicators obtained when searching for links between the mass of fish and the duration of induction of their state of anaesthesia, indicate the feasibility of conducting a second experiment.

Thus, according to the results of the experiment, it can be stated that the hot method of preparing an aqueous emulsion of clove oil for fish anaesthesia (on the example of clary catfish), in comparison with alcohol and cold methods, demonstrated its advantages, both in the shorter duration of fish entry into the state of anaesthesia and in the time of fish withdrawal from this state. This conclusion is generally consistent with the conclusions of other researchers (Ross, 2008; Aydın, 2020) regarding the feasibility of preparing a mother's liquor of a fine clove oil emulsion in high-temperature water (about 50°C), due to which, the drug is quite easily dispersed during intensive shaking. Therewith, this method loses its advantage when it is necessary to work with fish in conditions of low water temperatures (below 10°C), for example, when manipulating cold-loving fish species (salmon, whitefish) or with heat-loving objects, but at low water temperatures (for example, when conducting spring bonitation of breeding carp fish species). Under such working conditions, an alcohol method for preparing a mother liquor of clove oil, followed by bringing the concentration of the drug in water to working values can be proposed (Javahery, 2012; Öğretmen, 2013; Aydın, 2015).

Unfortunately, the authors of the study were not able to compare the results of an experiment to test the effectiveness of using clove oil from different methods of preparing a mixture for anaesthesia of 1.5-month-old clary catfish juvenile fish with materials from similar studies by other researchers due to the lack of relevant information. The following is a likely explanation for this state of affairs: physiological features of the clary catfish, as a representative of lungfish with exceptional survivability and a wide range of ecological adaptations (Weyl *et al.*, 2016), primarily due to oxygen deficiency, until recently did not encourage researchers to search for ways to anaesthetise this strong and hardy fish during technological manipulations or lifetime laboratory tests with it. Only the growing attention to the subject of the humane treatment of animals throughout the civilised world and the introduction of appropriate national and international standards and protocols for working with biological material have led to certain changes in this issue.

Conclusions

As a result of the experiment, new data were obtained on the technique of using clove oil for anaesthesia of African clary catfish, which have no analogues according to the search data in sources of scientific and technical information. The anaesthetic effect of using three methods of preparation of this anaesthetic, hot, cold, and alcohol, on 4-5-month-old juvenile fish of clary catfish was investigated and the influence of individual fish mass on the duration of different stages of the anaesthesia process was evaluated.

It was identified that the induction of the state of anaesthesia in clary catfish juvenile fish and the recovery of fish from the state of anaesthesia occurs most quickly using a fine aqueous emulsion prepared using a hot method of diluting clove oil. In the version with the alcohol method of preparing a solution of clove oil for anaesthesia, the second result was obtained in terms of the rate of introduction of fish into the state of anaesthesia and the third – in terms of the duration of recovery of fish from this state.

A direct relationship was identified between the duration of entry of clary catfish into the state of anaesthesia and the individual body weight of fish, with the degree of connection, depending on the method of preparation of clove oil for anaesthesia: high – for hot and alcoholic methods, medium – for cold method. Regression coefficients are calculated for each method of preparing clove oil for anaesthesia, which is of practical importance – it allows predicting the time of this stage of anaesthesia for each object of influence, depending on its individual body weight.

Divergent data were obtained on various variants of the experiment regarding the nature of the influence of individual body weight of clary catfish on the duration of the stage of withdrawal of clary catfish from the state of anaesthesia: from weak positive – for the cold method of preparation of the anaesthetic mixture, to average inversely proportional – for the alcohol method. This result makes it necessary to conduct additional research on this issue.

A promising area for deepening the research of the use of clove oil as a natural anaesthetic for various fish species should be considered the assessment of the consequences for fish health from repeated use of the anaesthesia procedure with this drug, in particular, for the implementation of reproductive function in the mother material of the valuable aquaculture objects.

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Вплив способу приготування емульсії гвоздичної олії на анестезуючий ефект у риб (на прикладі *Clarias gariepinus)*

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Анотація. В аквакультурі набуває популярності використання гвоздичної олії в якості природного анестетика для гідробіонтів. Існує декілька способів приготування водної емульсії препарату для анестезії, що створює для технологів проблему вибору найбільш ефективного. Метою дослідження була порівняльна оцінка анестезуючого впливу гвоздичної олії за трьох способів приготування препарату на молодь африканського кларієвого сома. Дослідження проведено у Центрі водних біоресурсів та аквакультури Національного університету біоресурсів і природокористування України. Методи дослідження – іхтіологічні та статистичні. Оцінено активність препарату гвоздичної олії, приготовленого на водній основі за трьома способами: гарячим, холодним і спиртовим. Дослідний матеріал – цьоголітки кларієвого сома з індивідуальною масою тіла від 22 до 285 г. Умови експерименту: дослідний матеріал до початку дослідження утримували без годівлі протягом доби; анестезію риб проводили у воді однакової температури +26 °С; тривалість маніпуляцій з кожною рибою була мінімальною. Ефективність різних способів приготування препарату оцінювали за тривалістю входження риби у стан анестезії і виходу із цього стану. Встановлено перевагу гарячого способу приготування водної емульсії гвоздичної олії над двома іншими: тривалість входу риби в анестезію була меншою у порівнянні з холодним способом на 22 %, із спиртовим способом – на 12,3 % ($p \leq 0,05$). Тривалість виходу риби зі стану анестезії за гарячого способу підготовки препарату також виявилася меншою: у порівнянні з холодним способом – на 18 % ($p \leq 0,05$), із спиртовим способом – на 43,2 % ($p \leq 0,001$). За допомогою кореляційного аналізу встановлено зв'язок між тривалістю входження риби у стан анестезії та індивідуальною масою тіла риби. Ступінь зв'язку: високий для гарячого ($r=0,606$) та спиртового способів ($r=0,529$) і середній – для холодного способу підготовки препарату анестетика ($r=0,385$). Отримані результати становлять практичну цінність для удосконалення методу анестезії риб з використанням гвоздичної олії

Ключові слова: аквакультура, анестезія, водна емульсія, кларієвий сом

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 13(4), 39-49
Received: 20.09.2022 Revised: 22.10.2022 Accepted: 01.11.2022

UDC 006.83:637.52:598.261.7
DOI: 10.31548/animal.13(4).2022.39-49

The quality of quail meat cans depending on storage conditions and time of consumption

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Suggested Citation:
Macari, V., Pavlicenco, N., Rotaru, A., Pistol, G., Alexandruc, M., & Israelian, V. (2022). The quality of quail meat cans depending on storage conditions and time of consumption. *Animal Science and Food Technology*, 13(4), 39-49.

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Abstract. Physicochemical and biochemical changes may occur in products of animal origin under the influence of internal and external factors, depending on the storage conditions. For the products made from the meat raw materials of various origins, it is important to know the optimal duration and modes of storage, which will preserve the optimal indicators of product quality and safety. The study aimed to determine the effect of different temperature regimes during storage on the quality and safety indicators of canned quail meat. Three series of experiments were conducted on the canned quail meat. In the first experiment, the quality indicators of the freshly prepared product were evaluated. In the second and third experiments, the parameters of the product were determined after aging for one year at temperatures of 2-4°C and 18-20°C. Organoleptic properties and physico-chemical and microbiological parameters were evaluated in the canned goods. The following methods of research were used: during the organoleptic assessment, it was considered the following properties: appearance, color, cross-section, smell, taste; fat content – by the Soxhlet method; hydrogen index (pH) – by the potentiometric method; microbiological indicators – by the horizontal method of counting colonies of microorganisms. It was found that the storage temperature did not affect the bacteriological indicators of the samples, which indicates the high quality of sterilization and excludes biological influence on the quality of canned goods. It has been established that, regardless of the storage temperature, the organoleptic indicators of canned food 12 months after production meet the state standard and the manufacturer's requirements, and they do not differ significantly. After one year of storage, the physico-chemical indicators of canned quail meat are within the limits of indicators established by the standard, regardless of the storage temperature. At the same time, certain changes were detected in the canned goods, which depended on the temperature conditions of storage. Considering the trends in physico-chemical parameters, the desired storage temperature for canned quail meat is 2-4°C, although the increase in temperature to 18-20°C does not lead to significant changes and it is permissible. Research is scientifically based on establishing the safety and quality of canned minced quail meat during long-term storage conditions, to create and produce high-quality and safe food products, which in turn allows for expanding the assortment of canned meat raw materials

Keywords: bones, collagen, peroxide index, sterilization, the bloat of cans

Introduction

In modern society, special attention is paid to proper nutrition, balanced in nutritious and safe substances. Nutritionists and food industry professionals are concerned about the issue of functional nutrition. The quality and safety of foodstuffs depend on the quality of the raw and auxiliary materials used, the conditions of processing, and storage. It is known that during the technological flow and the period of storage, there are permanent physico-chemical and biochemical changes, which lead more or less to the loss of nutritional and hygienic quality (Ostachowicz *et al.*, 2019).

Food storage is an important stage of the technical and economic circuit. During the storage of products, qualitative changes may occur, the intensity and meaning of which depend on the interaction between internal and external factors of the products. The prevention of these changes requires constant monitoring of the storage regime and action, and if necessary, to ensure and maintain a balance between action and mutual dependence on internal and external factors of the products (Umaraw *et al.*, 2020). The most important physical changes are mainly related to the fluctuating action of the air parameters, respectively the relative temperature and relative humidity. The influence of these parameters is the basis for both physical changes and all other types of changes (Riabovol & Bal-Prylypko, 2021).

The quality of canned quail meat is of particular interest, depending on the storage conditions and the term of consumption, having a high nutritional value; they can be consumed in its pure form, without any additional preparation. These products can be stored for a certain time, in conditions of adequate microclimate, constituting the supplement of the basic food for all categories of consumers (Abdel-Atty *et al.*, 2020).

The technology of manufacturing canned meat using the method of sterilization is primarily aimed at obtaining safe products, free from microorganisms that can lead to spoilage of the product and poisoning of consumers. Currently, this problem remains relevant. In particular, in the studies conducted in Egypt, the *Clostridium perfringens* was isolated from 28% of canned meat samples from beef and poultry (Bal-Prylypko *et al.*, 2022). This indicates the possibility of bacterial contamination due to violations during the receipt and storage of meat raw materials and technological requirements for the production of product, which affects changes in the canned meat during storage.

As a result of the long-term storage of sterilized canned meat, changes occur, which are associated with corrosion of the outer and inner surface of cans and changes in the sensory and physicochemical properties of the product. In particular, it was detected the color changes, accumulation of greenish-blue crystals, an unpleasant smell, the smell of stale meat, and a bitter aftertaste (Stojanovic *et al.*, 2021). An increase in the content of Fe in the product is also noted (Rashid & Khidhir, 2021), which leads to a deterioration of sensory evaluation due to a pronounced metallic aftertaste. The effect on canned products is determined not only by the duration, but also by the temperature of storage. In the example of canned tuna, which was stored for 120 days at temperatures of 5°C, 15°C, and 25°C, a connection was established (Sabow, 2021) between elevated storage temperatures and an increase in pH (up to 8.5 at

25°C) with the content of total volatile base nitrogen, free fatty acids, and thiobarbituric acids. During the storage of canned quail meat, chemical changes related to the fatty acid profile of fats can be expected, since this product contains a significant amount of unsaturated fatty acids. It has been found (Bal-Prylypko *et al*., 2016) that in some parts of the quail carcass, more than one-third of all fatty acids are oleic acids.

The purpose of the study is to determine the influence of temperature parameters on the quality and organoleptic indicators of the canned quail meat during the storage process. To fulfill the goal, the following tasks were defined: to conduct a literature search on the sterilization regimes of canned goods and the influence of microorganisms on the quality of ready-made canned goods during the storage, and to investigate the organoleptic, physico-chemical and microbiological indicators of product quality depending on the temperature and storage period.

Materials and Methods

The research was conducted on the canned stuffed quail meat produced in the Republic of Moldova. The first stage of the research consisted of evaluating the quality indicators of the freshly prepared product. In the second and third stages, it was conducted the studies of the complex characteristics of the quality of finished products after the aging for one year at temperatures of 2-4°C and 18-20°C; in particular, organoleptic, physico-chemical and microbiological parameters were determined.

There were 3 series of research on this product between 25.11.2020-13.10.2021 with the aim of researching the quality of canned quail meat depending on the storage conditions and the term of consumption and safety of the product. The assessment of quality of the researched product and the changes that took place under the influence of storage conditions, namely the temperature at 2 regimes (2-4°C and 18-20°C) and the term of consumption were made within the Institute Publica "Centrul Republican de Diagnostic Veterinary". Six samples of the investigated material were taken. All assessed quality indices and indices indicating changes in the quality depending on the storage conditions and shelf life of the product were analyzed by the national regulatory requirements in force and the company standard for this product. The organoleptic evaluation of the finished product was carried out according to DSTU 4823.2:2007. The main quality indicators were considered during the evaluation: appearance, color, cross-section, smell, and taste. Determination of physicochemical parameters, namely: hydrogen index (pH) – by the potentiometric method according to DSTU ISO 2917-2001 (2003), the mass fraction of the total fat content was determined by the Soxhlet method according to DSTU 8380:2015 (2017), sodium chloride content in canned meat was carried out according to DSTU 4939:2008. The determination of microbiological changes in canned goods was evaluated by the horizontal method using the quantitative counting of colonies of microorganisms following DSTU 8446:2015 (2017), and ISO 11290-1:2017 (2017). According to DSTU 7992:2015 (2017), DSTU 7963:2015 (2017), the sampling and determining the quality and safety of food products, packaging and labeling were carried out according to the rules and methods of analysis established by the current legislation for this type of product.

Results and Discussion

The evaluation of quality of the canned quail meat, depending on the storage conditions and the consumption term, was started with the sensory (organoleptic) analysis of the investigated product. Sensory analysis is very important when considering the quality of the end product (Fu & Chen, 2019). In the Table 1, there are the results of the organoleptic indices investigated in the first series of research.

Table 1. *The values of the organoleptic indices of canned quail meat*

Parameter analyzed	Test methods	Obtained results
Canned meat. Quail meat	DSTU 4823.2:2007, PS- CSA-PRO-01	Glass jar, tightly closed, uncombed. They can have pieces of meat, without tendons, bones and connective tissue. The meat is not juicy, rough, or uncooked, it does not crumble when removing from the jar. The color of the flesh is yellowish-cream, without spots. The unheated broth is clear light yellow. Pleasant odor, characteristic of the product, with the aroma of spices, without foreign odor. Pleasant taste, suitable for salting, and moderately spicy, without foreign taste

Source: (2009)

The results in the Table 1 regarding the organoleptic characteristics of the product investigated in the first series of research correspond to all the requirements presented in the company standard for this product and the National Legislation (GD No. 624 of 19.09.20 on the approval of the Quality Requirements for preparations and products meat), and no deviations from the norm regarding organoleptic indices have been detected. In the second series of research – the researched product was kept at a temperature of 2-4°C for 1 year. The organoleptic indices of the canned quail meat are shown below in the Table 2.

Table 2. *Organoleptic indices of the canned quail meat in the second series of research*

Sample name	Obtained results	Normative requirements	Test methods
Canned meat Quail meat	Glass jar, tightly closed, uncombed. Canned meat pieces, compactly placed, without tendons, bones and connective tissue. The meat is not juicy, rough, or uncooked, it does not crumble when removing from the jar. The color of the flesh is yellowish-cream, without spots. The unheated broth is clear light yellow. Pleasant odor, characteristic of the product, with the aroma of spices, without foreign odor. Pleasant taste, suitable for salt, without foreign taste	SF MD 41213475-002: 2019 (2019)	DSTU 4823.2:2007, PS- CSA-PRO-01

Table 2 clearly shows that the organoleptic indices of the investigated product (in the second series of research, which was kept at a temperature of 2-4°C, for 1 year) correspond to all the requirements presented in the company standard.

It is important to note that in the third series of research, the analyzed product has been kept at a temperature of 18-20°C for 1 year. Organoleptic indices in this series of research are given below in the Table 3.

Table 3. *Organoleptic indices of the canned quail meat in the third series of research*

Sample name	Obtained results	Normative requirements	Test methods
Canned meat Quail meat	Glass jar, tightly closed, uncombed. Canned meat pieces, compactly placed, without tendons, bones and connective tissue. The meat is not juicy, rough, or uncooked, it does not crumble when removing from the jar. The color of the flesh is yellowish-cream, without spots. The unheated broth is clear light yellow. Pleasant odor, characteristic of the product, with a slight aroma of spices, no foreign odor. Pleasant taste, suitable for salt, without foreign taste	SF MD 41213475-002: 2019 (2019)	DSTU 4823.2:2007, PS- CSA-PRO-01

From the data presented in Table 3, it can be stated that the organoleptic indices investigated in the product under study (in the third series of research, which was kept at a temperature of 18-20°C for 1 year) correspond to all the requirements presented in the company standard. At the same time, the assessment of product quality and safety has been determined by several specific aspects of the food products, which are carried out (determined) in laboratory conditions by physico-chemical indices of the canned stewed meat, namely:

- pH;
- mass fraction of salt, %; max
- mass fraction of fat, %,
- mass fraction of proteins, %;
- peroxide index ½ O mmol/kg;
- acidity index, mg KOH mg/g;
- bone mass fraction, %; MAX
- mass fraction of meat, %; min
- mass fraction of jelly sauce, %; MAX
- hydroxyproline content, %;
- mass fraction of proteins, %; min.

Performing the physico-chemical analysis of the canned quail meat studied during the three (3) series of research and compared with the requirements of the company standard, a wide range of results have been obtained, which are presented in the Figure 1.

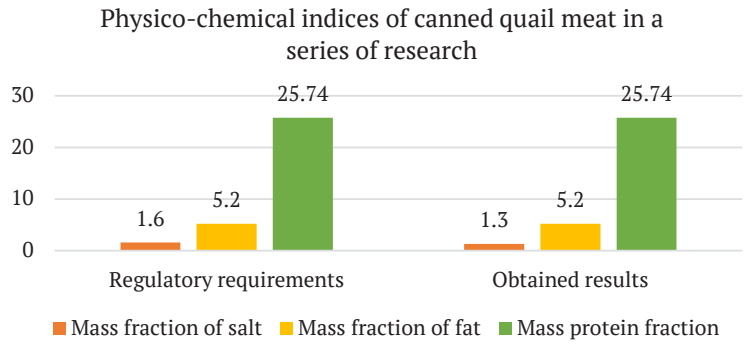

Figure 1. Physico-chemical indices of the stewed canned quail meat with regulatory requirements (1st research series)

According to the standard, the mass fraction of salt, %, max in the stewed canned quail meat amounted to 1.3%, which corresponds to the normative requirements. The mass fraction of fat, %, max in the canned quail meat is 5.2%, and the mass fraction of protein, %, max 25.74%, which corresponds to the requirements and rules in force. The results of the physico-chemical parameters obtained in the second series of investigations after keeping the researched product for 12 months at a temperature of 2-4°C are presented in the Figure 2-4.

