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03041, вул. Героїв Оборони, 15, м. Київ, Україна
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Editors office address:

National University of Life and Environmental Sciences of Ukraine
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Preliminary assessment of the safety of genetically modified food products

Yuriy Balji*

PhD in Veterinary Sciences

S. Seifullin Kazakh Agro Technical Research University

010000, 14-a Kerei i Zhanibek Handar Str., Astana, Kazakhstan

<https://orcid.org/0000-0002-5006-3224>

Abstract. Numerous studies on genetically modified food products indicate their safety and their potential to alleviate hunger worldwide. However, the issue of GM products and feeds remains relevant in the realm of food security. The purpose of this study is to present a comprehensive scheme of methods for determining the safety of GM food products and to develop a new method for assessing the safety of GM food products. This new method is based on the use of a biotest and the employment of *Paramecium caudatum infusoria*. In the study, GTS 40-3 and GTS 40-3-2 (Venus variety) soybeans, MON 00603 (NK 603) maize line, and non-GMO soybeans and maize were used as controls. Infusoria were prepared by culturing them in room temperature water (18-23°C) without direct sunlight, with the addition of non-GMO yeast powder as a nutrient solution and senna extract. Subsequently, the initial material containing approximately 100 infusoria was transferred to 100 ml flasks and incubated for 2-3 days. At the initial stage of the experiment, from day 1 to day 15, daily records and visual assessments were conducted. The assessment included the quantity of infusoria, motility (chemotaxis), size, shape, and the percentage of dead infusoria. Venus soybeans (GTS 40-3-2) exhibited a toxic effect on the protozoa, which died in repeated trials on the 14th-15th day of feeding. It was observed that infusoria fed with soybeans containing the GTS 40-3-2 (Venus) transformation event changed their shape after 3 days from the start of the experiment. Over the course of 10 days from the beginning of the study, a decrease in movement, motility, and the quantity of infusoria was noted. On the 14th-15th day, mortality was observed. Negative changes were also observed in infusoria that were fed with MON 00603 (NK 603) maize. The application of this method in the practice of assessing the safety of GM food products can provide a preliminary evaluation of long-term effects over a relatively short period

Keywords: feeds; biotest; infusoria; toxicity; long-term effects

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*Corresponding author



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Introduction

Nowadays, food and feed safety issues receive growing attention. Whether transgenic products which were created to ensure food and feed security are safe is a particularly sensitive issue. The issue of simplifying safety controls for products derived from genetically modified animals is currently being raised (Fan *et al.*, 2022). In a 90-day feeding study of phytase-transgenic maize 11TPY050, authors found no unexpected adverse effects in SD rats. Y. Li *et al.* (2021) investigated the effects of genetically modified (GM) rice BPL9K-4 in rats and found no toxicity.

Accumulating evidence points to potential and actual biological risks associated with the commercial use of GM plants. The American organisation “Center for Food Safety” (CFS) (n.d.) has highlighted six key negative impacts of genetic modifications that could affect consumer health. These include concerns related to toxicity, allergic reactions, antibiotic resistance, immune system suppression, potential cancer risks, and inadequate nutrient absorption. While researchers continue to debate the risks associated with consuming GM foods, many ordinary consumers in certain countries unwittingly participate in a “global experiment” by including GM products in their diets over several years. The long-term consequences of this experiment may only become apparent in future generations, possibly 200-300 years from now. In contrast, in many Asian countries like Kazakhstan, consumers do not have the luxury of choice because food market sellers do not disclose whether products are genetically modified or not.

Currently, the examination of GM-containing foods is conducted in several areas: medical and genetic evaluation (study of the claimed gene embedded at the molecular and cellular level and its impact on the plant and other objects); technology assessment (study of sensory, consumer, and other properties); medical and biological assessment, the results of which

are in clinical trials, issued as an opinion on the quality and safety of GM products (Ahmad *et al.*, 2021).

A stepwise safety and quality assessment of genetically modified sources is also implemented. At the core of this approach is the principle of comparative or real equivalence, which entails comparing GM foods with their conventional counterparts. This involves analysing the chemical composition of the product and comparing it to the conventional version, assessing basic nutrients, anti-nutritive and toxic substances, allergens specific to the food type, and examining the properties of the transferred genes. If the compositional equivalence evaluation reveals no differences between the GM food and the conventional counterpart, it is classified as first-class safety, indicating it is considered entirely safe for consumer health. In cases where differences are detected (second safety class) or there is a complete mismatch with conventional counterparts (third safety class), the safety assessment of genetically modified food should be further investigated. Stages of food safety research include the study of nutritional and toxicological characteristics of the product (Kopko *et al.*, 2022).

Evaluation of the properties of food involves the study of nutritional value of the new product; consumption rates; methods of use in nutrition; bioavailability; the introduction of specific nutrients (especially if the expected nutrient intake exceeds 15% of the daily requirement); impact on the intestinal microflora (if the genetically modified source contains living organisms). Toxicological characterisation involves determining the following indicators: toxicokinetics; genotoxicity; the potential allergenicity; potential colonisation in the gastrointestinal tract (in the presence of genetically modified live microorganisms as a source); the results of subchronic (90-day) toxicological experiments on laboratory animals and studies on human volunteers.

Such a quality assessment system and safety evaluation of GM food sources, which is based on the principle of compositional equivalence, can be recommended for products devoid of proteins and DNA (Wang *et al.*, 2022). The development of control methods and effective protocols, including the detection of new foreign proteins in raw materials and finished products that may trigger allergies, is a significant concern for improving product quality and, most importantly, ensuring human health safety (Kopko *et al.*, 2022). One solution to this issue involves the development of new methods for assessing food safety, including the study of long-term effects on dozens of generations of model organisms.

The application of biological test methods for assessing substance safety is grounded in the fact that living organisms require specific chemical compositions for their life, growth, reproduction, and functioning. Any alterations in this composition lead to immediate or delayed responses in test organisms, such as changes in behaviour (movement), morphological characteristics, reproductive function, or even death. B.S. Bandarra *et al.* (2023) employed biological test subjects to assess the ecotoxicity of industrial waste in their studies. A. Mehl & G.E. Morlock (2023) also proposed the use of bioassays to detect antibiotic residues in food, as this method can identify compounds that may remain undetected by other modern techniques like HPLC-MS.

The aforementioned studies support the feasibility of using protozoa as bioassays to assess the overall toxicity of food. In this regard, patents for inventions were developed (Balji & Adilbekov, 2022). The purpose of this study is to explore the potential of conducting preliminary safety assessments of GM products using model bioassays involving *Paramecium caudatum* infusoria.

Materials and Methods

The analyses were conducted at two different laboratories: the Food Safety Laboratory

within the Veterinary Sanitation Department of Kazakh Agro-Technical University, named after S. Seifullin, and the Laboratory of Food Product Analysis at the Republican State Enterprise (RSE) under the Right of Economic Management (REM) called the “National Reference Center for Veterinary Medicine”, affiliated with the Ministry of Agriculture of The Republic of Kazakhstan. The experimental studies utilised soybeans of the GTS 40-3 and GTS 40-3-2 (Venus variety) varieties, maize from the MON 00603 (NK 603) line, and non-genetically modified (GM) soy and maize as control samples. Real-time PCR was employed to analyse the test samples.

The analysis involved the determination of contaminants of anthropogenic and biogenic origin in both GM and non-modified samples. The concentration of toxic elements such as arsenic, cadmium, lead, and mercury was determined through Foodstuffs’ Determination of Trace Elements using Inductively Coupled Plasma Mass Spectrometry (ICPMS) after pressure digestion, following the BS EN 15763:2009 (2009) standard. Sample preparation adhered to the All-Union standard 31671:2012 (2012), titled “Food Products: Determination of Trace Elements with Sample Preparation Involving Mineralization at Elevated Pressure”. Detection was conducted using the Agilent Technologies 7700 ICPMS with inductively coupled plasma mass spectrometry.

For the determination of aflatoxin B1 concentration in fodder, an enzyme-linked immunosorbent assay (ELISA) was employed, utilising the CELER AFLA B1 kit (Tecna R & D Diagnostics Biotechnology, Italy). Nitrates were detected using the potentiometric method as specified in the All-Union Standard 13496:19-93 (1997) titled “Fodder, Mixed Fodder, Animal Feed Raw Material: Methods for Determination of Nitrates and Nitrites”. Total radioactivity (α , β , γ -rays) was assessed using the MKS-151 radiometer-dosimeter. Initial preparation of infusoria involved cultivating them in settled

water at room temperature (18-23°C), shielded from direct sunlight, with the addition of yeast powder (non-GM) as a nutrient solution and hay extract. After approximately 7-10 days, the concentration of paramecium reached approximately 10,000 u/ml. Following two weeks, starting material was extracted from the infusoria, consisting of around 100 infusoria in a 10 ml volume, and transferred to flasks containing 100 ml of water for 2-3 days. Subsequently, daily records and visual assessments were carried out from the 1st to the 15th day, focusing on the following parameters:

1. The amount of test objects.
2. The motility (chemotaxis) of test objects.
3. The size of test objects.
4. The form of the test objects.
5. The percentage of test objects death.
6. Reproduction (reproductive function) of test objects.