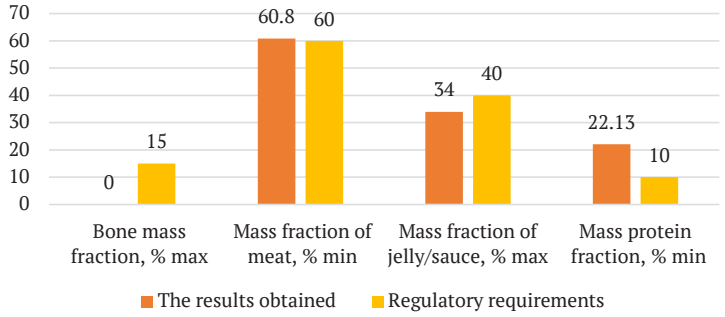

Figure 2. Test results after storage of the product for 12 months at 2-4°C following regulatory requirements

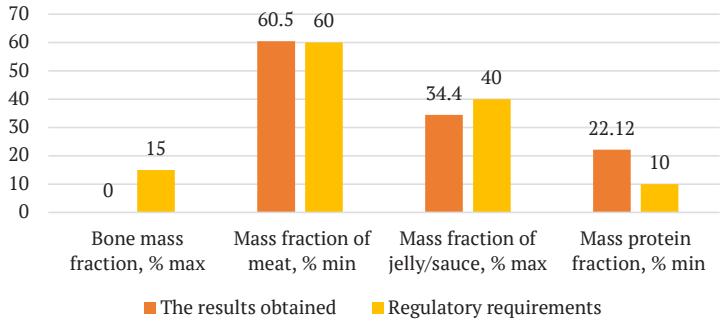

Figure 3. Test results after storage of the product for 12 months at 18-20°C following regulatory requirements

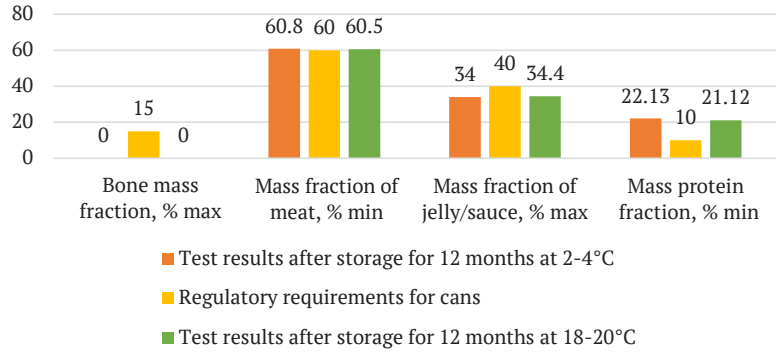

Figure 4. Test results after storage of the product for 12 months at 2-4°C and 18-20°C according to regulatory requirements

The pH is the degree of acidity or alkalinity of a food. Foods naturally contain organic acids. These acids are essential in preserving the taste, color, luster and quality of food. Due to improper sterilization, thermostable microorganisms are not destroyed during this technological process, and at a certain pH value, they can develop during storage, which can cause biological bloat of cans.

Results of pH examination after storage of the product for 12 months at 2-4°C and 18-20°C are presented in the Figure 5.

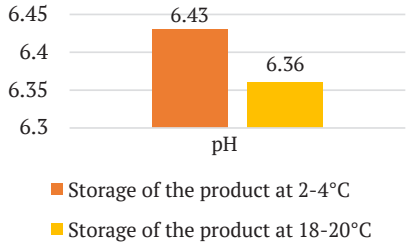

Figure 5. Results of pH examination after storage of the product for 12 months at 2-4°C and 18-20°C

After analyzing the obtained results (Fig. 5), it has been established that the pH of the product stored at 2-4°C is 6.43, and the pH of the product stored at 18-20°C is more acidic and has a value of 6.36. According to the results of conducted research, it has been established that the pH value also depends on the storage temperature of canned quail meat, but its value has decreased insignificantly (by 0.07). Nevertheless, this change did not affect the product's quality, as evidenced through the sensory qualities of the product (Pleasant taste, corresponding to the given product, without foreign taste).

However, during the storage of meat products, oxidation processes of fats can take place. These are the most important factors that determine the loss of quality and implicitly of flavor, texture, nutritional value and color: the lipid oxidation products acting as a substrate for protein oxidation. Oxidation products affect the product's quality, including the organoleptic aspects – rancid odor (Mushtruk *et al.*, 2022).

Results of the examination of the peroxide index and acidity index after storage of the product for 12 months at 2-4°C and 18-20°C are presented in the Figure 6.

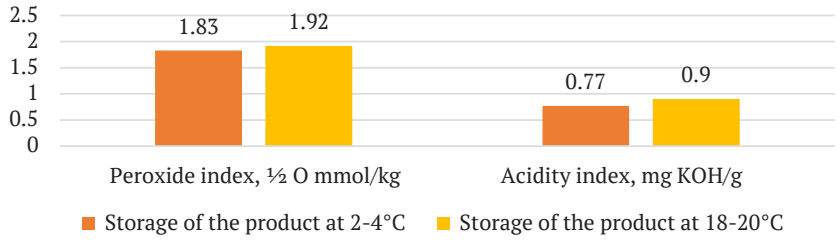

Figure 6. Results of examination of peroxide index and acidity index after storage of the product for 12 months at 2-4°C and 18-20°C

The peroxide index shows the oxidation degree of the product. Thus, analyzing the Figure 6, it can be concluded that the peroxide index of the product stored at 2-4°C is 1.83 and the peroxide index of the product stored at 18-20°C increased by 0.9 to 1.92. Thus, the high storage temperature contributed to the product's oxidation.

The acidity index is a parameter that shows the degree of fats' rancidity. Therefore, analyzing the figure 6, it has been found that the product stored at 2-4°C has an acidity index of 0.77 mg KOH/g, and the product stored at 18-20°C has significantly increased the acidity index, by reaching 0.9 mg KOH/g. Therefore, the temperature influences the value of acidity index in the investigated product. Thus, during the storage period of meat products, the oxidation of fats in products was practically minimal, which was proven by the low value of peroxide and acidity index and the insignificant variation of their values over time, values that did not negatively influence the appearance, aroma and color; it has been confirmed the stability of organoleptic indices during storage of the product, even at different temperatures.

Determination of the hydroxyproline content in the canned meat is a commonly used parameter for assessing the meat quality (Punia *et al.*, 2020).

The hydroxyproline content obtained in the meat products under research is 0.12%, which indicates a good quality of the canned food and demonstrates the use of quality raw material; this fact is confirmed by the meat content of 60.0-60.8% and zero value (0%) of bone content. The mass fraction of bones is an index that reflects the percentage of bones in a certain amount of bone-in meat. This is a parameter used to assess the quality of meat. The mass fraction of bones significantly influences the change of product during storage, because the bones contain a large amount of collagen, which shows the fact that the product will degrade more rapidly during storage.

Results of examination of the mass fraction of bones, meat, jelly/sauce and protein after the product's storage for 12 months at 2-4°C and 18-20°C are presented in the Figure 7.

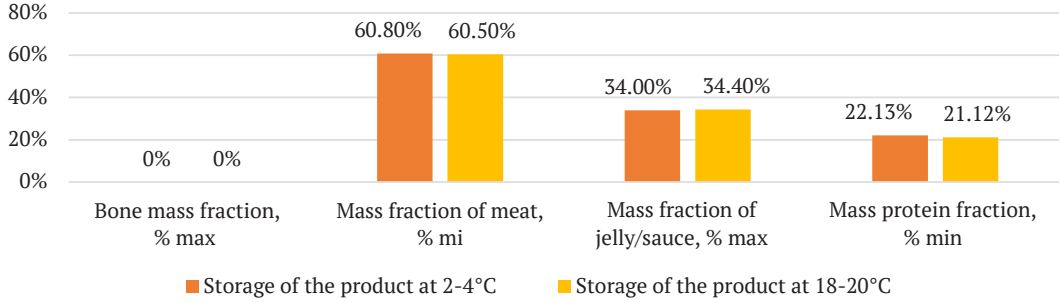

Figure 7. Results of examination of the mass fraction of bones, meat, jelly/sauce and protein after storage of the product for 12 months at 2-4 °C and 18-20 °C

The mass fraction of meat is an important index that shows the quality of canned quail meat. According to the study, analyzing the Figure 7, it has been found that the mass fraction of meat represents 60.8% for the product stored at 2-4°C, and the mass fraction of meat decreased insignificantly by 0.3%, reaching the mark of 60.5% for the product stored at 18-20°C.

The mass fraction of jelly/sauce is an indicator of quality, especially for the canned meat. After conducting the analysis, the Figure 7 shows that the mass fraction of jelly/sauce is 34.0% for the product stored at a temperature of 2-4°C, and the mass fraction of jelly/sauce has increased, by reaching the value of 34.4% for the product stored at a temperature of 18-20°C. Thus, the high temperature directly influences the formation of jelly and meat sauce.

The mass fraction of protein in a meat product restores its quality, as the value of meat mainly consists of the protein. In the course of the study, it has found that the mass fraction of protein is 22.13% for the product stored at 2-4°C, the mass fraction of protein has decreased significantly by 1.01%, reaching the mark of 21.12% for the product stored at 18-20°C (Figure 7).

After analyzing the obtained results, it is possible to conclude the following: all the physicochemical properties of canned quail meat are within the parameters set by the standard, and their value may vary depending on the storage conditions and the period of consumption, depending on the storage temperature that does not affect the quality of the product (Barreira *et al.*, 2019). It should be noted that the most important requirement of the food is its compliance with the rules of microbiological indices (Bal-Prylypko & Nikolayenko, 2018). It is pointed out in the cited sources that the microbiological safety of consumer is an important issue in ensuring the complete sterility of these types of cans (Creydt & Fischer, 2019), Holembovska *et al.*, 2021).

The microbiological indices analyzed in the researched canned quail meat (III research series) are presented in the Tables 4, 5, and 6, respectively.

Table 4. *Microbiological indices of the stewed canned quail meat in the first research series*

Analyzed parameter	Test methods	Normative requirements	Obtained results
Listeria monocytogenes in 25 g	ISO 11290-1:2017 [14]	Not Admitted	Not detected
Industrial sterility	DSTU 8446:2015 [13]	Not Admitted	Mesophilic aerobic viable microorganisms in 0.1 g were not detected. Viable mesophilic anaerobic microorganisms in 0.1 g were not detected. It corresponds to the industrial sterility

Table 5. *Microbiological indices of the stewed canned quail meat in the second series of research*

Sample name	Obtained results	Normative requirements	Test method
Listeria monocytogenes in 25 g	Not detected	Not Admitted	ISO 11290-1:2017 [14]
Industrial sterility	Mesophilic aerobic viable microorganisms in 1.0 g were not detected. Viable mesophilic anaerobic microorganisms in 1.0 g were not detected. It corresponds to the industrial sterility	Not Admitted	DSTU 8446:2015 [13]

Table 6. *Microbiological indices of the stewed canned quail meat in the third research series*

Sample name	Obtained results	Normative requirements	Test method
Listeria monocytogenes in 25 g	Not detected	Not Admitted	ISO 11290-1:2017 [14]
Industrial sterility	Mesophilic aerobic viable microorganisms in 1.0 g were not detected. Viable mesophilic anaerobic microorganisms in 1.0 g were not detected. It corresponds to the industrial sterility	Not Admitted	DSTU 8446:2015 [13]

Analyzing the data obtained from the laboratory expertise of microbiological indices (*Listeria monocytogenes*) and Industrial Sterility in all series of research, it is clear that the product is safe and meets the requirements in force. Mesophilic aerobic viable microorganisms in 1.0 g and Mesophilic anaerobic viable microorganisms in 1.0 g were not detected, Listeria monocytogenes in 25 g was not detected; moreover, their presence is not even allowed according to the company standard and GD no. 221/2009 "Rules on microbiological criteria for food", (Cherednichenko *et al.*, 2020), (Costachescu *et al.*, 2018).

The production of canned meat from quail is one of the methods of preserving meat, which allows keeping stocks of highly nutritious, high-quality and safe meat products ready

for consumption for a considerable period. In the studies (Suchenko *et al.*, 2017), it is stated that the biochemical processes occur due to the development of residual anaerobic microflora as a result of non-compliance with sterilization regimes or insufficient time for its implementation. All this leads to the rapid spoilage of the product.

Significant attention is paid to the safety of raw materials included in the canned products. M. Nikolaienko & L. Bal-Prylypko, (2020) have identified the dangerous factors in the production of stewed meat. The researchers have established that the risks of biological origin exist in every technological operation. O.M. Bergilevich, V.V. Kasyanchuk (2018) and O.A. Hitska (2018) note that the control of manufacturing process and especially the storage of canned meat should be risk-oriented and should be carried out at all stages of the production and circulation.

This requires a scientific-based assessment of risks, especially microbiological ones, which arise during the storage of canned meat (Roşca *et al.*, 2018; Zheplinska *et al.*, 2021).

The author of scientific work (Sofos, 2014) has conducted a study of various types of hazards that can be in the composition of meat products, and which can include chemical substances and biological agents (bacteria, viruses and parasites, and abnormal prions, as well as various mechanical impurities). According to the author, it follows that the formation of microorganisms in meat and meat products is a normal process, since various types of microorganisms are present in the animals and their environment. The presence of microbes and their growth is the reason of rapid spoilage of products and decrease in the safety. The quality and safety of meat and meat products must be maintained by an integrated preventive approach at all stages during production, including producers, processors, distributors, sellers, catering establishments, as well as consumers (Sofos, 2014).

The team of authors (Šopík *et al.*, 2022) have conducted a series of experimental studies regarding the development of indicators that have a significant impact on changes in the quality of food products and safety of the selected canned (Szeged goulash, canned chicken meat, pork pate, canned tuna) and dehydrated (goulash) food products during two years of storage. Experiments were conducted at four different temperature regimes. Storage temperatures have been chosen to reflect the following climate zones: arctic (-18°C), temperate (5°C), subtropical (25°C) and tropical (40°C), where such food is likely to be stored during humanitarian and military missions. The authors proposed methods for determining the number of microorganisms, depending on the storage temperature, and methods for determining the dry matter, fat and proteins. According to the results of experiments, it was observed an increase in the level of ammonia in all food products during 24-month storage, and the loss of individual amino acids during storage reached the mark from 5% regarding the calculated content of amino acids in a month "0" up to 15%. At storage temperatures above the freezing point, the indicators of hardness decreased with an increase in the storage temperature. Furthermore, at temperatures -18°C, the hardness development measured as the "rate of decline" was significantly higher compared to the absolute values (Šopík *et al.*, 2022).

Based on the above information, it has been proven that the researched products are of high quality, harmless, and safe for the consumer's health, as well as meet the requirements of industrial sterility and hygiene.

Conclusions

The stewed canned quail meat under research has demonstrated qualitative characteristics suitable for the manufacture for public consumption, complying with all the requirements and norms of the company standard and the requirements of the National Legislation (GD No. 624/2020) for this type of product.

Organoleptic parameters, such as color, taste and smell, which have been investigated for the canned quail meat depending on the storage conditions and shelf life, have demonstrated characteristics that correspond to the requirements of this product throughout the storage period at different temperatures.

The investigated physical-chemical parameters showed the following results: insignificant variation of the Ph values did not significantly influence the product's quality, this was also confirmed by the stability of the sensory indices, taste and smell throughout the storage period at different temperatures; temperature influences the value of peroxide index and the acidity index when storing canned quail meat, but their low values and insignificant variation during storage did not affect the quality of the product, the smell and taste remained stable and they were not identified as foreign smell and taste; as the temperature increases, the mass fraction of meat decreases, this is explained by the fact that the jelly, sauce and fat in the composition of meat decompose or melt. Thus, the mass fraction of meat in the canned quail influences the quality of product stored at different temperatures; as the storage temperature of the product increases, the content of jelly/sauce in the cans increases, which is explained by its decomposition from the meat. Therefore, the mass fraction of jelly/sauce in the canned quail meat stored at different temperatures influences the product's quality; however, as the temperature of product increases, the mass fraction of protein in the product decreases. The variation of storage temperature of the product has a great influence on the mass fraction of protein and the quality of canned quail meat, as protein is the most important substance in meat.

Microbiological indices and industrial sterility are the aspect that reflects safety and harmlessness of the food produced for the public consumption. According to the results of microbiological examination of the researched cans, no viable aerobic and anaerobic microorganisms have been detected in 1.0 g of the product, which confirms the quality and safety of the investigated product.

The prospects for further research are related to the calculation of nutritional value, determination of stages of

organising the supply, and quality control of the canned food, which includes the selection of control points according to the system of risk analysis, dangerous factors and control of critical points of HACCP, as well as the optimization of production processes and determination of rational equipment parameters for the production of canned meat from land and waterfowl.

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Якість консервів з перепелиного м'яса залежно від умов та часу зберігання

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Анотація. Під впливом внутрішніх і зовнішніх факторів залежно від умов зберігання, в продуктах тваринного походження можуть відбуватися фізико-хімічні та біохімічні зміни. Для продуктів виготовлених з м'ясної сировини різного походження важливо знати оптимальні тривалість і режими зберігання, за яких зберігатимуться оптимальні показники якості та безпечності продукту. Метою дослідження було визначити вплив різних температурних режимів під час зберігання на показники якості і безпечності консервів з м'яса перепелів. Проводили три серії дослідів на консервах з м'яса перепелів. В першому досліді оцінювали якісні показники свіжоприготовленого продукту. В другому і третьому дослідах визначали показники продукту після витримки протягом одного року за температури 2-4 °С і 18-20 °С. В консервах оцінювали органолептичні властивості, фізико-хімічні та мікробіологічні показники. Використовували такі методи досліджень: при органолептичній оцінці враховувалися: зовнішній вигляд, колір, вид на розрізі, запах, смак; вміст жиру – методом Сокслета; водневий показник (pH) – потенціометричним методом; мікробіологічні показники – шляхом горизонтального методу підрахунку колоній мікроорганізмів. Виявлено, що температура зберігання не вплинула на бактеріологічні показники зразків, що свідчить про високу якість стерилізації і виключає біологічний вплив на якість консервів. Було встановлено, що незалежно від температури зберігання органолептичні показники консервів через 12 місяців після виготовлення відповідають державному стандарту та вимогам виробника і суттєво не відрізняються. Через рік зберігання фізико-хімічні показники консервів з м'яса перепелів не залежно від температури витримування знаходяться в межах встановлених стандартом показників. У той же час в консервах було виявлено окремі зміни, які залежали від температурних режимів зберігання. З урахуванням тенденцій у фізико-хімічних показниках, бажана температура зберігання консервів з м'яса перепелів становить 2-4 °С, хоча підвищення температурних режимів до 18-20 °С не призводить до суттєвих змін і є допустимою. Дослідження є науково обґрунтованими щодо встановлення безпечності та якості фаршевих консервів з перепелиного м'яса під час тривалих умов зберігання, з метою створення і виробництва якісних і безпечних продуктів харчування, що в свою чергу дозволяє розширити асортимент консервів із м'ясної сировини

Ключові слова: кістки, колаген, перекисний індекс, стерилізація, бомбаж банок

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 13(4), 50-58
Received: 14.09.2022 Revised: 15.10.2022 Accepted: 31.10.2022

UDC 636.2.034.082.2:575.113
DOI: 10.31548/animal.13(4).2022.50-58

Assessment of the genetic parameters and breeding value of bulls-producers of the Ukrainian black speckled milky breed by the main characteristics

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Abstract. Evaluation and selection of breeding bulls based on economically important characteristics is the main tool in modern systems of genetic improvement of dairy cattle. The purpose of the study was to analyse the influence of environmental and genetic factors on the traits of milk productivity, reproduction, productive longevity, and the difficulty of calving, to assess the genetic parameters and breeding value of breeding bulls based on these traits. The research material was data on the indicators of productivity, reproduction, and productive longevity of cows of the Ukrainian black speckled milky breed of the agricultural cooperative "Vostok" of the Kharkiv region. A multidimensional linear-threshold model was used to calculate genetic parameters (heritability coefficients and genetic correlations) and breeding values of breeding bulls. A probable phenotypic negative association was identified between calving difficulty and milk yield for 305 days (r= -0.2244±0.0266, P>0.999); an increase in calving difficulty by 1 point leads to a decrease in milk yield for 305 days of lactation by 1281.0 kg. Calculated estimates of genetic correlation indicate the presence of a fairly high relationship between milk yield for 305 days of lactation and the service period (over +0.5), a negative relationship between the service period and productive longevity (about -0.37), and a positive relationship between the service period and calving difficulty (about +0.26). This data indicates the presence of genetic antagonism between milk productivity and the level of reproduction of dairy cows, the negative impact of the extended service period on the length of stay of cows in the dairy herd, and the impact of calving difficulty on the deterioration of cow fertility. Estimates of the breeding value of breeding bulls by milk yield, service period, productive longevity, and calving difficulty were calculated. The results obtained indicate the expediency of including traits of reproduction, productive longevity, and difficulty of calving cows in the breeding index, which is used to evaluate and select bulls-producers of the Ukrainian black speckled milky breed

Keywords: dairy cattle, selection, threshold model, heritability, genetic correlation, calving difficulty

Suggested Citation:
Ruban, S., & Danshyn, V. (2022). Assessment of the genetic parameters and breeding value of bulls-producers of the Ukrainian black speckled milky breed by the main characteristics. *Animal Science and Food Technology*, 13(4), 50-58.

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Introduction

Evaluation and selection are powerful driving forces in modern animal husbandry, including dairy cattle breeding. For example, in the United States dairy cattle industry, milk production has increased by 80% since 1944, while the number of dairy cows has decreased by 65% (Mueller & Van Eenennaam, 2022). Selection breeding plays a substantial role in this. Unlike feeding and production technologies, which require constant costs, the genetic improvement achieved in one generation is passed on to subsequent generations of animals, that is, it has a cumulative effect. Therefore, improving methods for the genetic assessment of animals is of great practical importance in terms of accelerating genetic progress.

Modern breeding of dairy cattle is based on the genetic assessment of animals based on economically important traits. The range of breeding traits in dairy cattle breeding is constantly expanding and now includes, in addition to milk productivity, many other traits, such as reproduction rates, longevity, etc. (Cole *et al.*, 2021).

An important breeding feature in dairy cattle breeding is the difficulty of calving (dystocia). The difficulty of calving is due to losses associated with increased mortality of calves and cows, reduced milk yields, poor reproductive capacity, and veterinary care costs. D. Malašauskien *et al.* (2022) identified that heavy calving leads to an increase in the incidence of lameness in dairy cows. A study by Morek-Kopec *et al.* (2021) showed that heavy calving increases the frequency of forced culling, especially in primiparous animals, which negatively affects the productive longevity of animals. Similar results were obtained by Fodor *et al.* (2020). The difficulty of calving is genetically related to another economically important trait – the birth of dead calves, which leads to a decrease in the number of valuable heifers (Sigdel *et al.*, 2022).

Mammi *et al.* (2021) identified that problematic calving is associated with reduced rumination and rest time for cows. The negative impact of heavy calving on the feed dry matter intake was also identified (Reshalaitihan & Hanada, 2019). A study by Antanaitis *et al.* (2021) showed that heavy calving causes a decrease in the concentration of lactose in milk and increases the risk of mastitis.

In the United States, estimates of breeding bulls on the difficulty of calving began to be officially published in 1978 (Miglior *et al.*, 2017). Today, in the United States, the difficulty of calving, along with the birth of dead calves, is included in the "calving ability" composite trait (Van Raden *et al.*, 2021). In the total merit index (TMI), which is used to evaluate and select the Norwegian Red breed, the ease of calving takes 2.9% (About Norwegian Red…, 2022).

Calving difficulty is expressed in points on 3-7-point scales (Tomka, 2018). In general, the difficulty of calving is characterised by low heritability – from 0.04 to 0.20 (Alam *et al.*, 2017; Silvestre *et al.*, 2019; Probo *et al.*, 2022). Genetic assessment of calving difficulty is performed using a linear or threshold model. The threshold model, which was first proposed by S. Wright, is theoretically more reasonable given the nature of this trait since it allows obtaining estimates of breed value in terms of a quantitative trait with a normal distribution (Ruban & Danshin, 2019), although its practical use is associated with certain technical problems, such as time spent on calculations, the convergence of estimates, etc. The optimal solution is to use a mixed linear-threshold multidimensional model to simultaneously estimate quantitative traits (e.g., milk productivity) and calving difficulty, considering genetic and environmental relationships between traits, which improves the accuracy of the estimate (Tomka, 2018).

The purpose of the study was to examine the influence of systematic environmental and genetic factors on the indicators of milk productivity (milk yield for 305 days of lactation), reproduction (service period), productive longevity, and difficulty of calving, an assessment of genetic parameters (heritability and genetic correlation coefficients) and breeding value of breeding bulls for these indicators.

Materials and Methods

The research material was the data of breeding accounting for indicators of productivity, reproduction, and productive longevity of cows of the Ukrainian black speckled milky breed of the agricultural cooperative "Vostok" of the Kharkiv region for the period from 2009 to 2021. The farm keeps 1,500 cows. They are kept in boxes with manure removal by mobile means and milking cows on two Parallel installations, 2×16. Cows are fed according to lactation phases (first phase – 1-120 days, second phase – 121-211 days, third phase – over 212 days) using total mixed ration (TMR). With an average milk yield of 28-34 litres per day, cows are fed 28-35 kg of silage, 12-15 kg of alfalfa haylage, 3-6 kg of hay, and 8-12 kg of grain mixture with minerals. One kilogram of dry matter of the total mixed ration contains 11.8-12.3 MJ of metabolic energy, 12-14% protein at the level of acid-detergent and neutral-detergent fibre of 19-20% and 35-38%, respectively.

All cows with completed lactation for 305 days (371 cows, 1346 lactations) were selected for the study. The assessment of breeding value was conducted for producers, daughters of which gave at least 10 lactations (a total of 30 breeding bulls were evaluated). Milk yield for 305 days of lactation was calculated using the interval method according to ICAR recommendations (Procedure 2 of Section 2…, 2020). The service period was calculated as the interval between the calving date and the date of fertilisation. Productive longevity was calculated as the interval between the date of first calving and the date of exclusion of the cow from the herd. The difficulty of calving was determined on a 3-point scale: 1 point – normal calving, 2 points – calving with minor complications, and 3 points – difficult calving.

Statistical data analysis (descriptive statistics, variance analysis, correlation, and regression analysis) was performed using the R version 3.6.0 environment (the R foundation of statistical computing).

The degree of influence of factors in the analysis of variance was determined by the formula:

$$\eta^2 = \frac{SS_{effect}}{SS_{total}} \quad (1)$$

where, SS_{effect} – sum of squared deviations for the effect under study; SS_{total} – total sum of squared deviations.

Calculations of genetic parameters (heritability coefficients and genetic correlations) and breeding values of breeding bulls were performed using the TM v.1.0 programme (Legarra & Varona, 2011). The following multidimensional linear threshold model was used:

$$y = Xb + Zs + e \quad (2)$$

where y – vector of observations (milk yield for 305 days of lactation, duration of the service period, productive longevity, and difficulty of calving of cows); b – fixed year-calving season effects vector and calving number; s – vector of additive genetic (breeding) values of breeding bulls; X and Z – plan matrices; e – balance vector.

Breed value estimates were calculated using the BLUP "breeder model" method (Schaeffer, 2019).

Reliability of breed value assessments (R) was calculated using the formula:

$$R = 1 - PEV/\sigma_a^2 \quad (3)$$

where PEV – prediction error variance; σ_a^2 – additive genetic trait variance.

These methods allow for assessing the probability and degree of influence of the main environmental and genetic factors on the characteristics under study, obtaining reliable values of genetic parameters, and calculating estimates of the breeding value of breeding bulls.

Results and Discussion

Tables 1 and 2 provide descriptive statistics on milk yield for 305 days of lactation, duration of the service period, productive longevity, and difficulty of calving of cows in SK "Vostok".

Table 1. *Descriptive statistics of productivity, reproduction, and productive longevity in SK "Vostok"*

Feature	n	M±m	σ	Cv, %
1 lactation				
Milk yield for 305 days of lactation, kg	371	7891.0±83.9	1615.5	20.5
Service period, days		133.1±2.2	42.0	31.6
2 lactation				
Milk yield for 305 days of lactation, kg	318	8512.6±104.6	1865.9	21.9
Service period, days		133.5±2.6	46.3	34.7
3 lactation				
Milk yield for 305 days of lactation, kg	274	8591.9±112.7	1866.0	21.7
Service period, days		136.0±2.8	46.1	33.9
4 lactation				
Milk yield for 305 days of lactation, kg	180	7974.3±139.3	1869.1	23.4
Service period, days		139.3±3.6	48.2	34.6
5 lactation				
Milk yield for 305 days of lactation, kg	95	8317.3±183.4	1787.6	21.5
Service period, days		135.2±4.7	45.5	33.7
6 lactation				
Milk yield for 305 days of lactation, kg	48	8283.2±265.4	1839.0	22.2
Service period, days		138.2±6.7	44.8	32.4
7 lactation				
Milk yield for 305 days of lactation, kg	28	6722.2±340.0	1798.9	26.8
Service period, days		139.8±8.8	46.6	33.4
8 lactation				
Milk yield for 305 days of lactation, kg	20	6585.0±370.3	1656.1	25.2
Service period, days		140.7±11.5	51.5	36.6
9 lactation				
Milk yield for 305 days of lactation, kg	7	7320.6±529.0	1399.6	19.1
Service period, days		121.7±11.8	31.3	25.7
10 lactation				
Milk yield for 305 days of lactation, kg	3	6543.7±983.3	1703.1	26.0
Service period, days		131.7±12.7	22.1	16.7

Table 1, Continued

Feature	n	M±m	σ	Cv, %
11 lactation				
Milk yield for 305 days of lactation, kg	2	7588.5±555.5	785.6	10.4
Service period, days		97.0±54.0	76.4	78.7
On average for all lactation periods				
Milk yield for 305 days of lactation, kg	1346	8185.6±49.9	1831.0	22.4
Service period, days	1346	135.1±1.2	45.2	33.5
Productive longevity, months	746	69.0±1.0	27.1	39.3

Notes: n – number of observations, M – arithmetic mean, m – standard error of the arithmetic mean, σ – mean square deviation, Cv, % – the coefficient of variation

Animals of the herd under study are generally characterised by a fairly high milk productivity. Milk yield increases in 305 days from the first to the third lactation, after which it decreases.

The duration of the service period gradually increases from the first to the fourth lactation, then decreases slightly, but then increases again and reaches its maximum value at the eighth lactation. In general, the value of the service period is too high, since it exceeds the duration of the service period in more productive cows of the Holstein breed in the United States, which is 114 days (Norman *et al.*, 2020).

The productive longevity of cows in this herd is about 5.8 years, which is a fairly high indicator for dairy cattle breeding. Thus, according to the data of de Vries & Marcondes (2020), the productive longevity of cows in countries with a high level of dairy productivity is from 3 to 4 years, and in a number of countries, such as France, Italy, Poland, and Canada, there is a downward trend in this indicator (Dallago *et al.*, 2021).

Table 2. *Descriptive statistics on the difficulty of calving in SK "Vostok"*

Calving difficulty, points	Number of calving sessions	Share, %
1	1224	91.3
2	105	7.8
3	11	0.9
Total	1340	100

The majority of calvings (91.3%) of primiparous animals and cows pass without complications, in 7.8% of cases they have minor complications, and only in 0.9% of cases difficult calving is observed (Table 2). For comparison, in Holstein cows in Italy, 79.5% of calvings are without any complications and 20.5% require the intervention of specialists (Probo *et al.*, 2022).

The correlation analysis identified a likely phenotypic negative relationship between calving difficulty and milk yield over 305 days (r = -0.2244±0.0266, P>0.999). Regression analysis showed that an increase in calving difficulty by 1 point leads to a decrease in milk yield for 305 days of lactation by 1281.0 kg. These results correspond well with the data of Atashi *et al.*, (2022), according to which heavy calving not only reduces milk yield in the early stages of lactation but also has a prolonged negative effect on milk productivity in general.

Table 3 shows the results of a variance analysis of the influence of factors "year – calving season", lactation number, father, and ease of calving on milk yield for 305 days of lactation, service period, and productive longevity.

Table 3. *Influence of environmental and genetic factors on milk during 305 days of lactation, service period, and productive longevity*

Factor	Milk yield of 305 days of lactation		Service period		Productive longevity	
	F	η^2	F	η^2	F	η^2
Year – calving season	23.177***	41.2	3.658***	11.8	8.379***	16.4
Lactation number	13.717***	4.7	0.993	-	-	-
Father	3.044***	8.3	0.979	-	24.332***	15.6
Ease of calving	4.208*	0.3	0.371	-	2.322	

Notes: F – Fischer criterion, η^2 – degree of influence, %, * – P>0.95, ** – P>0.99, *** – P>0.999

The "year – calving season" factor was highly likely to affect all the traits under study, while the lactation number had a likely effect only on milk yield for 305 days of lactation. The father of the cow substantially affected milk yield and productive longevity, but not the service period, which can be explained by the low heritability of this trait.

Similar results were obtained in other studies. For example, the likely effect of the season of the year and lactation number on milk productivity was established by Habibi *et al*. (2022). Ukita *et al*. (2022) identified a likely effect of the year and month of calving on the inflammation rate of Holstein cows in Japan. A study by Nejad *et al*. (2021) established the dependence of the risk of culling (and, accordingly, the duration of productive life) of cows on the season of the year.

Table 4 shows estimates of the genetic parameters (heritability coefficient and genetic correlations) of the traits under study.

Table 4. *Estimates of genetic parameters of milk yield for 305 days of lactation, service period, productive longevity, and difficulty of calving*

Feature	Heritability rate	Genetic correlation		
		service period	productive longevity	calving difficulty
Milk yield of 305 days	0.3341	+0.5229	+0.0943	+0.085
Service period	0.0830	-	-0.3688	+0.2571
Productive longevity	0.2045		-	
Calving difficulty	0.1787		+0.0410	-

The milk yield for 305 days of lactation had the highest value of the heritability coefficient. Productive longevity and calving difficulty were characterised by low heritability, while service period heritability was the lowest. The resulting assessment of the heritability of calving difficulty substantially exceeds the estimates obtained in the study by Sigdel *et al*. (2022), which were 0.06-0.07.

Regarding the genetic correlation coefficients, a fairly high relationship between milk yield for 305 days of lactation and the service period (over +0.5), a negative relationship between the service period and productive longevity (about -0.37), and a positive relationship between the service period and calving difficulty (about +0.26) are notable. These results indicate a known genetic antagonism between milk production and the reproduction rate of dairy cows (Canaza-Cayo *et al*., 2018; Yamazaki *et al*., 2020; Martinez-Castillero *et al*., 2020), the negative impact of the extended service period on the length of stay of cows in the dairy herd and the impact of the difficulty of calving on the deterioration of cow fertility (Probo *et al*., 2022).

Based on the use of a mixed multidimensional linear-threshold model, estimates of the breeding value of breeding bulls by milk yield, service period, productive longevity, and calving difficulty were calculated (Table 5). Breeding values of bulls by calving difficulty are expressed in terms of a quantitative trait with a normal distribution (average value is 0.00569, variance – 0.18166).

Table 5. *Estimates of the breeding value of breeding bulls by milk yield for 305 days of lactation, service period, productive longevity and difficulty of calving*

Breeding bull	Number of lactations	Milk yield for 305 days of lactation, kg		Service period, days		Productive longevity, months		Calving difficulty	
		BV	R	BV	R	BV	R	BV	R
FR3535222528	16	+722	0.62	+6.70	0.40	-1.40	0.09	-0.26	0.30
NL736463357	26	-290	0.71	-2.80	0.51	-0.90	0.48	-0.02	0.54
NL761829452	17	+385	0.62	+1.10	0.37	+0.30	0.13	-0.31	0.33
UA1800619813	14	+459	0.60	+2.30	0.42	-0.60	0.71	-0.24	0.30
UA3200801725	114	+4	0.88	+2.10	0.75	+0.10	0.93	+0.67	0.84
UA3200822444	50	-279	0.80	-0.10	0.66	+0.20	0.93	+0.28	0.77
UA3200822461	16	-130	0.64	-2.90	0.51	+2.80	0.88	+0.05	0.58
UA5600607819	13	-103	0.60	+0.60	0.44	-3.80	0.80	-0.37	0.14
UA5600607838	101	-104	0.85	-2.90	0.72	-3.20	0.87	-0.02	0.77
UA5900260662	124	-521	0.88	+0.90	0.75	-0.40	0.94	+0.27	0.83
UA6300108374	18	-364	0.62	-7.70	0.53	+4.70	0.87	-0.18	0.66
UA6300109103	19	-78	0.58	-1.40	0.51	+0.40	0.83	-0.12	0.61
UA6300109105	13	+135	0.53	+0.60	0.49	+6.20	0.81	+0.66	0.58
UA6300109106	37	-36	0.72	-1.50	0.61	+2.40	0.88	+0.37	0.80
UA6300109107	12	-92	0.55	-1.30	0.46	+0.40	0.83	+0.11	0.60

Table 5, Continued

Breeding bull	Number of lactations	Milk yield for 305 days of lactation, kg		Service period, days		Productive longevity, months		Calving difficulty	
		BV	R	BV	R	BV	R	BV	R
UA6300109108	11	+70	0.53	-5.60	0.46	+11.10	0.83	+0.58	0.64
UA6300309551	10	-93	0.48	-1.20	0.46	+1.60	0.81	+0.01	0.60
UA6300309555	32	+246	0.71	+0.50	0.57	+1.90	0.88	+0.32	0.77
UA6300376295	21	-158	0.69	-3.40	0.55	+2.10	0.88	-0.02	0.61
UA6300376594	40	-12	0.79	-1.20	0.65	+1.60	0.91	-0.08	0.72
UA6300446717	153	-1008	0.90	-6.40	0.76	-2.40	0.94	-0.19	0.77
UA6300447275	104	-146	0.88	+1.90	0.73	-2.00	0.92	+0.04	0.76
UA6300661596	21	-42	0.69	+1.20	0.53	+0.60	0.60	+1.03	0.66
UA6300755786	110	-240	0.87	-2.60	0.70	-4.00	0.90	-0.93	0.35
UA6300765661	32	+595	0.75	+3.70	0.53	-2.80	0.87	-0.64	0.26
UA6300777972	41	+280	0.79	+2.00	0.61	-2.30	0.75	-0.65	0.21
UA8000358181	12	+583	0.57	+4.40	0.30	+0.40	0.06	-0.09	0.14
UA8010591081	13	-183	0.60	+0.30	0.30	-1.80	0.02	-0.20	0.14
UA8010785745	22	-239	0.69	+0.20	0.44	-3.00	0.48	-0.37	0.30
UA8011167205	20	+493	0.69	+9.20	0.51	-2.20	0.69	+0.47	0.56

Notes: BV – assessment of breed value, R – reliability of assessment of breed value

The highest assessment of breeding value by milk yield for 305 days of lactation was given to FR3535222528 (+722 kg), UA6300765661 (+595 kg), and UA8000358181 (+583 kg) breeding bulls. According to the estimates of breeding value, the duration of the service period in bulls UA6300108374 (-7.7 days), UA6300446717 (-6.4 days), and UA6300109108 (-5.6 days) are notable. In terms of productive longevity, the best estimates of breeding value were given to bulls UA6300109108 (+11.1 months), UA6300109105 (+6.2 months), and UA6300108374 (+4.7 months). Regarding the difficulty of calving, bulls UA6300755786 (-0.93), UA6300777972 (-0.65), and UA6300765661 (-6.4) can be highlighted.