Transitions were observed over a period of 6 months, twice a week. The foliar application was conducted with the GM soybean, maize and soy; as a control with soy and maize which do not contain genetic alterations 2 times per week.

The experimental setup was organized into groups, with flasks containing bioassay objects as follows:

Flask 1 – Control. Infusoria were fed with non-genetically modified soybeans.

Flask 2 – Control. Infusoria were fed with non-genetically modified maize.

Flask 3 – Infusoria were fed with GM soy from transformation event GTS 40-3.

Flask 4 – Infusoria were fed with GM soy from transformation event GTS 40-3-2 (*Venus grade*).

Flask 5 – Infusoria were fed with GM maize from the MON 00603 (NK 603) line.

The feed for infusoria was provided daily in the form of powder, with a quantity of 150 mg per flask.

Results are presented as the mean observations per flask. All analyses were performed in duplicate. Due to the limited number of

samples, no statistical analyses were performed (Customs Union Commission..., 2010).

Results and Discussion

Initially, both GM soybean, maize, and control samples were examined for the presence or absence of contaminants of biogenic and technogenic origin, such as toxic elements, aflatoxin B1, pesticides, and nitrates, which could potentially affect the test organisms. Background radiation levels were also determined for all samples, and they did not exceed the natural background radiation, measuring no more than 0.14 microns per hour.

Bioassay objects, *Paramecium caudatum*, were exposed to the investigated GM product samples; for control in a parallel experiment, similar soy and maize samples without GM modifications were used as controls. The long-term effects on the infusoria, spanning 5-10 generations or more, were visually assessed over a relatively short period ranging from 15 days to 6 months. A notable factor favouring the selection of these bioassays is that the culture of *Paramecium caudatum* can be utilised for extended periods due to their periodically changing reproductive pathways (sexual reproduction and fission). Certain changes that occur with the test bioassays were established. Morphological alterations result in distinct shapes and sizes compared to the control groups.

Specifically, infusoria fed with soybeans containing the transformational event GTS 40-3-2 (*Venus*) exhibited changes in their shape after 3 days from the start of the experiment. These experimental infusoria became more rounded in contrast to the control group, as depicted in Figure 1. Within 10 days of the experiment in this group, a slow movement of infusoria was observed, along with reduced motor activity and population size. On the 14th to 15th day of the experiment, infusoria mortality was observed. Similar results were obtained in duplicate.

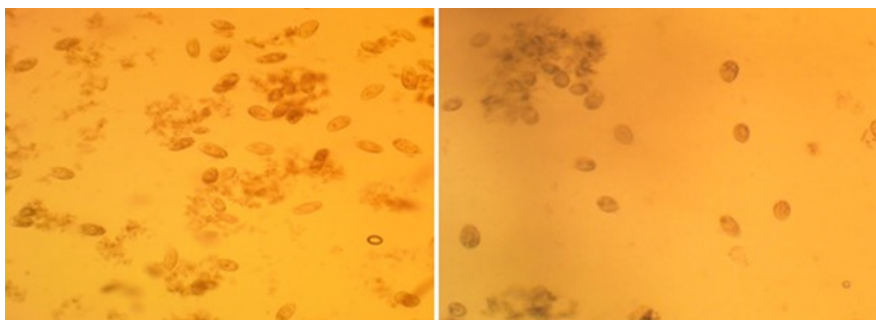


Figure 1. Changes in the shape of infusoria after feeding non-GM soy (left panel) and GM soy (right panel)

Source: author's development

Maize from the MON 00603 (NK 603) line induced the following changes in infusoria. After 3 days from the start of the experiment, infusoria slightly increased in size and took on an oval shape.

Subsequently, every 7 days, their population decreased. After 60 days of the experiment, slow movement of protozoa was observed, and after 4 months, mortality occurred in all bioassays (Fig. 2).

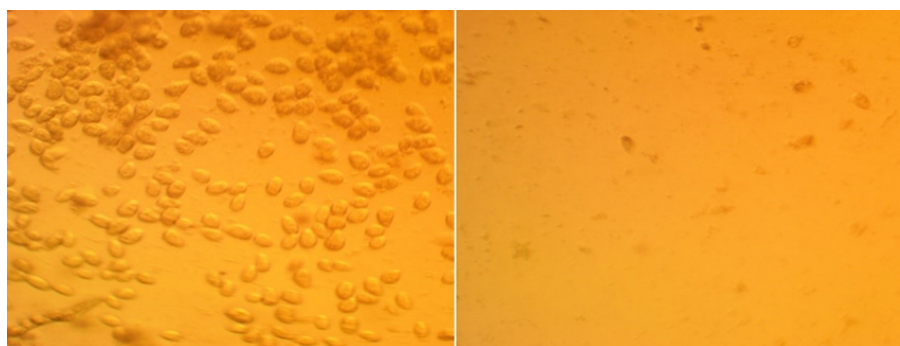


Figure 2. Changes in the shape of infusoria after feeding with GM maize (right panel) at the beginning of the experiment (left panel) and at the end of the experiment (right panel)

Source: author's development

In the control groups, no morphological changes were observed over the course of 6 months. In the control group, there was positive chemotaxis of infusoria, and even after 4 months of observation, the number of protozoa decreased to one-third. However, after 6 months from the start of feeding, no complete deaths of infusoria were observed in the control groups.

The study also examined the long-term impact on the reproductive function of the

protozoa. However, it remained unclear from this study whether infusoria fed GM soybean and maize had altered the morphological characteristics and lifespan of the protozoa. It may be assumed that different genetically modified events in the studied cultures have varying effects on the lifespan of *Paramecium caudatum* protozoa. In the control group, where infusoria were fed non-GM soy and maize, the duration of reproductive function extended beyond 6

months, although the population decreased in both GM-fed and non-GM-fed groups. Nevertheless, complete infusoria death was not observed in the control groups. The experimental studies were concluded after 6 months, as the protozoa in the control groups ceased to exist after 15 days (GTS 40-3-2 Venus) or after 4 months of initial GM feeding.

Various biological organisms are employed to assess the safety of GM foods, with experiments commonly conducted on rats and mice. For instance, a 90-day subchronic feeding study assessed the food safety of stacked trait GM maize GH5112E-117C, which contains insect-resistance gene Cry1Ah and glyphosate-resistant gene G2-aroA, in comparison to non-GM Hi-II maize fed to Sprague-Dawley rats. The study used three different dietary concentrations (12.5%, 25%, and 50% w/w) of GM maize or its non-GM counterpart. The results indicated no biologically significant differences in clinical outcomes, body weights, food consumption, haematology, clinical chemistry, organ weights, and histopathology between the stacked trait GM maize groups and the non-GM maize groups. The results of the 90-day subchronic feeding study demonstrated that the stacked trait GM maize GH5112E-117C is as safe as the conventional non-GM maize Hi-II. Safety tests deemed the GM variety to be “substantially equivalent” to conventional soybeans. Feeding rats diets containing genetically modified or conventional (native) maize did not affect their final body weight or growth. These findings are confirmed by the results obtained by I. Kosieradzka *et al.* (2008).

A 49-day feeding study was conducted to assess the impact of genetically modified (GM) maize strain C0030.3.5 on Japanese quails (*Coturnix japonica*), focusing on body performance and egg quality. Furthermore, the bodily fats of transgenic proteins in the Japanese quails were investigated. The findings indicated that male and female quails that consumed GM diets exhibited normal parameters in terms of

body weight, haematology, serum chemistry, relative organ weight, and histopathological appearance, and no discernible differences in laying performance or the nutrient composition of eggs between the groups with varying diets (Zhang *et al.*, 2021).

Y.-Ch. Wang *et al.* (2023) delved into the impact of GM-Lac on juvenile Asian seabass. This study aimed to explore the potential of a combination of lactic acid bacteria (LAB), specifically *Lactobacillus* and *Bifidobacterium*, as a probiotic supplement in the field of aquaculture. In summary, GM-Lac, which includes four LAB strains (GMNL-93, -141, -277, and -550), was proposed as a natural antimicrobial agent suitable for prophylactic use and as a probiotic feed supplement in fish aquaculture practice for enhancing growth performance and intestinal health, reducing the host susceptibility to pathogenic infection, and lowering the food conversion ratio (FCR). Moreover, the rise in muscle amino acid levels was regarded as a promising advantage offered by GM-Lac in enhancing the taste of freshwater-cultured fish. These studies confirm the effective use of GM microorganisms in aquaculture. There is a widespread scientific consensus regarding the safety of approved foods and feed products derived from genetically modified (GM) plants, fish, livestock, poultry, and animals fed GM diets. This consensus is based on assessments by regulatory agencies, independent scientists, and scientific organisations from various countries (Vergolyas & Goncharuk, 2016; Blair & Regenstein, 2020).