The obtained estimates of heritability coefficients are close to those obtained by other researchers, in particular, Stefani *et al*. (2021), although the heritability of milk yield at 305 days of lactation in this controlled study was higher (0.33 vs. 0.15). The use of a mixed linear threshold model allowed for a higher heritability coefficient for calving difficulty (0.1787) than when using linear models (Tomka, 2018; Silvestre et al., 2019), which allowed increasing the reliability of estimates of the breeding value of breeding bulls based on this feature. The decrease in milk yield for 305 days of lactation due to the difficult calving of cows is consistent with the data of other authors (Stefani *et al*., 2021).

The calculated values of genetic correlations confirm the well-known genetic antagonism between milk production and reproduction of dairy cows, which has recently been confirmed by genomic studies, the results of which showed that over 40 years (from 1964 to 2004), intensive breeding for milk productivity in the Holstein breed led to a deterioration in the reproduction rate of cows (Ma *et al*., 2019). 234 regions of the genome with selection signatures were identified, of which 125 regions either contained or were located near 198 genes affecting reproductive function.

Notably, the low level of heritability of reproduction indicators, including the service period, and the presence of an unfavourable genetic relationship between them and the milk productivity of cows does not mean that their genetic improvement by breeding methods is impossible. Weller *et al*. (2022) analysed phenotypic and genetic changes in milk productivity (milk yield, fat, and protein) and reproduction (fertilisation status, which is the inverse of the number of inseminations per fertilisation, expressed as a percentage) in the Holstein livestock population of Israel for the period from 1980 to 2018. The authors identified that in parallel with the increase in milk productivity, there was a decrease in the status of fertilisation from 55.6% in 1983 to 46.5% in 2018, but the average assessment of the breeding value of animals by fertilisation status increased from -1.9 to -0.1. These data indicate that the deterioration in reproduction rates was caused not by genetic, but by environmental factors. This is supported by the results of the study by Morton *et al*. (2018), according to which the proportion of non-genetic component covariance between reproduction traits and milk productivity is up to 67%.

According to Lucy (2019), breeding is a tool that allows for quickly improving the level of reproduction of dairy cows. In the Nordic countries (Denmark, Sweden, and Finland), reproduction rates began to be included in the breeding programmes of dairy breeds as early as the 1960s, which allowed for avoiding their deterioration with increased milk productivity (Muuttoranta *et al*., 2019). In the Holstein population of the United States, between 1960 and 2000, there was a steady decline in both the breeding value and the factual (phenotypic) values of the cow pregnancy level, then from 2000 there was a gradual increase in the factual values of the cow pregnancy level, and since 2010 (after the introduction of genomic assessment

of animals), there has been some genetic progress (Berry, 2018). Currently, two-thirds of Interbull member countries conduct a genetic assessment of dairy cattle based on reproductive traits (Interbull, 2022).

Thus, as a result of research, a substantial influence of environmental factors (especially the "year – calving season" factor) on the milk yield, service period, and productive longevity of cows of the herd under study was established. The presence of genetic antagonism between milk productivity and the level of reproduction of cows of the Ukrainian black speckled milky breed and the negative impact of heavy calving on the duration of the service period were confirmed.

Conclusions

The conducted studies indicate a substantial influence of the "year – calving season" factor on the indicators of milk productivity, reproduction, and productive longevity of cows, which makes it necessary to include this factor in the model for assessing the genetic parameters and breeding value of breeding bulls and cows based on the examined traits.

Heritability estimates (from 0.0830 to 0.3341) indicate the possibility of genetic improvement of all these traits through targeted breeding work.

The calculated values of genetic correlations confirm the existence of an unfavourable relationship between milk yield for 305 days of lactation and the service period (+0.5229), a negative relationship between the service period and productive longevity (-0.3688), and a positive relationship between the service period and calving difficulty (+0.2571). Such results indicate the negative consequences of breeding only for milk productivity for the level of reproduction of dairy cows of the Ukrainian black speckled milky breed and indicate the need to include traits of reproduction, productive longevity, and difficulty of calving of cows in the economic breeding index, based on which the assessment, selection, and breeding of animals will be conducted.

The use of a mixed multidimensional linear threshold model allows simultaneously assessing the breeding value of breeding bulls based on quantitative characteristics and those expressed in qualitative categories (calving difficulty). Using such a mixed multidimensional linear-threshold model, estimates of the breeding value of bulls-producers of the Ukrainian black speckled milky breed by milk yield, service period, productive longevity, and calving difficulty were calculated. The obtained estimates should be used in practice for the genetic improvement of this breed.

The obtained estimates of genetic parameters can be used in further studies when modelling possible genetic changes in the breeding traits under study (direct and correlated response to selection) under different selection scenarios and variants of breeding programmes in the Ukrainian black speckled milky breed.

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Оцінка генетичних параметрів і племінної цінності бугаїв-плідників української чорно-рябої молочної породи за основними ознаками

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Анотація. Оцінка та відбір бугаїв-плідників за економічно важливими ознаками є головним інструментом в сучасних системах генетичного покращення молочної худоби. Метою досліджень було проведення аналізу впливу середовищних і генетичних факторів на ознаки молочної продуктивності, відтворення, продуктивного довголіття і важкості отелень та здійснити оцінку генетичних параметрів і племінної цінності бугаїв-плідників за цими ознаками. Матеріалом досліджень були дані по показниках продуктивності, відтворення і продуктивного довголіття корів української чорно-рябої молочної породи сільськогосподарського кооперативу «Восток» Харківської області. Для розрахунку генетичних параметрів (коефіцієнти успадковуваності і генетичні кореляції) і племінних цінностей бугаїв-плідників використовувалась багатомірна лінійно-порогова модель. Виявлено вірогідний фенотипічний негативний зв'язок між важкістю отелення і надоєм за 305 днів (r= -0,2244±0,0266, P>0,999); збільшення важкості отелення на 1 бал призводить до зниження надою за 305 днів лактації на 1281,0 кг. Розраховані оцінки генетичної кореляції свідчать про наявність досить високого зв›язку між надоєм за 305 днів лактації і сервіс-періодом (більше +0,5), а також негативного зв'язку між сервіс-періодом і продуктивним довголіттям (біля -0,37) та позитивного зв'язку між сервіс-періодом і важкістю отелення (біля +0,26). Ці дані свідчать про наявність генетичного антагонізму між молочною продуктивністю і рівнем відтворення молочних корів, негативний вплив подовженого сервіс-періоду на термін перебування корів у молочному стаді і вплив важкості отелення на погіршення плодючості корів. Були розраховані оцінки племінної цінності бугаїв-плідників за надоєм, сервіс-періодом, продуктивним довголіттям і важкістю отелення. Отримані результати свідчать про доцільність включення ознак відтворення, продуктивного довголіття і важкості отелення корів в селекційний індекс, за яким здійснюється оцінка та відбір бугаїв-плідників української чорно-рябої молочної породи України

Ключові слова: молочна худоба, селекція, порогова модель, успадковуваність, генетична кореляція, важкість отелення

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 13(4), 59-68
Received: 07.09.2022 Revised: 10.10.2022 Accepted: 29.10.2022

UDC 636.09:615.356:598.278:591.366
DOI: 10.31548/animal.13(4).2022.59-68

Preclinical study of the effects of vitamin-mineral complex use during normal pregnancy in white rats

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Abstract. Vitamin-mineral deficiency is quite common in the world, which negatively affects the growth and development of children, provokes the development of chronic pathologies, allergic conditions. Children and pregnant women are particularly sensitive to this condition. Considering the vitamin and elemental composition of each of the drugs, it becomes necessary to examine the effect of various complexes on the body. The purpose of the study is to examine the morphological state of organs from the use of a vitamin-mineral complex during normal pregnancy in white rats and their offspring and examine the elemental analysis of target organs and whole blood. The experiment was conducted on pregnant female white laboratory rats and their offspring to achieve this goal. Histological, ultramicroscopic,

Suggested Citation:
Tkach, G., Shvets, O., Bal-Prylypko, L., Slobodyanyuk, N., & German, S. (2022). Preclinical study of the effects of vitamin-mineral complex use during normal pregnancy in white rats. *Animal Science and Food Technology*, 13(4), 59-68.

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morphometric, atomic-absorptic, functional methods, and statistical analysis are used for the study. A positive effect of the vitamin-mineral complex of the selected composition on both pre- and postnatal development of offspring is identified. The average offspring rate increased by 9.79% (p=0.0443), while the stillbirth rate decreased by 81.82% (p=0.0324). The absence of negative side effects of the complex on the development of basic reflex and behavioural reactions, motor activity, and cognitive activity of offspring is proved. A positive effect on the morphological characteristics of the liver, brain, heart, and kidneys is evident: the number of full hepatocytes in mature animals increased by 8.17% (p=0.0482), and in young animals – by 5.03% (p=0.0137), in the brain tissue of 7-day-old animals, the number of neurons increased by 7.70% (p=0.0438), and the number of functionally defective neurons decreased. Microelement analysis of organs identified a tendency to increase the amount of a number of trace elements, which was reflected in the improvement of the antioxidant systems pf the body. A positive effect of the complex on the processes of hematopoiesis is noted. The results obtained can become morphological evidence for the choice of correction of macro-, microelement, and vitamin balance, prevention of its deficiency in pregnant women, and prevention of the development of deficient conditions in infants

Keywords: animals, offspring, vitamins, morphology, macro- and microelements

Introduction

Vitamin-mineral complexes are widely used all over the world, in particular, in well-developed countries, which is associated with the desire of the population to prevent the development of chronic diseases, hypervitaminosis and avitaminosis, improve health, level the absence of certain nutritional components in the diet, and the impact of adverse environmental factors (Vdovichenko & Ostroglyad, 2017). Vitamin-mineral deficiency is quite common in the world and dangerous for public health, especially for children and pregnant women (Horobets, 2019).

Deviations in macro-, microelement, and vitamin supply lead to changes in metabolism, reduced performance, contribute to the deterioration of physical and mental health, especially in children, and lead to the development of chronic pathology, allergies, and a decrease in reproductive potential (Babaei *et al.*, 2012, Trachtenberg *et al.*, 2013, Sheremeta *et al.*, 2015). The ability of the immune system to counteract pathogenic factors and adverse environmental influences decreases (Horodetska & Blavatska, 2019).

The main causes of vitamin deficiency are alimentary insufficiency, changes in the normal intestine microbiota, impaired assimilation and metabolism, and an increased need for vitamins (Nyssen *et al.*, 2022). The rapid growth of the population leads to a shortage of food, and simultaneously, to a decrease in the supply of elements and vitamins of the body (Nikolaenko & Bal-Prylypko, 2020). The addition of macro-, microelements, and vitamins to the diet has a positive effect on well-being, especially for pregnant women, and as a result, provides high birth rates for healthy offspring (Kotsyubenko, 2012). During pregnancy, there is an additional need for macro-, microelements, and vitamins, which is associated with increased consumption of the latter, increased metabolism, and fetal development (Cetin, *et al.*, 2009).

Researchers around the world now recommend the introduction of dietary supplements. Today, manufacturers offer a huge selection of vitamin-mineral complexes. Having knowledge about the variety and effects of supplements, the doctor can correctly introduce them to the diet to improve metabolic processes and body functions, considering their individual characteristics (Horobets, 2019, WHO technical report..., 2007).

Numerous foreign studies showed that taking vitamin-mineral complexes reduces the risk of cataracts, heart diseases, diabetes, and oncological diseases such as breast, lung, colon, and prostate cancer (Gaziano *et al.*, 2012, Fortmann *et al.*, 2013, Park *et al.*, 2010). In addition, nutraceuticals have a positive effect on the cognitive system, reduce the risk of infectious processes in the elderly (Stephen & Avenell, 2006).

Thus, considering the vitamin, macro-, and microelement composition of each of the additives, and the variety of effects of each component, it becomes necessary to examine the effect of various complexes on the body. The experiment was performed on pregnant rats to ensure the safety of the drug and the absence of side effects, which also identified the effect of taking supplements on the offspring. In addition to the morphofunctional state of organs, it is important to identify the micro- and macronutrient composition, hidden deficiencies or elemental imbalances since the lack, excess, or incorrect ratio of macro- and microelements substantially affects the state of the antioxidant system of the body.

The purpose of the study is to examine the effects of using the vitamin-mineral complex during normal pregnancy in white rats and their offspring, assess the morphofunctional state of organs after a course of taking the vitamin-mineral complex.

The main objectives of the study were:

1. Simulate and perform an experiment during normal pregnancy in white rats.

2. Monitor the overall physical development of the offspring, in particular, examine the rate of occurrence of basic sensory-motor reflexes during feeding, emotional-motor behaviour, the ability to learn, and the memory of animals.

3. Assess the morphofunctional state of target organs (brain, liver, kidneys, heart) using morphometry and light, raster, and transmission electron microscopy.

4. Examine the morphofunctional state of the organs of laboratory animals (brain, heart, kidneys, liver), provided that a vitamin-mineral complex is used as a complementary food.

5. Perform macro- and microelement analysis of target organs and whole blood to assess the functioning of the antioxidant system, and evaluate the main indicators of the general blood test.

Materials and Methods

The experiment was conducted on 10 pregnant female white laboratory rats aged six to twelve months and the offspring obtained from them (20 rats).

Experimental animals were cared for in a vivarium in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), "General Ethical Principles of Animal Experiments", adopted by the First National Congress on Bioethics (Kyiv, 2001), and the Law of Ukraine "On the Protection of Animals from Cruelty" (dated 21.02.2006) (European Convention for..., 1986, Reznikov, 2003).

The animals were divided into the following groups:

- Animals in the control group received dry granulated feed and drinking water as food.
- Animals of the experimental group received dry granular feed and a vitamin-mineral preparation at the appropriate dose, dissolved in drinking water, the composition of which is presented in Table 1.

Calculation of the dose of the additive for animals: the weight of one tablet of the vitamin-mineral complex is 756 mg. According to the instructions, the drug is prescribed 4 tablets a day (2 tablets in the morning and 2 tablets in the evening) for adults, which is 3024 mg. The daily dose per kilogram of adult body weight is 43.2 mg/kg. Formula (1) was used to calculate the amount of vitamin supplement for pregnant rats:

$$\text{Rat dose} = (r \times \text{human dose}) / R \quad (1)$$

where r – the coefficient of species endurance for a rat=3.62; R – the coefficient of species endurance for humans=0.57.

According to the formula, the daily dose for a pregnant animal was 274.4 mg/kg. Considering the average weight of a pregnant rat (200 g), the daily dose is 55 mg. The experimental group consisted of 10 pregnant animals who received 55 mg of vitamin supplements daily for 24 days. The total amount of the supplement per experiment is 1320 mg, which corresponds to 17 tablets of the drug. The content of trace elements and vitamins in the daily dose for the animal is shown in Table 1.

Table 1. *Content of trace elements and vitamins of the complex in the daily dose for the animal*

Trace elements and vitamins	Content in the daily dose (55 mg) for the animal
Zinc	0.08 mg
Iron	0.11 mg
Manganese	0.02 mg
Copper	6.2 μg
Chrome	0.76 μg
Magnesium	1.64 mg
Beta-carotene	0.055 mg
Vitamin B1	0.018 mg
Vitamin B2	0.022 mg
Vitamin B6	0.022 mg
Vitamin B9 (folic acid)	3.64 μg
Vitamin B12	0.018 μg
Vitamin C	1.1 mg
Vitamin D3	0.055 μg
Vitamin E	0.12 mg
Vitamin B3 (PP, nicotinamide)	0.22 mg
Flavanoids	1.46 mg

The weight of pregnant and newborn rats was determined using ACOM JW electronic scales with an accuracy of 0.01 g.

The absolute and relative mass of the liver, heart, brain, and kidneys was determined.

The following organometric parameters were also determined:

- When examining the liver – the greatest length, width, and thickness;
- During the examination of the heart – the total height, width, thickness, and volume of the heart muscle were calculated;
- During the examination of the kidneys – the length, width, thickness, and volume of each of the kidneys;
- During the examination of the brain – width and length.

The above-mentioned linear dimensions were examined using a calliper with an accuracy of 0.1 mm.

For the histological preparations, pieces of the liver, brain, heart, and kidneys were put in a 10% solution of neutral formalin. The samples were then prepared and processed as described earlier in (Rtail *& et al.*, 2020, Dudchenko *& et al.*, 2020). When examining liver preparations, attention was paid to the structure of classic

lobules, perilobular zones, perisinusoid spaces, sinusoids; the number of normal hepatocytes in the visual field was determined; the relative areas occupied by liver beams in the organ of adult and newborn animals were calculated.

During the examination of the brain, the structure of all layers of the organ was visually evaluated, the width of the inner pyramidal layer was determined, its cellular composition was established; the number of normal neurons in the visual field, the glial-neuronal index, and the average diameter of the capillaries of the organ were identified. During the examination of heart preparations, the morphological organisation of the heart muscle at the light-optical level was visually evaluated; attention was paid to the structure of cardiomyocytes.

During the examination of histological preparations of the kidney, its histoarchitectonics were visually examined; morphometrically, the area of the renal body, the diameter of the proximal and distal renal tubules, and the diameter of glomerular capillaries were determined, as the most substantial indicators of the functionality of the organ. Material sampling for electronomicroscopic examination of the structural components of the tissues of the organs under study was performed as described earlier in (Rtail *et al.*, 2020, Dudchenko *et al.*, 2020).

According to the obtained data from transmission electron microscopy of the liver, the morphology of hepatocytes, their main organelles, sinusoids, and bile capillaries was investigated. The diameter of hepatocytes and the hepatocyte nuclei were determined, and the nuclear-cytoplasmic ratio was calculated. During the examination of the brain, the structural organisation of neurons, the brain glia, was investigated using transmission electron microscopy. The area and diameter of neurons in the inner pyramidal layer were determined morphometrically. The nuclear-cytoplasmic ratio was calculated.

During the ultramicroscopy of cardiac preparations, attention was paid to the morphology of cardiomyocytes. The diameter of cardiomyocytes of the left and right ventricles and their relative volume were determined. The three-dimensional organisation of the ventricular myocardium was investigated using raster electric microscopy. During the examination of kidney preparations, the ultamicroscopic organisation of the renal body and its main components were investigated.