Within the available literature, there is substantial data supporting the safe utilisation of GM products. There is a lot of information about the beneficial properties of genetically modified foods, for example, that GM crops are more productive than conventional crops, they reduce the use of toxic chemicals, pathogen infestation, the need for production costs, have a longer shelf life of perishable products, etc. (Udovic *et al.*, 2013; Ahmad *et al.*, 2021). In addition, GM crops can contribute to increased

biodiversity by incorporating new traits into field crops, expanding the range of germplasm used in cultivation (Schulman, 2020). In general, the literature presented shows the enormous economic, environmental, and health benefits of GM crops. However, there is evidence stating that most of the studies on the safety of GM products are methodologically incorrect and unreliable. Of the 37 identified adverse events associated with GM consumption, approximately 59.46% were categorised as serious. These serious adverse events encompassed risks such as mortality, tumour development or cancer, a significantly reduced number of pup deliveries, diminished learning and reaction capabilities, and structural abnormalities in organs, including the stomach, intestinal adenoma, mammary glands, pituitary, liver, and kidney. The interventions or exposures in the studies related to adverse events predominantly focused on GM soybeans, maize, and rice, specifically in the context of certain GM events.

In some developing countries, there is no legal framework regulating the control of the safety of GM products, their circulation and use. Study by S.S. Al Mazrooei & D.R. Alreshidi (2023) introduced and improved a PCR-screening system for detecting GMO regulatory elements p35S and Tnos. This system uses a modified in-house DNA extraction method and appropriate control samples. This screening is seen as a prerequisite for the regulation of GMOs in biotech-derived products. Their findings revealed the presence of GMOs in food and feed products available in the Kuwaiti market for both human and animal consumption. Animal studies are at the lowest hierarchy of evidence, and there are flaws in study design making the results not convincing. The evidence on the effect of GM consumption on humans is still insufficient. Research results indicated that in European countries, over 62.5% of the population holds a negative attitude toward the use of GM products, while in North America, Latin America, and Asia, this sentiment is below 50%.

Further clinical trials and cohort studies focusing on the effects of GM food consumption in human populations are deemed necessary. It is essential to thoroughly investigate safety before approving GM products for food consumption. It also suggests the necessity of labelling GM food so that consumers can make their own choices (Shen *et al.*, 2022).

Using ciliates like *Paramecium caudatum* as test subjects for assessing the safety of GM foods offers several advantages over traditional subjects like rats, mice, quails, or earthworms:

1. **Simplicity and availability:** Ciliates are single-celled organisms with a straightforward structure, making them easy to culture and examine in a laboratory. Their short life cycle allows for quicker experiments and faster results.

2. **Sensitivity to toxic substances:** Ciliates are highly responsive to environmental changes, including the presence of toxic substances. This sensitivity makes them valuable as indicators or biomarkers for evaluating the potential toxicity of GM foods and other substances.

3. **Cost efficiency:** Ciliates are much smaller and require fewer resources (food, space, etc.) to keep compared to larger animals such as rats or quails. This cost-effectiveness makes them a practical choice for large-scale safety studies of GM products.

4. **Ethical considerations:** Using ciliates as test subjects avoids ethical concerns associated with more complex animals like rats or mice, which may require special care and protection.

However, it should be noted that the use of ciliates has some limitations. Their simple structure might restrict the study of complex biological processes or higher-level effects on organisms. In addition, research results from ciliates may not always directly apply to human health or the environment.

In general, the use of infusoria instead of rats, mice, quails, or earthworms can be useful for simple and cost-effective experiments to determine the safety of GM products, but requires additional research and evaluation in the

context of the specific goals and requirements of the study. The increased utilisation of bioassay methods through the use of simple organisms has long been a goal in the food industry for assessing the overall toxicity of food products and their impact on reproductive properties over multiple generations. The subjects of control and monitoring in food processing facilities are complex due to their variable nature, characterised by numerous multi-ingredients that can undergo changes in their properties under the influence of external factors. As chemical and biochemical reactions progress, the formation of new compounds with potentially harmful biological activity can occur, which may not be easily detectable using modern analytical equipment. Altering the genetic structure of a product can also lead to the emergence of new properties that may not be favourable for reproductive functions within the body.