For a general blood test, haematological parameters were determined in whole blood: haemoglobin content – by the acetoncyanhydrin method (g/l); haematocrit (%), the number of red blood cells (10^{12}/l), white blood cells (10^{9}/l), and platelets (10^{9}/l), content (%) of lymphocytes, MID (eosinophils, monocytes), reticulocytes, granulocytes. Analysis of elements in blood and organ samples was performed by atomic absorption spectrometry with electrothermal and flame atomisation, as described earlier (Rtail & Tkach, 2020). The content of K, Na, and Ca was determined on an S-115-M1 AT "Selmi" spectrophotometer with flame atomisation in the emission mode (Table 2). The Mg, Fe, Mn, Zn, and Cu content was analysed on a CAS-120.1 atomic absorption machine with an A-5 electrothermal atomiser and a Carl Zeiss Jena graphite furnace in the adsorption mode (Rtail & Tkach, 2020), the characteristics of which are given in Table 3.

Table 2. *Spectral measurement and atomisation conditions for K, Na, Ca*

Element	Wavelength, nm	Spectral gap, nm	Combustible gas	Oxidiser	Temperature flame	Flame type
K	769.9	0.4	C_2H_2	air	2300	oxidising
Na	589.0	0.4	C_2H_2	air	2300	oxidising
Ca	422.7	0.4	C_2H_2	N_2O	2950	stoichiometric

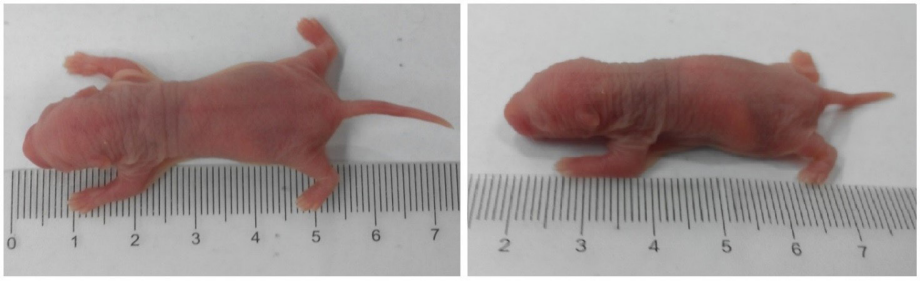
Table 3. *Spectral and temperature-time measurement modes*

Element	Wavelength, nm	Gap width, Nm	Pyrolysis		Atomisation	
			T, ˚C	τ, s	T, ˚C	τ, s
Mg	202.6	0.4	1000	10	2200	5
Fe	372.0	0.4	1000	10	2500	5
Mn	279.5	0.4	1000	10	2500	4
Zn	213.9; 307.4	1.0	600	10	2100	5
Cu	324.7	0.4	1000	10	2500	5

Statistical data processing was performed using a package of applied statistical computer programmes – MS Excel 2016 and SPSS-17. The validity of differences between the two samples was determined using the Student's parametric t-criterion. The difference was considered reliable if the probability of a random difference did not exceed 0.05 ($p \leqslant 0.05$).

Results and Discussion

When using the vitamin-mineral complex as a complementary food for pregnant rats, an increase in the average size of the offspring was identified by 9.79% (p=0.0443), while the stillbirth rate of animals decreased by 81.82% (p=0.0324) relative to the control group of animals. No developmental abnormalities were detected in the comparison groups (Fig. 1 (a, b)).



a) b)

Figure 1 – three-day-old animals whose mothers consumed dry granulated feed as food during pregnancy (a) and dry granulated feed with a vitamin-mineral complex (b)

The weight of newborn animals in the control group increased by 21.11% (p=0.0396) on day 7 of postnatal development, and by 15.69% (p=0.0311) and 5.88% (p=0.0438) on days 14 and 21, respectively. The weight of newborn rats, whose mothers received the vitamin-mineral complex, tended to increase by 28.48% (p=0.0324), 16.56% (p=0.0171), and 5.52% (p=0.0580), respectively, on days 7, 14, and 21 of postnatal development. This allows asserting the positive effect of the complex on the course of pregnancy and postnatal development of the offspring.

The main indicators of the overall development of the offspring in the postnatal period (detachment of the auricle, appearance of primary hair, eruption of incisors, opening of the eyes, omission of testes, and opening of the vagina) were within the physiological norm in both groups. During the examination of the main sensory-motor reflexes of offspring whose mothers consumed a vitamin-mineral complex during pregnancy, it was identified that the timing of their appearance corresponds to the norm and is fully fulfilled. In particular, it was noted that the main reflexes (turning over on a flat surface, avoiding a potential fall, pendulum reflexes) tended to appear earlier, compared to the control group of animals.

When investigating emotional and motor behaviour and fine coordination of movements of newborn rats of the experimental group, no pathological disorders were identified, the animals showed good abilities, not inferior to the animals from the control group. The ability of the offspring of the experimental group to learn was at a high level, which was manifested in such experiments as passive and active avoidance with negative (pain) reinforcement and learning in a maze with positive (food) reinforcement.

Macroscopic examination of internal organs (liver, brain, kidneys, heart) did not identify any congenital developmental abnormalities in 7-day-old animals and pathologies in mature females. Microscopic examination of organ tissues identified a number of positive effects from the use of the vitamin-mineral complex in animals of both age categories. Thus, morphological examination of the liver visually observed a decrease in apoptosis-altered and necrotic cells in mature animals. Therewith, there were almost no functionally defective hepatocytes in 7-day-old animals (Fig. 2). It was identified that when using the drug, the number of full hepatocytes in mature animals increases by 8.17% (p=0.0482), respectively, and in young animals – by 5.03% (p=0.0137), in particular, in the latter cells had signs of high functional activity. Ultramicroscopic examination confirmed the normal architectonics of animal liver tissue (Fig. 3).

Microscopic examination of the liver of rats in the control group of both age categories showed that the classic liver lobules had a polygonal shape. In the corners between the classic hepatic lobules were layers of loose connective tissue that contain interparticle of blood and lymphatic vessels and bile ducts. In 7-day-old animals, interparticle connective tissue septa were not pronounced. Central veins are located in the centre of the classic hepatic lobules. The cytoplasm of most hepatocytes is evenly colored, light. The nuclei are normochromic, located in the centre of the cells. Apoptotically altered or necrotic cells and dilated sinusoid capillaries were detected in the centrilobular zone of classic hepatic lobules in animals of both age categories of the control groups (Figures 4 and 5).

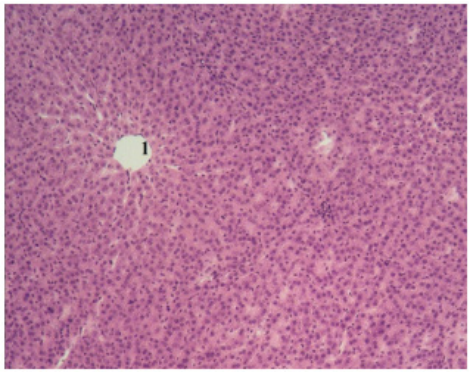


Figure 2 – liver of 7-day-old animals whose mothers received a vitamin-mineral complex during pregnancy: 1 – lumen of the central vein. Magnification x200

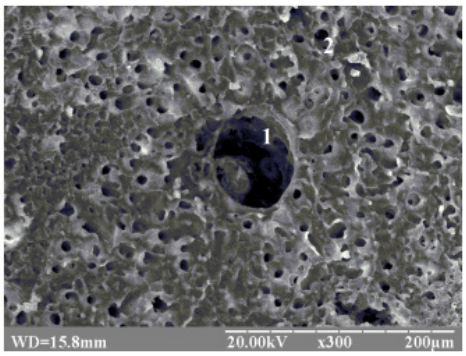


Figure 3 – ultrastructure of the liver of 7-day-old animals whose mothers consumed a vitamin-mineral complex during pregnancy: 1 – lumen of the central vein; 2 – sinusoid capillaries

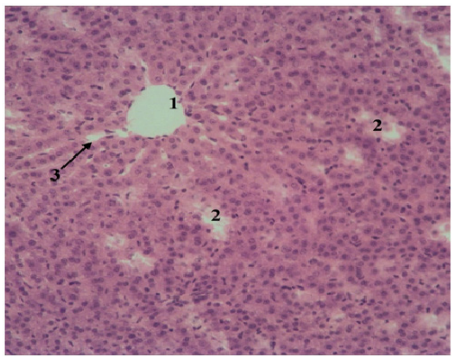


Figure 4 – liver of 7-day-old animals of the control group. Hematoxylin-eosin staining: 1 – central vein lumen; 2 – destroyed hepatocytes; 3 – dilated sinusoid capillaries. Magnification x400

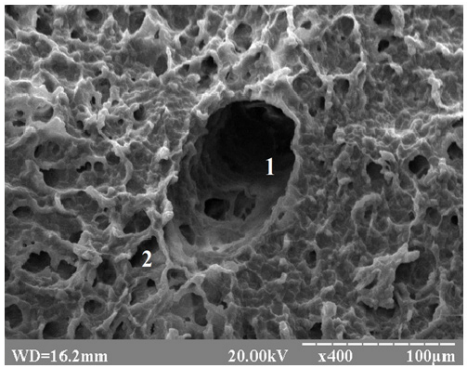


Figure 5 – ultrastructure of the liver of 7-day-old animals of the control group: 1 – lumen of the central vein; 2 – sinusoid capillaries.

In the brain tissue of 7-day-old animals, an increase in the number of neurons was identified by 7.70% (p=0.0438) compared to the control group, and a decrease in the number of functionally defective neurons in the visual field, which was presumably reflected in high learning and memory indicators (Fig. 6). Morphological examination of the heart did not identify any deviations from the norm in both young and mature animals, which indicates the benefit of the vitamin-mineral complex. Microscopic examination of the kidneys identified a decrease in the number of defective glomeruli, tubules, and there was no pathological dilation of capillaries, which was quite often observed in young animals of the control group (Fig. 7).

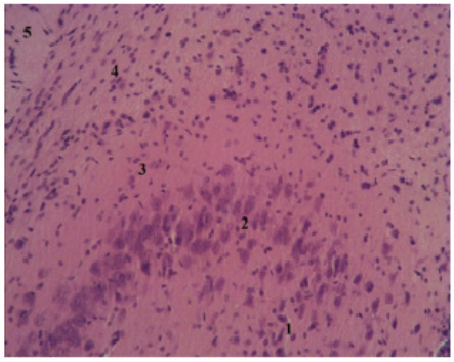


Figure 6 – the brain of 7-day-old rats whose mothers consumed a vitamin-mineral complex during pregnancy. Hematoxylin-eosin staining: 1 – molecular layer; 2 – outer granular layer; 3 – outer pyramidal layer; 4 – inner granular layer; 5 – inner pyramidal layer. Magnification x400

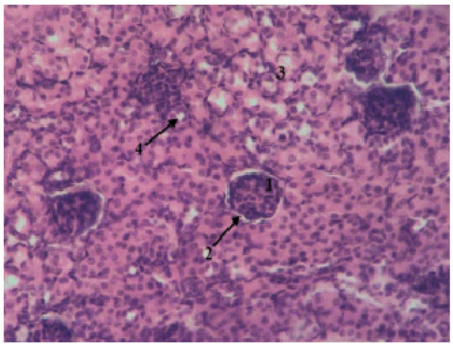


Figure 7 – the cortical substance of the kidneys of 7-day-old animals whose mothers consumed a vitamin-mineral complex during pregnancy. Hematoxylin-eosin staining: 1 – renal glomerulus; 2 – glomerular capsule lumen; 3 – proximal convoluted tubules; 4 – distal convoluted tubules. Magnification x400

In the molecular layer and outer pyramidal layer of the cerebral cortex of 7-day-old rats, a lower density of neurons and microglial cells was observed during the microscopic examination, compared with a group of 7-day-old rats whose mothers consumed a vitamin-mineral complex during pregnancy (Fig. 8). Microscopic examination of the heart of 7-day-old rats in the control group identified a lower expression of connective tissue compared to their mothers. No altered cardiomyocytes in animals of both age categories were observed. During the histological examination of kidney preparations in adult animals of the control group, the capsule, cortical, and medulla substances were clearly detected. In the cortical substance of the kidneys of 7-day-old animals in the control group, morphofunctional glomeruli were identified, the lumen of the capsule of which was not always visualised. Some proximal and distal convoluted tubules tended to narrow. Sometimes dilated blood vessels were observed (Fig. 9).

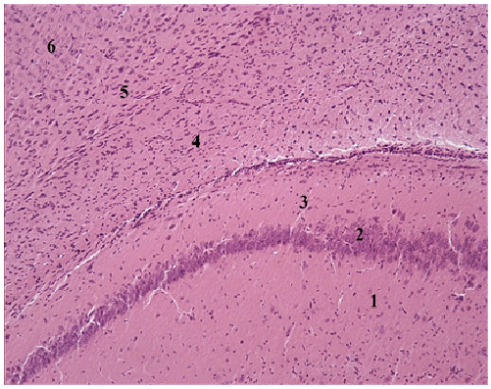


Figure 8 – brain of 7-day-old rats of the control group. Hematoxylin-eosin staining: 1 – molecular layer; 2 – outer granular layer; 3 – outer pyramidal layer; 4 – inner granular layer; 5 – inner pyramidal layer; 6 – polymorphic cell layer. Magnification x200

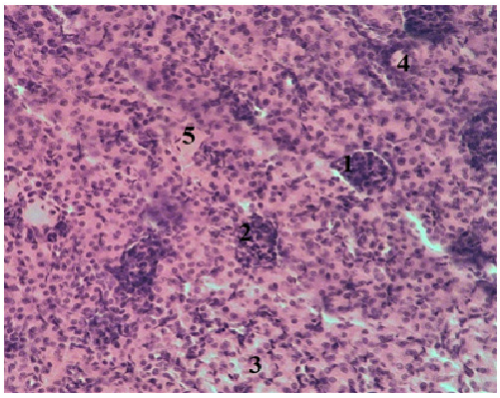


Figure 9 – the cortical substance of the kidney of 7-day-old rats of the control group. Hematoxylin-eosin staining: 1 – renal glomerulus; 2 – morphofunctional glomerulus; 3 – narrowed proximal tubules; 4 – distal renal tubule; 5 – dilated capillary. Magnification x200

Using functional research methods, a tendency to increase the content of zinc, iron, cobalt, magnesium, and copper was identified, to a greater extent in the liver and kidneys. This is presumably reflected in the improvement of the antioxidant systems of the body and the reduction of morphofunctional necrotic cells. Microelement analysis of whole blood showed an increase in iron concentration.

Among the main indicators of the general blood test, the level of haemoglobin and the number of red blood cells experienced the greatest changes. Thus, in animals that consumed a vitamin-mineral complex during pregnancy, the haemoglobin level increased by 3.45% (p=0.0238), and the number of red blood cells – by 11.6% (p=0.0292), respectively, in the control group. In their offspring, the level of haemoglobin increased by 19.05% (p=0.0483), and red blood cells – by 41.75% (p=0.0311) compared to newborn rats whose mothers did not consume the vitamin-mineral complex. Therewith, there was an increase in the color index. In animals that consumed the vitamin-mineral complex during pregnancy, it increased by 14.29% (p=0.0152), and in their offspring – by 11.63% (p=0.0382) according to the control indicators, which allows asserting the positive effect of the complex on the processes of hematopoiesis.

Thus, it was identified that when using a vitamin-mineral complex as a complementary food for pregnant rats, the average size of the offspring increases, while the stillbirth rate of animals decreases. Therewith, no abnormalities in the development of offspring were detected. Bryan *et al.* (2004) also observed an increase in pregnancy cases in cows due to the use of chromium-methionine as a supplement to the main feed. In the studies of Shtapenko (2019), it was shown that organic complexes of manganese, zinc, and chromium in low doses do not have a cytotoxic effect on cultures of cells of reproductive organs. The author notes the high survival rate of cells and an increase in their proliferation rate.

Analysis of the results of the studies by O. Shtapenko (2019) also showed that the use of chromium-methionine in rabbits for 1.5 months before fertilisation contributed to their reproductive function, increased fertilisation and embryo implantation. This allowed getting the maximum number of fetuses and the lowest preimplantation mortality compared to the control. O. Shtapenko (2019) notes that the blood counts of rabbits that consumed chromium-methionine were normal. She highlights an increase in red blood cells and haemoglobin, which indicates the activity of hematopoiesis, increased oxygen uptake by tissues, which indicates a higher favourability of females to scurrility, which positively correlates with reproductive function.

In the study, in the indicators of the general blood test, the level of haemoglobin, the number of red blood cells, and the colour indicator increased both in pregnant females and in their offspring, which allows asserting the positive effect of the vitamin-mineral complex on the processes of hematopoiesis. In addition, an increase in the content of zinc, iron, cobalt, magnesium, and copper, to a greater extent in the liver, kidneys and iron in whole blood was identified, which was identified in improving the antioxidant systems and reducing morphofunctional, necrotic cells.

E. Biletska *et al.* (2014) researched the histomorphological state of the placenta and features of placentogenesis under the influence of zinc chloride and identified a positive effect on placentogenesis and the development of a mature fetoplacental complex. In the study, a decrease in apoptosis-altered and necrotic liver cells in females was observed. In addition, hepatocytes had high functional activity. In 7-day-old animals, there were no functionally defective hepatocytes at all, which confirms the positive effect of adding a vitamin-mineral complex of this composition to the diet of pregnant females.

Conclusions

Thus, after applying the presented vitamin-mineral complex, a positive effect was identified on both pre- and postnatal development of offspring: the average indicators of offspring increased by 9.79% (p=0.0443), while the stillbirth rate decreased by 81.82% (p=0.0324). The absence of

negative side effects of the complex on the investigated target organs (liver, brain, heart, kidneys), and a positive effect on their morphofunctional characteristics is proved: the number of full-fledged hepatocytes increases in mature animals by 8.17% (p=0.0482), and in young animals – by 5.03% (p=0.0137), in the brain tissue of 7-day-old animals, an increase in the number of neurons by 7.70% (p=0.0438), and a decrease in the number of functionally defective neurons, which probably affects the development of basic reflex and behavioural responses, motor activity, and cognitive activity. In addition, the results of functional research methods, in particular, microelement analysis of organs identified a tendency to increase the amount of zinc, iron, cobalt, magnesium, copper, which was reflected in the improvement of the antioxidant systems of the body; there was also a positive effect on the processes of hematopoiesis: the level of haemoglobin increased by 3.45% (p=0.0238), and the number of red blood cells – by 11.6% (p=0.0292).

The results obtained can become morphological evidence for the choice of correction of macro, microelement, and vitamin balance and prevention of their deficiency in pregnant women and prevention of the development of deficient conditions in infants. The prospects for further research are to examine the morphological state of organs from the use of various vitamin-mineral complexes during normal pregnancy in white rats and their offspring, to examine the elemental analysis of target organs and whole blood, and to conduct a comparative-statistical analysis of the influence of vitamin-mineral complexes of different composition.

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Доклінічне дослідження ефектів від застосування вітамінно-мінерального комплексу під час нормальної вагітності у білих щурів

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Анотація. Вітамінно-мінеральний дефіцит є досить поширеним явищем у світі, що негативно позначається на рості й розвитку дітей, провокує розвиток хронічної патології, алергічних станів. Особливо чутливим до даного стану є дитяче населення та вагітні жінки. Беручи до уваги вітамінний та елементний склад кожного з препаратів, виникає необхідність дослідити ефект різних комплексів на організм. Метою дослідження було вивчити морфологічний стан органів від застосування вітамінно-мінерального комплексу під час нормальної вагітності у білих щурів та їх потомстві та дослідити елементний аналіз органів-мішеней й цільної крові. Для досягнення поставленої мети експеримент був проведений на вагітних самках білих лабораторних щурів та отриманому від них потомстві. Для дослідження використовували гістологічний, ультрамікроскопічний, морфометричний, атомно-абсорбційний, функціональний методи та статистичний аналіз. Було виявлено позитивний вплив вітамінно-мінерального комплексу вибраного складу, як на пре- так і постнатальний розвиток потомства. Середні показники приплоду збільшились на 9,79 % ($p = 0,0443$), водночас зменшився на 81,82 % ($p = 0,0324$) показник мертвонароджуваності. Доведено відсутність негативної побічної дії комплексу на формування основних рефлекторних та поведінкових реакцій, рухову активність, когнітивну діяльність потомства. Показано позитивний вплив на морфологічні характеристики печінки, мозку, серця, нирок: збільшилась кількість повноцінних гепатоцитів у зрілих тварин на 8,17 % ($p = 0,0482$), а у молодих тварин – на 5,03 % ($p = 0,0137$), у тканині головного мозку 7-денних тварин виявлено збільшення кількості нейронів на 7,70 % ($p = 0,0438$), а також зменшення кількості функціонально неповноцінних нейронів. Мікроелементний аналіз органів виявив тенденцію до збільшення кількості низки мікроелементів, що знайшло відбиток у покращенні антиоксидантних систем організму. Відмічено позитивний вплив комплексу на процеси кровотворення. Отримані результати можуть стати морфологічним доказом для вибору корекції макро-, мікроелементного, вітамінного балансу, профілактики його дефіциту у вагітних та попередження розвитку дефіцитних станів у немовлят

Ключові слова: тварини, потомство, вітаміни, морфологія, макро- та мікроелементи

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 13(4), 69-77
Received: 07.09.2022 Revised: 13.10.2022 Accepted: 02.11.2022

UDC 636.2.082.2:612.118
DOI: 10.31548/animal.13(4).2022.69-77

Lifetime productivity of bulls depending on the similarity of their parents by antigens of the B blood group system

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Abstract. During the selection of parents of future bulls of meat breeds, the problem of improving the main signs of meat productivity is a priority. Search for techniques that would additionally influence the effectiveness of using future bulls using conventional breeding methods determines the relevance of this study. The purpose of the study was to determine the influence of the selection of parent pairs based on antigens of the B blood group system on the results of lifelong use of bulls of Ukrainian beef cattle, created by the method of the complex reproductive crossing of four different breeds. The study was conducted by comparing the performance of bulls assigned to different groups by the antigenic similarity index (r_{as}) of their parents. By r_{as} value over 0.150 formed a group of progenies from a homogeneous selection of parents (n=5). In the group from a heterogeneous selection of parents (n=5), the indicator r_{as} less than 0.150. The libido of bulls was tested in the arena by the time of manifestation of sexual reflexes when receiving sperm on an artificial vagina. Within the groups, the age of bulls at the time of first sperm collection, life and productive use, the number of ejaculates received and culled, and the quality and fertilising ability of sperm were determined. It was identified that in bulls obtained from the homogeneous selection of parents by r_{as} compared to peers from heterogeneous mating, there is a tendency to increase the age of initiation of sperm collection by 33.9%. They also have a longer productive use expectancy by 6.3% and a longer life expectancy by 21.5%. Bulls obtained from heterogeneous selection during use have more leads to the scarecrow by 13.6% and predominate in the number of ejaculates obtained. The percentage of ejaculate culled is twice as high. Bulls from homogeneous selection have a higher (by 15.3 points) share of active manifestation of sexual reflexes, a larger volume of ejaculate, better indicators of sperm motility and concentration, fewer culled ejaculates, and a higher fertilisation capacity of sperm by 18.2 points. The results obtained should be used in the selection of bulls for potential mothers of valuable bulls of the Ukrainian beef breed and in other livestock populations created by the method of complex reproductive crossing

Keywords: Ukrainian beef cattle breed, red blood cell antigens, duration of productive use, ejaculate, reproductive crossing, sexual activity

Suggested Citation:
Ugnivenko, A., & Natalych, O. (2022). Lifetime productivity of bulls depending on the similarity of their parents by antigens of the B blood group system. *Animal Science and Food Technology*, 13(4), 69-77.

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Introduction

In meat cattle breeding, the use of artificial insemination of cows and heifers with the sperm of valuable bulls accelerates breeding progress compared to the use of natural mating. The effectiveness of using bulls largely depends on their productivity on the characteristics associated with the collection and accumulation of cryopreserved sperm. It is known that the genetic improvement of reproduction traits can be achieved by the optimal use of sperm from the best bulls in artificial insemination (Suprayogi *et al.,* 2020). Many bulls with genetically high productivity during use in the artificial insemination programme are culled for signs of growth, due to injuries and previous diseases, poor libido, unsatisfactory sperm quality, and its unsuitability to be frozen (Dangar *et al.,* 2021). The fertility of bulls is given less attention than that of cows (Fair & Lonergan, 2018) because it is complex and multi-factorial. Understanding the reasons for the variability of bull fecundity is a key prerequisite for achieving a reliable assessment of their sperm fertilisation capacity.

In the classical theory of selection, heterogeneous mating of parents is used to improve traits, which contributes to the manifestation of the effect of heterosis. In the Ukrainian beef breed of cattle, there is a need to justify effective selection methods, the use of which would contribute to improving the reproductive capacity of bulls. One of the strategies to improve the performance characteristics of cattle is to introduce methods for selecting pairs using polymorphic erythrocyte antigens of the system in blood groups. Their use has a certain effect on the signs of growth of bulls in the period from 8 to 18 months of age (Ugnivenko & Natalych, 2021). To date, there is a lack of published studies on the specific features of the use of bulls, meat breeds created in Ukraine, obtained from the selection of parents by blood group factors.

Signs of reproduction of bulls are divided (Savchuk, 1985) into indirect (testicular size, scrotum girth, age of puberty) and direct (sexual activity, sperm production, fertilisation capacity of sperm). The physiological maturity of bulls lasts from 2nd to 5th year of age (Naumenko *et al*., 2009). They show five unconditional basic sexual reflexes: attraction, climbing, erection, mating, and ejaculation. Sexual desire and climbing reflex bulls show not only to the female but also to the artificial cow (an artificially created figure of a cow that reproduces the shape of their body). This ensures that sperm is collected on the artificial vagina. Not all bulls mount on an artificial vagina, which is manifested by conditioned reflexes. It is known that the sexual behaviour of sire is not affected by their sperm mass and physical and morphological aspects (De Oliveire *et al.,* 2007). It is affected by parameters (length, width, depth, thickness, circumference of scrotum, and total testicular volume (Gippolito *et al.,* 2019; Novotná *et al.,* 2022), body structure type, and expressiveness of the meat forms (Ugnivenko & Koropets, 2021).

When selecting parents in meat breeds, special attention is paid to their heterogeneity. Using the system B blood groups has been one of the methods of predicting the productivity of cattle for a long time. Analysis (Vagonis & Vinikas, 1981) of cow insemination showed that for an increase from 0.40 to 0.81 of the antigenic similarity index (r_{as}) between cows and mated bulls, the number of inseminations per fertilisation increases from 1.49 to 3.08. Fertilisation is substantially higher when mating heterogeneous cows and bulls than when combining homogeneous individuals (Shadmanov, 1981). When mating heterogeneous pairs from the first insemination, from 52.2 to 60.2% of cows are fertilised, and homogeneous pairs – from 37.2 to 41.5 %.

Analysis of studies on the livestock of the Ukrainian meat breed indicates ambiguous results of the relationship between blood group factors in males on the one hand and signs of their productivity on the other. Thus, under the conditions of heterogeneous selection of parents, the volume of ejaculate of the Cisdnieper type is 7.4% larger than that of peers from homogeneous selection (Koropets, 2003). Producers of the Chernihiv type of Ukrainian meat breed have an advantage of 9.1%. Bulls obtained from parents with a lower antigenic similarity index outperform their peers in terms of sperm motility and concentration. The opposite feature was obtained (Ugnivenko & Natalych, 2021) in males based on growth characteristics. By increasing the similarity of blood group antigens between parents with large differences in pedigrees, their sons show a tendency to increase the average daily weight gain, live weight, and improve in the expressiveness of meat forms. In the Ukrainian meat breed, the features of lifelong use of bulls obtained by considering the similarity of erythrocyte antigens of their parents are not sufficiently covered.

Thus, the purpose of the study is to determine the influence of the selection of parental pairs based on antigens of the B blood group system on the results of lifelong use of first- and second-generation bulls, which have a low degree of similarity in polymorphic proteins, of the Ukrainian beef breed created by complex reproductive crossing.

Materials and Methods

The analysis of the lifelong use of bulls of the Ukrainian beef breed, which were kept in the breeding plant "Volia" of the Zolotonosha District of Cherkasy region, was conducted in 2022. The raising of experimental bulls was conducted using the technology of beef cattle breeding. Until 6-7 months of age, bulls were kept wit regulated suckling. After weaning, from 6-7 to 8 months, they were accustomed to a typical diet and conditions of being on a leash. Evaluation of bulls by their productivity was conducted from 8 to 15 months of age by growth rate, live weight, feed consumption per 1 kg of growth, and the expressiveness of meat forms. At the 14th month of life, bulls began to be used to collect sperm on an artificial vagina. After cryopreservation, it was sent for artificial insemination of breeding stock. The conditions of detention and use of bulls during the study met the requirements of the Order Ministry of Agrarian

Policy and Food of Ukraine No. 652 dated 25.10.2012, "On approval of the Procedure for using animals in agriculture".

The selection of bulls into groups was conducted based on the results of checking for the reliability of origin, considering the number of available antigens of the B blood group system of cattle, published in the catalogue "Blood types of bulls-producers and cows used in the breeding of dairy and meat breeds of cattle" (Podoba *et al.,* 1987). 10 animals were left to determine the lifetime productivity of bulls, which, according to the results of the assessment of their productivity, had a comprehensive breeding index "A" over 101. Bulls were distributed according to the value of the index of antigenic similarity of parents. The first group (n=5) included animals obtained with a relatively homogeneous selection of parents, in which the value of the antigenic similarity index is over 0.150. The second group (n=5) includes bulls who came from a heterogeneous selection of parents whose antigenic similarity index is less than 0.150.

Red blood cell antigens of the B system of bovine blood groups were used to calculate the index of antigenic similarity of parents. It was determined by the formula (1) of Zhivotovsky-Mashurov, quoted from the paper (Ugnivenko & Natalych, 2021):

$$r_{as} = \frac{S}{n_1 + n_2 - S} \quad (1)$$

where: r_{as} – index of antigenic similarity of parents; S – the total number of identical antigens in the father and mother; n_1 – the number of red blood cell antigens detected in the mother; n_2 – the number of red blood cell antigens detected in the father.

Based on the results of testing and using bulls, their age at the time of first sperm collection, life expectancy, productive use in days, and the number of ejaculates received and culled were determined.

Evaluation of bull ejaculates was performed immediately after they were received. The assessment included an analysis of the presence of inclusions, sperm volume, and concentration. Sperm motility was evaluated before cryopreservation of sperm.

The use of sperm of the bulls under study was evaluated on the farm based on the results of artificial insemination of breeding stock. The analysis was conducted according to zootechnical accounting data. In particular, the number of cows and heifers that were inseminated with the sperm of bulls of experimental groups was determined, including the percentage of those fertilised after the first insemination.

The degree of sexual activity (libido) of bulls was evaluated in the arena for obtaining bull sperm after bringing the animals to the artificial cow (an artificially created figure of a cow that recreates the shape of their body). According to the manifestation of libido, bulls were divided into three groups: active – ejaculation is conducted from 10 to 60 seconds; moderate – from 60 to 120 seconds, and calm – the time before ejaculation lasted over 120 seconds. The life expectancy of bulls (days) was determined by the period between the date of exclusion of the bulls from the herd and their birth date. The duration of productive use of bulls (days) was determined by the period between the dates of the first and last sperm collection from them.

Research data were processed to determine the average value for groups and its statistical error, and the relative indicators of individual traits as a percentage.

Results and Discussion

In breeds that were bred a long time ago and have a high degree of similarities by main traits, supporting the genetic diversity is attempted by using different variants of heterogeneous selection of parental pairs. In newly created breeds, stabilising selection is used, which allows obtaining animals of the desired type. This selection gradually reduces the level of genetic variation in the main breeding traits, while other traits can preserve genetic diversity and be used in breeding work. In the process of breeding the Ukrainian meat breed by complex reproductive crossing, the genes of the four original breeds were combined. This is caused a great genetic diversity of livestock. The effects of the heterogeneous and homogeneous selection of parents on the productivity of bulls of the newly created Ukrainian meat breed were investigated. The degree of similarity of parents by antigens of the B blood group system of cattle is one of the signs of genetic diversity of their offspring. The smaller the antigenic similarity of parents, the higher the theoretical degree of heterozygosity of their offspring for alleles that determine productivity, reproduction, immunity, and other traits. It was identified that bulls obtained from parents with varying degrees of antigenic similarity differ in the results of lifelong use (Table 1).

Table 1. *Results of lifelong use of bulls obtained from different selection of parents according to the index of antigenic similarity of the B blood group system*

Feature	Antigenic similarity index (r_{as})			
	over 0.150		less than 0.150	
	n	M±m	n	M±m
Age of first sperm collection, days	5	671±158.2	5	501±71.4
Duration of productive use, days	5	542±158.8	5	510±71.9
Life expectancy, days	5	1227±232.2	5	1010±64.6
Number of leads to the artificial cow, times	5	154±39.4	5	175±34.8
Number of ejaculates received, pcs.	5	124±29.5	5	147±36.8
-//-, %	5	82.3±2.8	5	82.8±8.3

In bulls from homogeneous selection of parents according to the index of antigenic similarity of the B blood group system (r_{as} over 0.150), the average age of receiving the first ejaculate using an artificial vagina was 671 days. In bulls obtained by heterogeneous mating of father and mother (r_{as} less than 0.150), there is a tendency to decrease the age of the first sperm collection by 33.9%. Within the groups, there was a large variability in the age of the beginning of reproductive use of bulls, which is due to the need for long-term rearing to assess meat productivity and other production factors associated with the accumulation of cryopreserved sperm and breeding work on the farm. In terms of life expectancy and productive use, bulls obtained from homogeneous selection had slightly better indicators, but no substantial difference in these characteristics between the groups was established. The number of attempts to collect sperm (leads to the mount) and ejaculates obtained during life was less in bulls obtained from homogeneous selection of parents by 13.6% and 18.5%, respectively, and the proportion of successful attempts to obtain ejaculate did not differ between animals of both groups. In general, according to the results of lifelong use, slightly better indicators can be noted in bulls obtained from heterogeneous parents for erythrocyte antigens, which is reflected in the earlier beginning of reproductive use and a larger number of ejaculates obtained. However, the increased intensity of use may be associated with the need for additional sperm production from heterogeneous bulls, so other signs of their reproductive ability were analysed.

The results of sexual activity assessment were analysed in bulls of experimental groups (Table 2).

Table 2. *Sexual activity of bulls obtained from different selection of parents according to the index of antigenic similarity of the B blood group system*

Feature		Antigenic similarity index (r_{as})			
		over 0.150		less than 0.150	
		manifestations	indicator by group	manifestations	indicator by group
Sexual activity on the mount	Active libido, %	53	76.8	88	61.5
	Moderate libido, %	11	15.9	33	23.1
	Calm libido, %	5	7.3	22	15.4
Time of libido manifestation	With active libido, seconds	53	8.9±1.23	88	12.8±1.37
	For moderate libido, seconds	11	73.6±23.29	33	81.8±14.46
	For a calm libido, seconds	5	720.0±360.0	22	649.1±141.64

It was identified that bulls from homogeneous selection (r_{as} over 0.150) were characterised by higher sexual activity. Active manifestations of libido in their group were 15.3 points more. In the group of bulls from heterogeneous selection (r_{as} less than 0.150), the proportion of moderate and calm libido manifestations was higher by 7.2 and 8.1 points, respectively. The time of manifestation of sexual reflexes within the classes for sexual activity also differed. Bulls obtained from a homogeneous selection of parents with libido rated as active gave ejaculate to an artificial vagina 4 seconds earlier than heterogeneous ones. In the class with moderate libido, homogeneous bulls also prevailed in terms of the speed of mounting and ejaculation by 8 seconds.

The data obtained indicate higher sexual activity of bulls from the homogeneous selection of parents by red blood cell antigens, but it does not explain why more ejaculates were obtained from heterogeneous bulls during their lifetime. This becomes clear after analysing the quality of native bull sperm and the results of its use (Table 3).

Table 3. *Quality of sperm production of bulls obtained from the heterogeneous selection of parents according to the index of antigenic similarity of the B blood group system*

Feature	Antigenic similarity index (r_{as})			
	over 0.150		less than 0.150	
	n	M±m	n	M±m
Ejaculate volume, cm^3	4	5.02±2.9	5	4.77±2.39
Sperm motility, points	4	7.52±4.34	5	6.98±3.49
Sperm concentration, billion/cm^3	4	1.40±0.81	5	1.24±0.62
Culled ejaculates, pcs.	5	11.5±6.2	5	18.9±6.5
-//-, %	5	7.5±4.0	5	15.0±7.4
Total inseminated cows and heifers, heads	3	582	3	299
Fertilised after the first insemination, %	3	51.0	3	32.8

Bulls from parents with a higher antigenic similarity index, against the background of better sexual activity, had a slightly larger ejaculate volume and prevailed over bulls obtained from heterogeneous selection for sperm motility by 0.54 points and sperm concentration by 0.16 billion/cm^3. These are the signs that determine the number of standard sperm doses that can be obtained from a single ejaculate. In addition to better sperm quality, bulls from a homogeneous selection of parents had a 7.5-point (2-fold) lower proportion of culled ejaculates after their analysis for inclusions and sperm quality. Thus, more sperm doses were obtained from each bull that descended from parents with higher similarity in red blood cell antigens, and therefore the need to receive additional ejaculates from them was reduced.

Costs of bull sperm obtained from the mating of heterogeneous parents (r_{as} less than 0.150) were higher. In particular, the fertilisation rate of breeding stock after the first insemination with their sperm was only 32.8%, which is 18.2 points less than the indicators of bulls obtained from the homogeneous selection of parents.

Thus, for most signs of reproduction, the best results were obtained in bulls descended from parents with a high index of similarity in erythrocytic antigens of the B blood group system. More ejaculates received from bulls whose parents had r_{as} less than 0.150 is due only to the need to replenish the reserves of standard sperm doses, which were received less from the ejaculate, and the costs due to low fertilisation capacity were higher.

According to Dahiya *et al.,* (2013), obtaining sperm from producers at a relatively early age is economically beneficial and reduces the time for evaluating the quality of the offspring. Of the genetic factors, the age of the bull during the first sperm collection is affected by the breed (*Naha et al.,* 2019), different types of crossbreeding (Vijetha *et al.,* 2014). A comparative analysis of results obtained with the conclusions of studies by other authors on the effect on the age of taking sperm from bulls from different types of parental selection shows that their heterogeneous mating leads to a decrease in the age of the beginning of sires using. Thus, in mixed bulls, the age of the first sperm collection is less than in purebred animals (Mukhopadhyay *et al.,* 2010).