One of the ways to solve this problem is to use biological methods, based on the fact that for the life, growth, reproduction, and functioning of living beings, there is a need for an appropriate environment with a strictly defined chemical composition. When altering the composition, such as by introducing an additional (specifically defined) compound or substance, the test organism typically provides a corresponding response over time, and sometimes, this response occurs almost immediately. Protozoa are single-celled organisms that possess all the essential functions of life, including metabolism, irritability, movement, reproduction, and more. As a result, they closely simulate the effects of chemical or complex biological components within ecosystems. The ultimate indicator of the effectiveness of an introduced substance on these organisms is their survival or death. Initially, researchers examined behavioural responses in infusoria, which represent one of the simplest types of reactions. Later, reproductive function in several generations was also studied, i.e., the ability to breed and maintain its population.

In the future, it is important to explore the impact of various GM products on protozoa, particularly assessing their reproductive capabilities over 7-10 generations. The results obtained do not show the dramatic negative impact of GM foods on the studied protozoa (except for GTS 40-3-2 Venus soy). When provided with a uniform diet, infusoria gradually decrease their active lifespan and reproductive function over time. Although vital functions of infusoria in experimental groups fed GM soy and maize ceased after 4 months, whereas in control groups, this occurred after more than 6 months, it may suggest a potential delayed impact of GM foods on protozoa. However, the author indicates that, when using a safety assessment of GM foods on the protozoa bioassay, it is needed to consider the results in the first 1-2 months. If no adverse effects are observed during this initial period, it may be inferred that the tested GM sample is safe at that time. The application of this developed rapid method for determining the safety of GM products through bioassays will empower food safety laboratories to expedite studies on GM products while providing a preliminary assessment of their long-term effects without the need for analytical equipment or laboratory animals.

Conclusions

Nearly all of the available results that negatively characterise GM foods have been disproven in scientific literature, often because researchers had flawed experimental designs. An additional alternative method for determining the safety of GM foods without the use of animals, including the impact on several generations of protozoa in a relatively short period of time was proposed. A new method based on the application of bioassay and *Paramecium caudatum* infusoria was developed in this study. Soybeans GTS 40-3 and GTS 40-3-2 (Venus variety) and maize line MON 00603 (NK 603) were tested as a feed for infusoria and showed some adverse effects. Those effects included changes in the shape,

reduced movement, reduced motor activity, and total number of infusoria. Feeding the infusoria with GM diets led to the death of the organisms within 15 days in the case of soybeans GTS 40-3-2 Venus and after 4 months with maize line MON 00603. These outcomes underscore the critical significance of safety testing for GM foods and feeds.

This method can be applied as a preliminary assessment of GM foods safety. Further, more comprehensive studies can be conducted based on the results from the method based on the application of bioassay and *Paramecium caudatum* infusoria. An advantage of this proposed method is its ability to conduct preliminary assessments of GM food safety across numerous generations of biological test subjects within a relatively short time frame and without incurring high costs. Future research should extend to higher animals and humans, notably human observational cohort studies, to mitigate any potential health risks for the

broader population. Furthermore, to address public concerns, information should be available in easily accessible and understandable formats.

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Conflict of Interest

None.

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Попередня оцінка безпеки генетично модифікованих харчових продуктів

Юрій Балджи

Кандидат ветеринарних наук

Казахський агротехнічний науково-дослідний університет імені С. Сейфулліна
010000, вул. Керей і Жанібек Гандар, 14-а, м. Астана, Республіка Казахстан
<https://orcid.org/0000-0002-5006-3224>

Анотація. Численні дослідження генетично модифікованих продуктів харчування показують, що вони безпечні та можуть захистити людей у всьому світі від голоду. Проте проблема ГМ-продуктів і кормів продовжує залишатися актуальною у сфері харчової безпеки. Метою даного дослідження було представлення узагальненої схеми методів визначення безпечності ГМ харчових продуктів та розробка нового методу визначення безпечності ГМ харчових продуктів. Цей новий метод заснований на застосуванні біотесту та використанні інфузорії *Paramecium caudatum*. У дослідженні використовували соєві боби GTS 40-3 і GTS 40-3-2 (сорт Венус), лінію кукурудзи MON 00603 (NK 603) і без ГМО сою та кукурудзу як контроль. Інфузорії готували культивуванням у воді кімнатної температури (18-23 °C) без прямих сонячних променів з додаванням дріжджового порошку (не ГМ) як поживного розчину та екстракту сени. Потім вихідний матеріал, що містив близько 100 інфузорій, переносили у колби з водою об'ємом 100 мл і витримували 2-3 доби. Далі на початковому етапі експерименту з 1-ї по 15-ту добу проводили щоденні записи та візуальні оцінки. Оцінка включала кількість інфузорій, рухливість (хемотаксис), розмір, форму та відсоток мертвих інфузорій. Соя сорту Венера (ГТС 40-3-2) виявила токсичну дію на найпростіші, які гинули в повторних дослідах на 14-15 добу після початку згодовування. Було помічено, що інфузорії, згодовані соєвими бобами, що містять трансформаційну подію GTS 40-3-2 (Venus), змінили форму через 3 дні від початку експерименту. Протягом 10 діб від початку досліду спостерігалось зниження руху, рухової активності та кількості інфузорій. На 14-15 добу досліду спостерігали загибель. В інфузоріях, яким підживлювали кукурудзу лінії MON 00603 (NK 603), також спостерігалися негативні зміни. Застосування цього методу в практиці оцінки безпеки ГМ харчових продуктів може дати попередню оцінку віддалених ефектів за відносно короткий час

Ключові слова: корми; біопроба; інфузорії; токсичність; віддалені ефекти



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Proteolytic processes in cheese analogues ripening

Svitlana Danylenko^{*}

Doctor of Technical Sciences, Senior Researcher

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroyiv Oborony Str., Kyiv, Ukraine

Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine

02002, 4a Yevhena Sverstiuka Str., Kyiv, Ukraine

<https://orcid.org/0000-0003-4470-4643>

Larysa Bal-Prylypko

Doctor of Technical Sciences, Professor

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroyiv Oborony Str., Kyiv, Ukraine

<https://orcid.org/0000-0002-9489-8610>

Taisia Ryzhkova

Doctor of Technical Sciences, Professor

State Biotechnological University

62341, 1 Akademichna Str., Mala Danylivka, Ukraine

<https://orcid.org/0000-0002-1029-8838>

Sergii Verbytskyi

PhD in Technical Sciences

Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine

02002, 4a Yevhena Sverstiuka Str., Kyiv, Ukraine

<https://orcid.org/0000-0002-4211-3789>

Oleksandr Panasiuk

PhD Student

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroyiv Oborony Str., Kyiv, Ukraine

<https://orcid.org/0000-0001-6439-6604>

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^{*}Corresponding author



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Abstract. The research relevance is predefined by the theoretic basics of their production processes not being sufficiently studied, including the proteolysis despite the wide production of cheese analogues incorporating vegetable substances. The research aims to determine the effect of the content of cheese analogues, namely milk fat substitutes, soy protein isolates, and lactobacilli species *Lactococcus lactis ssp. lactis* and *L. lactis ssp. cremoris*, *L. lactis ssp. lactis* *bv. diacetylactis* as well as *Leuconostoc mesenteroides* by the physical, chemical, and sensorial traits, and by amino acids content of cheese analogues. Determination of the physical and chemical parameters was fulfilled according to the standards: active acidity (pH) – DSTU 8550:2015; solids content (by weight) – DSTU 8552:2015 and ISO 5534:2004; non-casein nitrogen content (by weight) – ISO 17997-1:2004; total protein (by weight) – ISO 8968-1:2014 and ISO 8968-5:2001; casein content (by weight) – ISO 17997-1:2004. The fractional composition of proteins was determined using the Polyacrylamide Gel Electrophoresis. While in the cheese dough after analysing the differences in the spectrum of free amino acids between the variants were insignificant, in ripe cheese analogues the accumulation was significantly less than in the control cheeses, only one ripe cheese analogue showed the total amount of free amino acids and was almost equal to the control. During ripening, the content of these compounds increased in cheese analogues from 2.2 times to 3.3 times, in the control the content of free amino acids increased by 2.5 times, and individual free amino acids accumulated from 3-7 to 30-40 times. Active proteolytic processes led to the appearance of all ripe products of such essential amino acids as methionine and isoleucine, there was also an increase in the level of glycine. The practical significance of the study is determined by substantiating rationale proteolytic processes in the manufacture of cheese analogues to ensure consumer quality close to the quality of cheeses made of milk

Keywords dairy industry; dairy products; milk fat substitute; physical and chemical properties; amino acids