In addition to genetic factors, the age of puberty, and the first sperm collection from bulls are affected by the concentration of the hormone testosterone in the blood serum (Lunstra *et al.,* 1978), the season of their birth (Abdullah *et al.,* 2018), scrotum circumference (Suprayogi *et al.,* 2020). Testing shows (Waite *et al.,* 2019) that 98% of young bulls are suitable for use with a scrotum circumference ⩾27 cm and they have ⩾70% of normal sperm. The age of first sperm collection is lower in bulls that have higher average testosterone concentrations (Lunstra *et al.,* 1978), live mass of as newborns (Abdullah *et al.,* 2018), and are born in the fall, compared to their spring peers (Rendel, 2002). The effect of the birth season of bulls on their sexual development is a consequence of the photoperiod, which may be involved in the regulation of testicular function immediately after puberty (Tatman *et al.,* 2003).

Due to the complexity of the longevity trait in cattle, Zhang *et al.,* (2021) recommend making extensive use of two groups of its definitions, which either cover the entire life span or represent only part of it. According to the data of this study, the duration of productive use and life of bulls obtained from the homogeneous selection of parents according to the antigenic similarity index is 6.3 and 21.5% longer, respectively, than in peers born from heterogeneous mating. Consequently, the high heterogeneity of parents by erythrocyte antigens tends to negatively affect these traits in the offspring.

Bulls from homogeneous selection tend to reduce the number of leads to the mount and the total number of ejaculates received on the artificial vagina. The tendency to increase these signs of productivity in bulls obtained from parents with a lower index of antigenic similarity may be associated with an earlier start of their use, or a violation of signs of sperm productivity, which causes the need for more intensive use of bulls. The intensity and effectiveness of ejaculate selection from bulls are related to their sexual activity. In this study, a trend regarding the superiority of bulls obtained from a homogeneous selection of parents over peers from heterogeneous mating by the active manifestation of sexual reflexes was established. Bulls obtained from heterogeneous parents spent more time showing active and moderate types of sexual activity. Sexual behaviour is an indicator of hormonal activity, in particular, it was noted that exposure to certain factors that increase testosterone levels can simultaneously improve the sexual activity of bulls and sperm quality (Srivastava *et al.,* 2022). In our study, the concentrations of sex hormones were not investigated, but the results obtained indicate that the indicators of features that they affect are better in bulls obtained from a homogeneous selection of parents. In addition to a decrease in libido activity, bulls often completely refuse to mount and ejaculate on the artificial vagina. Refusal to ejaculate in bulls may be associated with seasonal stress, especially in summer (Bhakat *et al.,* 2009), their sexual lethargy or weak temperament (Singh, 2014). Most of the above reasons for the refusal of bulls to show sexual reflexes are due to natural conditions or technological factors. Since the proportion of refusals of bulls to mount in both groups was the same, this indicates the same impact on them of the conditions of keep and use. While the level of their sexual activity is determined by individual characteristics and, in the opinion of this study, was defined by the method of selecting parents.

The characteristics of the signs of sperm productivity of bulls are very different and depend on the type of selection of their parents by the antigenic similarity index. Sires originating from a homogeneous selection of parents compared to peers from heterogeneous mating are characterised by a larger ejaculate volume and sperm motility and concentration. There is a lot of data in the literature on the sperm productivity of bulls. In particular, it was noted that the sperm characteristics presented in this study are not similar to those given in the literature

(Koropets, 2003). They differ due to differences in the genotypes of the used bulls of the created Ukrainian meat breed of the third and fourth generations.

Bulls obtained from the homogeneous selection of parents by the antigenic similarity index have a 2-fold lower percentage of ejaculate culling. The quality of sperm in the ejaculate and the signs by which it can be culled are affected by various factors. The worst indicators of quantitative and qualitative signs of sperm are observed during puberty (Nugraha *et al.,* 2019). Young bulls secrete less sperm in their ejaculate than adults (Fair & Lonergan, 2018). At the age of 1 year, they have worse indicators of sperm production and sperm motility compared to older ones, although there is no difference in sperm motility after unfreezing cryopreserved sperm doses between younger and older bulls (Murphy *et al.,* 2018). One-year-olds selected for breeding have not only reduced sperm production but also an increased inflammatory response, leading to poor reproduction if used intensively in breeding (Snider *et al.,* 2022). Sexually mature bulls have better-quality sperm than young ones (Bhakat *et al.,* 2011). They have better libido. Spermatozoa are formed in the seminal tubules located inside the testes. Sperm production increases with age due to a positive correlation between sperm and testicular size (Asenabor *et al.,* 2015), which are 90% seminal tubules (Nugraha *et al.,* 2019). In this study, the use of bulls was conducted after the onset of puberty, so the negative impact of the early age of animals can be discarded, and the reasons for intergroup differences are considered to be the individual characteristics of the animals included in them, due to the origin from parents with different similarities in erythrocyte antigens.

Evaluation of sperm quality indicators is conducted to ensure effective fertilisation of cows and heifers. There is a very close genetic correlation between the percentage of live sperm and sperm motility before and after cryopreservation (Berry *et al.,* 2019). In bulls descended from parents with different antigenic similarity indices, a substantial difference was identified in the fertilisation capacity of sperm after the first insemination. The number of breeding stock fertilised from the first time was dominated by bulls obtained from a homogeneous selection of parents. The volume of ejaculate, the concentration and motility of sperm were higher in bulls of this group, so quantitative and qualitative indicators of their sperm productivity should be considered a likely reason for the better fertilisation capacity of sperm. The effectiveness of fertilisation of breeding stock is one of the most important characteristics of bulls and their sperm, as it affects the economy of beef production. The use of bulls with satisfactory fertility has the greatest impact on farm profits (Barth, 2018), so producers prefer older bulls (Brimlow & Doyle, 2014), which, due to physiological characteristics, have a better development of the reproductive system and reproduction indicators. For example, among farmers raising cattle and calves in the United States, in seventy-four per cent of cases, mature bulls are used, while only six are one-year-olds (USDA, 2020). Forty-seven per cent of farmers buy 18-month-old bulls, fourteen – 12-month-olds, and eight – two-year-olds (Banwarth *et al.,* 2021). The purchase of older bulls is justified by the need to ensure effective reproduction, although they have higher prices at the time of sale (Bacon *et al.,* 2017). One-year-old bulls, due to the worst development of the reproduction function, are not allowed to breed more than twenty-five cows (Engelken, 2008). These data highlight the economic importance of bull breeding traits.

Regarding the need to improve the productivity characteristics of meat cattle by breeding methods, one of the most common techniques is the use of a heterogeneous selection of parents. In commercial meat cattle breeding, crossbreeding is used, and during purebred breeding, animals that are remote in origin are selected to avoid inbreeding. According to Karoui *et al.* (2011), inbreeding does not lead to a deterioration in the signs of sperm production (ejaculate volume, motility, and sperm concentration), but previous attempts to use the selection of parents based on the index of antigenic similarity of erythrocyte antigens of the system in blood groups (Vagonis & Vinikas, 1981) identified that an increase in r_{as} it leads to a deterioration in fertility based on the characteristics of cows and bulls. Since then, heterogeneous selection by erythrocyte antigens has been thought to improve the productivity of livestock. Notably, these studies were conducted for purebred breeding on animals with high similarity of erythrocyte antigens and a negative result was obtained for r_{as} from 0.40 to 0.81. This study used data from bulls obtained at the stage of breeding and consolidation of the Ukrainian meat breed with substantially lower similarity of parents in terms of erythrocyte antigens. There is presumably an optimal level of parental similarity for r_{as}, which can affect the manifestation of various signs of livestock productivity. It is advisable to select parents with an antigenic similarity index greater than 0.150 to improve the signs of sperm productivity of bulls of the Ukrainian meat breed, such as the volume of ejaculate, the concentration and motility of sperm, and the fertilising ability of sperm after the first insemination. Excessive parental differences in red blood cell antigens will have a negative result.

Conclusions

In the bulls of the Ukrainian meat breed, obtained at the first stages of complex reproductive crossing from a heterogeneous selection of parents, according to the index of similarity of antigens of the system in blood groups, compared with peers from homogeneous mating, there is a tendency to reduce the age of sperm collection by 33.9%, lengthening productive use by – 6.3 and life expectancy – by 21.5%. However, they have a higher number of leads to the mount – by 13.6, received ejaculates – by 18.5%, and a lower percentage of sperm culling by 2 times.

There is a tendency of 15.3 points advantage of bulls obtained from the homogeneous selection of parents over peers from heterogeneous mating for the active

manifestation of sexual reflexes and a decrease in time by 44.6 and 11.1%, respectively, for the manifestation of active and moderate libido. Sires originating from the homogeneous selection of parents compared to their peers from heterogeneous mating are characterised by a 5.2% increase in ejaculate volume, 7.7% sperm motility, and 12.9% sperm concentration.

In the Ukrainian meat breed, the selection of parents should be conducted with the similarity index for erythrocyte antigens of the B blood group system (r_{as}) over 0.150, which will positively affect the most important signs of bull fertility. The results obtained should be considered when selecting sires for cows that are potential mothers of valuable bulls. There is a need for further research to determine the optimal difference between father and mother in the similarity of antigens of the B blood group system in a larger r_{as} range to substantiate the methods of selecting parental pairs aimed at improving the efficiency of using future sires. It is also advisable to check the results obtained in the study in other cross-breeding populations of cattle, which are bred according to the principle of the Ukrainian beef breed by a combination of remote source breeds.

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Довічна продуктивність бугаїв залежно від подібності їхніх батьків за антигенами системи В груп крові

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Анотація. Під час підбору батьків майбутніх бугаїв м'ясних порід, у першу чергу вирішують завдання з поліпшення основних ознак м'ясної продуктивності. Пошук прийомів, які за умов застосування традиційних методів селекції додатково впливали б на ефективність використання майбутніх бугаїв визначають актуальність цієї статті. Метою дослідження було визначити вплив підбору батьківських пар за антигенами системи В груп крові на результати довічного використання бугаїв української м'ясної худоби, створеної методом складного відтворювального схрещування чотирьох різних порід. Дослідження провели методом порівняння продуктивності бугаїв, віднесених до різних груп за індексом антигенної подібності (r_{as}) їхніх батьків. За величиною r_{as} понад 0,150 сформували групу плідників від гомогенного підбору батьків (n=5). У групі від гетерогенного підбору батьків (n=5) показник r_{as} менший за 0,150. Лібідо бугайців тестували у манежі за часом прояву статевих рефлексів під час отримання сперми на штучну вагіну. У межах груп визначали вік бугайців під час першого взяття сперми, тривалість життя та продуктивного використання, кількість отриманих та вибракуваних еякулятів, якість і запліднюючу здатність сперми. Виявлено, що у бугаїв, отриманих від гомогенного підбору батьків за r_{as}, порівняно з ровесниками від гетерогенного спаровування, проявляється тенденція до збільшення віку початку взяття сперми на 33,9 %. У них також більша тривалість продуктивного використання на 6,3 % та тривалість життя на 21,5 %. Бугаї, отримані від гетерогенного підбору за час використання мають більше підведень до опудала на 13,6 % і переважають за кількістю одержаних еякулятів. Відсоток вибракуваних еякулятів у них вдвічі вищий. У бугаїв від гомогенного підбору більша (на 15,3 пункти) частка активного прояву статевих рефлексів, більший об'єм еякуляту, кращі показники рухливості і концентрації спермій, менше вибракуваних еякулятів та вища на 18,2 пункти запліднююча здатність сперми. Отримані результати доцільно використовувати під час підбору бугаїв до потенційних матерів ремонтних бугайців української м'ясної породи та в інших популяціях худоби створених методом складного відтворювального схрещування

Ключові слова: українська м'ясна порода великої рогатої худоби, еритроцитарні антигени, тривалість продуктивного використання, еякулят, відтворювальне схрещування, статева активність

ANIMAL SCIENCE AND FOOD TECHNOLOGY

Journal homepage: https://animalscience.com.ua/en
Animal Science and Food Technology, 13(4), 78-86
Received: 09.09.2022 Revised: 11.10.2022 Accepted: 25.10.2022

UDC 637.051
DOI: 10.31548/animal.13(4).2022.78-86

Assessment of the quality and nutritional value of organic dried sausages

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Abstract. Organic production, which is focused on the production of high-quality, high-nutritional and environmentally friendly food products, is becoming increasingly relevant in developed countries, in particular, Ukraine. The purpose of the study is to determine the quality and nutritional value of organic dried sausages. For its implementation, physico-chemical, microbiological, organoleptic, and statistical research methods and approaches to abstraction, synthesis, analysis, systematisation, and generalisation of data are used. Three types of sausages are selected for the study: Sample 1, Sample 2, and Sample 3. As a result of the study, it is identified that all indicators of the investigated samples are controlled and meet the requirements of regulatory documents. During the organoleptic assessment, it is identified that all the investigated samples had a dry surface without damage, an elastic consistency, and a pleasant smell with the aroma of

Suggested Citation:
Khomenko, M., Slobodianiuk, N., Omelian, A., Seba, M., & Briukhachova, I. (2022). Assessment of the quality and nutritional value of organic dried sausages. *Animal Science and Food Technology*, 13(4), 78-86.

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spices. The difference was observed only in colour, it was from dark pink to dark red, which is due to different recipes of sausages. Microbiological analysis of the products shows that there was no pathogenic microflora in all samples. According to the results of the physico-chemical analysis, it is identified that the content of moisture, salt, and sodium nitrite was within the normal range. The difference was observed in protein and fat content. The first sample of sausage showed the lowest protein content (14%), while the second and third samples showed 16.2% and 19.2%, respectively. Substantial differences were also observed in the fat content of the finished product. The lowest level of this indicator was in Sample 3 and was 6.4%, which is 22.8% and 13.6% lower compared to Sample 1 and Sample 2. This difference in protein content is due to the different composition of raw materials of the investigated samples. The lowest caloric content (134 kcal) is identified in Sample 3 sausage, which had the highest protein levels and the lowest fat content. The results obtained practically allow assessing the dietary characteristics of the investigated assortment and orient the consumer in choosing a product in accordance with their individual preferences and needs, characteristics, and nature of the diet

Keywords: sausage fermentation, microbiological indicators, protein mass fraction, fat mass fraction, sodium nitrite, organoleptic assessment, pathogenic microorganisms

Introduction

In recent years, the problem of a clean environment in the world is quite relevant among more than 150 countries, including Ukraine. Due to the deterioration of the environmental situation and other adverse factors in people's lives, the problem of improving the safety of meat, in particular, sausage products, is of particular importance. Today, providing the population with high-quality, environmentally friendly and safe food products is quite an urgent task, due to a number of reasons, such as: environmental pollution, expanding the range of food products, creating new and improving existing production technologies, using food additives, etc. (Lupenko, 2013).

Every year in the developed countries of the world, the demand for organic products is growing, which is associated with a high level of consciousness in the population. Society is interested in healthy and safe nutrition, which is manifested by concern for health and the environment. The European Green Deal (Mission of Ukraine to the ..., 2021) is central to the policy agenda of the European Commission. Its main goal is a sustainable, climate-neutral Europe by 2050 (European Green Deal, 2021). The European Green Deal (2021) highlights that it is extremely important to manage the transition to a more sustainable food system aimed at combating climate change, protecting the environment, and preserving biodiversity. As part of its efforts to promote organic production, the European Commission is implementing large-scale activities in the production and marketing of food chains and food establishments (Communication from the Commission..., 2021). For example, Copenhagen became the first city where the share of organic food in public catering establishments reached 100%. Vienna has a network of urban organic gardens with an area of about 860 hectares, which also supply food to public catering establishments, in particular, to nurseries. Public catering establishments in Rome serve approximately 1 million organic meals a day (Communication from the Commission..., 2021). This shows the determination of Europe to switch to full organic production, which cannot be ignored by Ukraine, which is seeking official EU membership.

The organisation of organic production in Ukraine is relatively just beginning its activity. The Ukrainian market is not crowded with organic products, but still, the consumer can find them among the affordable assortment. At the beginning of the development of Ukrainian organic production, it is important to pay special attention to the examination of technologies and products directly, make sure that novice producers are conscientious and that organic business is effective. In addition, in the context of the desire of Ukraine to officially become a member of the European Union, it is important for researchers to focus not just on technology or production aspects. The main problem of the organic segment in Ukraine is the restructuring of capacities for product safety. One of the ways to solve it is to examine the Ukrainian organic product, namely, to determine the quality and nutritional value indicators, to identify and eliminate shortcomings. In the absence of the latter, it is necessary to publish the success of existing Ukrainian organic production facilities to popularise and develop the industry. It is important to scientifically substantiate the feasibility of conducting organic production, which encourages the change in the concept of conventional production towards organic.

Consumers of sausage products are almost 90% of the total population of Ukraine. Nine out of ten Ukrainians consume sausage, so their production is one of the most important in the meat industry. Due to advances in biotechnology, it is possible to obtain environmentally friendly and high-quality meat products with new sustainable quality indicators (Shevchik *et al.*, 2018).

Fermentation is one of the most relevant technologies among the methods of producing healthy food (Pilevar & Hossein, 2017). Under appropriate temperature and relative humidity conditions, fermented sausages are made by combining microbiological, biochemical, and physical activity. As a result of these important processes, the taste properties of the product change during maturation. Fermentation not only increases the shelf life and microbiological safety of products but also increases their digestibility (Caplice & Fitzgerald, 1999).

In the composition of fermented sausages, the main part is meat (at least 70-80%) (DSTU 4427:2005, 2006), which is a source of proteins, B vitamins, minerals, and

biologically active substances that play an important role in human nutrition, as they are involved in metabolism, growth, and development of the body (Tsiprian *et al*., 2007). Due to the fact that dried sausages are not subjected to heat treatment, the content of all nutrients in the finished product remains unchanged. Thus, the fermentation of sausages allows not only getting products that have a high nutritional value but also have a positive impact on human health.

In addition to technology, the quality of raw materials also plays an equally important role in obtaining high-quality sausage products. Due to the fact that the internal market is overflowing with low-quality food products, consumers are increasingly inclined to buy organic products. According to the results of the study (DSTU 4427:2005, 2006), about 7850 thousand kg of organic food products of Ukrainian production were sold on the internal market of Ukraine in 2020, which is more than 709 million UAH, which is the equivalent of 25.1 million US dollars at the NBU exchange rate as of 31.12.2020. One of the prerequisites for the development of organic production was also the promotion of a healthy lifestyle. In this regard, manufacturers are faced with the task of producing high-quality and environmentally friendly food products (Lupenko, 2013).

The analysis of papers available in the public space showed the interest of researchers in finding alternative components of sausages to replace conventional ones. Often, the goal is to develop cheaper, more cost-effective recipes while maintaining the quality of the product. Researchers from the Philippines C.J.A. Domingo, K.J. Sartagoda, N.J.C. Catandijan, and N.K. Yasin (2023) conducted a study on the introduction of vegetable fat into the recipe of sausages. The Chinese researchers also became interested in vegetable fat substitutes in sausages. C. Li, W. Xie, X. Zhang, J. Liu, M. Zhang, J. Shao (2023) showed the feasibility of using pea protein – chitosan, as a new fat substitute, to improve the quality of pork sausages. There are also studies on the organic production of raw meat using natural growth stimulants in animal feeding (Fraz *et al*., 2023). In the context of organic production of boiled meat products and sausages, researchers focus on replacing undesirable acidifiers (Chattopadhyay *et al*., 2023), preservatives (Yaghoubi *et al*., 2023), and emulsifiers (Adiamo *et al*., 2023) of a chemical nature. Despite the fact that the use of $NaNO_2$ to preserve the attractive commercial colour of meat products is still allowed in organic production, Turkish (Serdaroglu *et al*., 2023) and Polish (Szymański *et al*., 2023) researchers have begun work to replace it due to its high toxicity. M. Serdaroğlu, H. Can, B. Sarı, H.S. Kavuşan, F.M. Yılmaz (2023) published interesting results of using natural sources of nitrites from arugula and barberry extract in the production of heat-treated fermented sausages. Polish researchers R. Szymański, V. Łaszkiewicz, A. Kern-Jędrychowska, U. Siekierko, D. Kołożyn-Krajewska (2023) were interested in the ability of enzymes to perform the function of forming the desired colour in boiled meat products.

In Ukraine today, there is not enough scientific literature on the production of organic sausages, which is due to the beginning of its introduction. The scientific community should pay attention to this because the experimental justification of the feasibility of producing organic sausages can be a catalyst in the development of this food area.

The purpose of the study is the justification of the feasibility of producing organic dried sausages by determining their quality and nutritional value indicators for meeting the demand formed by a wide range of consumers with different nutritional needs.

Materials and Methods

The study was conducted in 2021 based on the Enterprise LLC "Organic Meat Product", which is part of the vertically integrated company PE "Galex Agro". All products of the company are certified by the international certification company Organic Standard LLC and meet the requirements of Bio Suisse and Naturland (Organic Meat Product, 2021). Organic Meat Product LLC produces products from its own raw materials, which are also certified as organic. From the range of sausages that are produced at this enterprise, three types of dried sausages were selected for research: Sample 1, Sample 2, and Sample 3. Organoleptic, microbiological, and physico-chemical analyses were performed in the studies.

First, an external inspection of each type of product was conducted. 10% of the total amount of products from the batch were selected (50 kg). For the following experiments, units of products were selected: 2 units for each experiment (DSTU 4427:2005, 2006).

For organoleptic studies, 2 single samples were first taken from different units of products weighing 400-500 g, from which a total sample of 800-1000 g was formed. During the organoleptic study, the following indicators were evaluated: appearance, consistency, type of minced meat (or sausage product) on the cut, taste, and smell. The assessment was conducted visually in accordance with DSTU 4427:2005 (2006).

A tasting assessment of compliance of dried sausages with the standard was conducted according to a five-point system. According to it, the main quality indicators are: appearance, appearance and colour on the cut, smell (aroma), taste, consistency (tenderness, hardness), and juiciness. Each indicator has 5 degrees of quality: for excellent quality – 5, very good, good – 4, average, acceptable quality – 3, acceptable quality, but not desirable – 2, unacceptable quality – 1.

For microbiological studies, 2 single samples with a length of 15 cm each from the edge of the sausage were taken with sterile instruments, which makes up the total sample. In accordance with the recommendations, the following indicators were evaluated: pathogenic microorganisms, in particular, bacteria of the genus *Salmonella* (DSTU EN 12824:2004, 2005); Escherichia coli bacteria (coliforms) (DSTU GOST 30726-2002, 2003); sulfitreducing clostridia (DSTU ISO 7937:2006, 2007); L. *Monocytogenes*; S. *Aureus* (DSTU ISO 11290-1:2003, 2004; DSTU ISO 6888-1:2003, 2004).

For physical and chemical studies, 2 single samples were first taken from different units of products weighing 200-250 g, and then a total sample of 400-500 g was formed (for each type of product separately) (DSTU 4427:2005,

2006). The mass fraction of fat, protein, and moisture was determined using Food Scan Meat Analyser. The salt concentration was determined by the Mohr method (DSTU 4427:2005, 2006), and the content of nitrites using the colourimetric method. The obtained research results are processed by statistical analysis methods.

The approaches of abstraction, analysis, synthesis, systematisation, and generalisation of data were used to conduct general scientific research. Abstraction included: fixing the specific features and principles of organic sausage production technology; forming and describing analytical, synthetic, and predictive information of the authors. Methods of analysis and synthesis were used in close unity, which allowed isolating and recording important data that were in the array of available information. In addition, the synthesis approach was actively used to describe the results of the observations of this study. Systematisation and generalisation of data were used both for logical and consistent presentation of the processed information, and for forming conclusions based on research materials (Danilyan & Dzoban, 2019).

Results and Discussion

During the quality assessment, first of all, the composition of the fermented sausages was examined. The composition of the sausages of Sample 1, organic top grade, includes: organic beef of the highest and first grade, organic low-fat and semi-fat pork, organic spine lard, nitrite-salt mixture, and organic spice blend. The composition of Sample 2, organic, top grade includes: premium organic beef, organic spine lard, nitrite-salt blend, and organic spice blend. The composition of Sample 3 sausage from organic beef of the top grade includes: organic beef of the highest grade (50%), organic beef of the first grade (50%), nitrite-salt mixture, organic sugar, organic ground black pepper, organic garlic, organic ground cinnamon. For the production of sausages, beef and pork of own production certified as organic are used. The organoleptic evaluation of sausages is shown in Table 1.

Table 1. *Organoleptic evaluation of organic sausages*

Parameter	Sample 1	Sample 2	Sample 3
Appearance	Sausages have a clean, dry surface, no damage or deposits of minced meat	The product is rounded in shape, the surface is dry and clean without damage	The products are square in shape, have a dry surface without damage or deposits of minced meat
Consistency	Elastic	Elastic	Elastic
Type of minced meat on the cut	The colour of minced meat is from dark pink to dark red without grey spots and voids	Dark red without grey spots and voids, minced meat is evenly mixed with the inclusion of small pieces of fat	Dark red without grey spots and cavities, minced meat evenly mixed
Taste and smell	Characteristic of this type of product with the aroma of spices, moderately salted	The smell is pleasant with the aroma of spices, moderately salty, without foreign taste and smell	Characteristic of this product with the aroma of spices, moderately salty without foreign taste

The results of the study showed that all the sausages were fresh, had a dry, whole surface without damage, deposits of minced meat, fat, mould, or mucus. In all samples on the section, both in the centre and on the periphery, the consistency was elastic and dense, the minced meat looked evenly mixed and without grey spots, the difference was in colour, fat content, and size. Sample 3 differed the moost, it was dark red in colour, which is due to the fact that the composition of this product includes only beef, according to the recipe, the composition of this sausage does not include lard and pork. The other two samples had a colour from dark pink to red with the inclusion of lard, in the sausage Sample 2 pieces of lard were the largest. The smell in all three samples was pleasant with the aroma of spices, in Sample 3 it was more pronounced.

Tasting assessment of experimental sausages was conducted on a 5-point scale. Results of the examination (Fig. 1) indicate that the samples meet the regulatory organoleptic parameters of the standard.

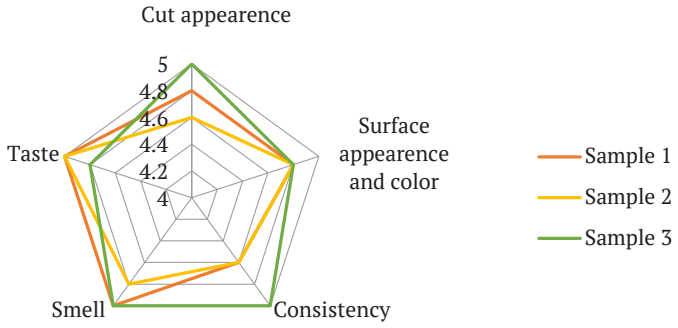

Figure 1. Tasting evaluation of experimental sausages

Sample 3 received the highest scores in terms of smell, cut appearance, and consistency.

The microbiological stability of fermented sausages depends on compliance with all technological operations during production and a number of factors that allow obtaining a high-quality and safe product. According to the results of numerous studies, it is known that the process of maturation of dried sausages is based on the vital activity of milk bacteria. These microorganisms are used as starter cultures that promote meat fermentation (Caplice and Fitzgerald, 1999). Lactic acid bacteria increase the safety and stability of the product, stabilise the colour, prevent rancidity, and release various aromatic substances. During the maturation of the product, they inhibit the development of unwanted harmful microflora (Papamanoli *et al.*, 2003; Francesca *et al.*, 2013). Therefore, a microbiological study was conducted, the results of which are shown in Table 2.

Table 2. *Microbiological quality indicators of dried sausages*

Parameter	Sample 1	Sample 2	Sample 3	Control methods
Pathogenic microorganisms, in particular, bacteria of the *Salmonella* genus	Not detected	Not detected	Not detected	DSTU EN 12824:3004
Escherichia coli bacteria (coliforms) in 0.01 g	Not detected	Not detected	Not detected	DSTU GOST 30726-2002
Sulfitreducing clostridium, in 0.01 g	Not detected	Not detected	Not detected	DSTU ISO 7937:2006
L. monocytogenes, 25.0 g	Not detected	Not detected	Not detected	DSTU ISO 11290-1:2003
S. aureus, d 1.0 g	Not detected	Not detected	Not detected	DSTU ISO 6888-1:2003

According to the results of the study, it was identified that in terms of microbiological indicators, all three samples met the standards of the current regulatory documentation, which is shown in Table 2. The finished product does not contain bacteria of the Escherichia coli group, *Salmonella* genus, sulfitreducing clostridium, L. *Monocytogenes*, or S. *Aureus*. Based on the results of microbiological analysis, it can be argued that the enterprise where the samples of the investigated sausages were made adhere to technological processes at all stages of maturation of dried sausages.

While examining sausages, in addition to microbiological analysis, it was necessary to conduct a physico-chemical analysis of the finished product, which will give an idea of the quality of the product, nutritional value, which depend on the ratio, moisture, fat, protein, salt, and sodium nitrite. The research results are shown in Table 3.

Table 3. *Physical and chemical parameters of dried sausages, %*

Parameter	Sample 1	Sample 2	Sample 3	Control methods
Mass fraction of moisture	27.6±1.13	28.2±0.92	26.9±1.32	DSTU 4427:2005
Mass fraction of table salt	5.76±0.20	5.66±0.21	5.75±0.22	
Mass fraction of sodium nitrite	0.0026 ±0.0003	0.0028 ±0.0002	0.0026 ±0.0003	
Mass fraction of protein	14.0±0.54	16.2±0.37	19.2±0.33	
Mass fraction of fat	29.2±0.44	20.0±0.33	6.4±0.27	

It is known that one of the most important indicators of the quality of readiness of dried sausages is the moisture index, which directly affects organoleptic, microbiological indicators, and, most importantly, the shelf life of the product. This indicator is strictly regulated by the requirements of DSTU 4427:2005 (2006), which is due to its substantial impact on a large number of indicators and, accordingly, on the quality and safety of the product. The moisture content of dried sausages is regulated according to the requirements in the range of 22-30%. If the indicator is below the limit, it will negatively affect the residual amount of beneficial microflora and indicators such as consistency and juiciness (Kapreliants *et al.*, 2015).

Analysis of the results of sausage examination showed that the moisture content in all three samples did not exceed the maximum permissible value and met the regulated requirements of DSTU 4427:2005 (2006). The highest moisture content was in Sample 2 – 28.2%, which is 0.6 and 1.3% higher than in Sample 1 and 2, respectively. All sausages in terms of table salt content met regulatory requirements (DSTU 4427:2005, 2006).

One of the important indicators, the content of which in sausages must be strictly controlled, is sodium nitrite. The properties of this additive do not allow for full replacement of it with food additives and components of natural or microbiological origin. Due to the fact that the exclusion of sodium nitrite from the formulation can lead to microbiological risks and deterioration of product quality, it is allowed in organic production (Oshchypko, 2019; Holck *et al.*, 2017). The assessment showed that the content of this indicator in all samples was within the normal range. In the first and third samples, it was 0.0026%, in the

second – 0.0028%, which meets the requirements of DSTU 4427:2005 (2006).

The biological value of sausage products is characterised by such an indicator as protein content. Analysis of the results showed that in Sample 3, the protein content was the highest and amounted to 19.2%. The high content of this indicator is due to the fact that the composition of this product includes only beef of the highest and first grade. From the literature data and scientific papers, it is known that beef contains more protein than pork, about 18-20%, while pork – 12-17% (Yancheva *et al*., 2021). The lowest protein content was in the first sample, which included pork in addition to beef, it was 14%, which is 2.2 and 5.2% lower, respectively, compared to the second and third samples.

One of the indicators that determine the energy value of the finished product is the fat content. The mass fraction of this indicator largely depends on the recipe and must meet the requirements of DSTU 4427:2005 (2006). As a result the examination, it was identified that the lowest fat content was in the third sample, only 6.4%, which is due to the recipe of this sausage, since it does not include lard and pork. The highest content of this indicator was in the first sample and amounted to 29.2%, which is 9.2 and 22.8% higher compared to the second and third. Analysis of energy value showed that the highest caloric content was identified in sausage Sample 1 – 322 kcal, while in Sample 2 and Sample 3 – 244 and 134 kcal, respectively.

The studies of the state and development of organic production and the organic products market in Ukraine are limited to agriculture. European suggestions of environmental challenges and threats are felt in the public space. Guz M.M. draws attention to the problem of organic farming management (Guz, 2021). Also of concern are possible problems with agricultural exports, which, without strict monitoring of chemical pesticide and antimicrobial residues, could be reduced by 50% by 2030 (Popova *et al*., 2022). Ukraine regularly conducts operational monitoring studies on the areas of agricultural land engaged in organic production (as of 31.12.2021, 422,299 hectares (1% of the total area of agricultural land in Ukraine) (Organic production in Ukraine, 2021), which allows assessing the degree of the development of organic grain, fruit, and vegetable production. The share of imported grain to Europe in 2021 is 47.3%, and fruit – 10.6% (Organic production in Ukraine…, 2022). Modern papers also maintain the tendency to cover the state of organic agricultural production, as evidenced by the papers by N. Novak, O. Davydenko, A. Salnikova, N. Makarenko (Novak, 2020; Davydenko, 2021; Salnikova, Makarenko, 2021). However, if currently Ukraine remains the leader in the production and export of organic grain crops, then there is no information on the production of sausage products, which was one of the motivational reasons for the authors to start the research on this topic.

Conclusions

The results of organoleptic evaluation showed that all samples had a dry, whole surface without damage, deposits of minced meat, fat, mould, or mucus, an elastic, dense consistency, evenly mixed minced meat without grey spots. Differences in smell, colour, fat content, and size were identified: Sample 3 had a more intense dark red colour and the most pronounced spice flavor; Sample 2 differed in the largest pieces of lard.

After conducting microbiological studies, no Escherichia coli bacteria, *Salmonella* genus, sulfitreducing clostridium, L. *Monocytogenes*, or S. *Aureus* were detected in these samples. Therefore, it can be concluded that the sausages of the highest grade under study met microbiological safety indicators since they did not contain pathogenic microflora. Analysis of the energy value identified that the most caloric was Sample 1, which had the highest mass fraction of fat – 29.2%. Sample 3 was identified as diet, it had the highest protein content (19.2%) and the lowest fat content (only 6.2%).

According to the results of the tasting assessment, Sample 3 received the highest scores for most indicators (smell, appearance on the cut, and consistency), but Sample 2 was the best in terms of taste properties. The results of the study confirm the feasibility of introducing organic sausage production to meet the demand formed by a wide range of consumers with different nutritional needs and individual preferences, such as diet, taste, appearance, etc., which contributes to the successful sale of products on the internal market. The possibility of organising organic production of dried sausages in Ukraine with a wide range of products that meet the quality and safety requirements, which allows entering foreign markets, was identified. The necessity of developing organic production of the Ukrainian food complex was highlighted.

The prospect of further research is to determine the quality and nutritional value of other types of organic products in Ukraine.

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Оцінка якості та харчової цінності органічних сиров'ялених ковбас

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Анотація. Все більшої актуальності у розвинених країнах, зокрема Україні, набуває органічне виробництво, яке сфокусоване на виготовлення якісних, з високою харчовою цінністю та екологічно безпечних, продуктів харчування. Метою досліджень є визначення показників якості та харчової цінності органічних сиров'ялених ковбас. Для її реалізації використано фізико-хімічні, мікробіологічні, органолептичні, статистичні методи досліджень та підходи абстракції, синтезу, аналізу, систематизації та узагальнення даних. Для проведення дослідження відібрано три види ковбас: «Зразок 1», «Зразок 2», «Зразок 3». У результаті досліджень було встановлено, що всі показники досліджуваних зразків контролюються та відповідають вимогам нормативних документів. Під час органолептичної оцінки встановлено, що всі досліджувані зразки мали суху без пошкоджень поверхню, пружну консистенцію, приємний запах з ароматом прянощів. Різниця спостерігалась тільки за кольором, він був від темно рожевого до темно червоного, що зумовлено різною рецептурою ковбас. Мікробіологічний аналіз виробів показав, що у всіх зразках була відсутня патогенна мікрофлора. За результатами фізико-хімічного аналізу встановлено, що вміст вологи, солі та нітриту натрію були в межах норми. Різниця спостерігалась за вмістом білку та жиру. У першому зразку ковбаси виявлено найнижчий вміст білку (14 %), тоді як другому та третьому – відповідно 16,2 % та 19,2 %. Значні відмінності спостерігались і за вмістом жиру в готовому продукті. Найнижчий рівень цього показника був у зразку 3 і становив 6,4 %, що на 22,8 % та 13,6 % нижче порівняно зі Зразком 1 та Зразком 2. Така різниця за вмістом білку зумовлена різним складом сировини досліджуваних зразків. Також встановлено, що найнижча калорійність (134 ккал) була у ковбасі Зразку 3, яка мала найвищий рівень білка та найнижчий вміст жиру. Отримані результати практично дають можливість оцінити дієтичність досліджуваного асортименту і зорієнтувати споживача у виборі продукту відповідно до його індивідуальних вподобань і потреб, особливостей і характеру дієти

Ключові слова: ферментація ковбас, мікробіологічні показники, масова частка білку, масова частка жиру, нітрит натрію, органолептична оцінка, патогенні мікроорганізми

ТВАРИННИЦТВО ТА ТЕХНОЛОГІЇ ХАРЧОВИХ ПРОДУКТІВ

Науковий журнал

Том 13, № 4. 2022

Заснований у 2010 р. Виходить чотири рази на рік

Оригінал-макет видання виготовлено у відділі науково-технічної інформації
Національного університету біоресурсів і природокористування України

Відповідальний редактор:
Д. Кокра

Редагування англомовних текстів:
С. Воровський, К. Касьянов

Комп'ютерна верстка:
М. Кришталь

Підписано до друку 23 листопада 2022 р. Формат 60*84/8
Умов. друк. арк. 10,2
Наклад 50 прим.

Адреса видавництва:
Національний університет біоресурсів і природокористування України
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ANIMAL SCIENCE AND FOOD TECHNOLOGY

Scientific Journal

Volume 13, No. 4. 2022

Founded in 2010. Published four times per year

The original layout of the publication is made in the Department of Scientific and Technical Information of National University of Life and Environmental Sciences of Ukraine

Managing Editor:
D. Kokra

Editing English-Language Texts:
S. Vorovsky, K. Kasianov

Desktop Publishing:
M. Kryshtal

Signed for print of November 23, 2022. Format 60*84/8
Conventional printed pages 10.2
Circulation 50 copies

Editors Office Address:
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https://animalscience.com.ua/en