Introduction

G. Mangia *et al.* (2022) state that milk and dairy products are of inevitable necessity for human nutrition the said products provide numerous advantages for the health of consumers. Medicine studies, for example, S.M. Vanderhout *et al.* (2020), postulate that milk product help minimizes the probability of obesity, low bone weight, heart attacks and several cancer types. Cheese occupies a special place among dairy products. It is a protein-fat concentrate that retains its properties for several months and even years. According to an evaluation made by L. Lebid (2021), on average, Ukrainian consumes 3.5-4 kg of hard cheeses per year which is significantly less than in the EU, where the average yearly consumption of cheese is 20 kg pro capita. It is predicted that world cheese

production will reach 27 million tons by the end of 2030. R. Kamath *et al.* (2022) state that many enterprises in the dairy industry, along with the traditional assortment, produce analogue products with a combined composition. An analogue cheese can be defined as a cheese-like product obtained by partially or completely replacing components such as milk, milk fat or milk protein, and incorporating vegetable substances, as well as additives such as emulsifying salts, hydrocolloids, preservatives, acidifiers and sometimes flavours (sodium chloride, cottage cheese flavour, etc.) (Dairy Industries, 2020).

According to B.N. Esen *et al.* (2020), cheese-like products (analogue/imitation cheeses) are generally defined as food products produced from a mixture of non-milk fats, milk proteins

or vegetable proteins in certain proportions. Cheese-like products can be categorized as milk-based, partially milk-based, and non-dairy-based. The category of a cheese-like product depends on the source of the protein and/or fat content. The part that uses vegetable protein and vegetable oil in its composition is expressed as milk-based or non-milk-based. Following R. Kamath *et al.* (2022) soy is a highly nutritious food material containing well-balanced amino acids and desirable fatty acids. It plays an important role as a source of protein for many people around the world. In addition, it is necessary to note that the cost of producing cheese analogues may be less than products obtained only from animal proteins. Various food formulations include soy proteins for various purposes, usually related to health benefits or used soy for dairy fortification to alleviate milk availability problems.

A. Pua *et al.* (2022) consider that the sensorial properties of the analogues made based on the technological procedures similar to the real products made of milk are not satisfactory as the analogues produce specific off-flavours while their texture was also significantly different. A special processing technique is necessary to prepare plant-based ingredients for the fermentation to be done. To make sensorial parameters, including texture, the plant ingredients of analogue milk products undergo bacterial, yeast or fungal fermentation. R.M. Caldeira

et al. (2022) studied the sensorial properties of analogue ice cream made with olive oil. Aroma, flavour, and several other properties appeared to correspond to the milk-based ice cream.

According to C.M. Galanakis (2021), the use of high-quality milk fat substitutes in combination with valuable nutritional supplements makes it possible to regulate the composition of the product, and therefore its properties, obtaining products with a balanced fatty acid composition that meets the principles of healthy nutrition. Competently balanced cheese analogues allow, in addition to expanding the range, to obtain products of improved quality in terms of nutritional value, shelf life and sensorial perception.

The above considerations predefine the research aim, which is to analyse the effect of the content of cheese analogues namely milk fat substitutes, soy protein isolates and lactobacilli species upon the physical, chemical, and sensorial traits, and amino acids content of these products.

Materials and Methods

The principal idea while planning the research its results being presented herewith was the closest possible simulation of the traditional manufacturing process of hard cheeses but with the above-mentioned substitution of raw materials. The objects of research are specified in Table 1.

Table 1. Composition and characteristic traits of the objects of research

Object of research	Composition and characteristic traits
Fermenting bacterial concentrate for hard rennet cheese with a low temperature of the second heating (FBC)	Contains cultures of lactobacilli species <i>Lactococcus lactis ssp. lactis</i> and <i>L. lactis ssp. cremoris</i> , which provided the necessary level of acid formation, as well as aroma-forming species <i>L. lactis ssp. lactis</i> <i>bv. diacetylactis</i> and <i>Leuconostoc mesenteroides</i> ; soy protein isolate (protein content – 90%, odourless, neutral taste, no dietary fibre)
MFS1	Melting point – 32-34°C, hardness according to Kaminsky - 140-180 g/cm
Milk Fat Substitutes (MFS)	MFS2 Melting point – 32-34°C, hardness according to Kaminsky – 80-120 g/cm
MFS3	Melting point – 32-36°C, hardness according to Kaminsky – 120-140 g/cm
MFS4	Melting point – 32-34°C, hardness according to Kaminsky – 120-140 g/cm

Source: author's development

Cheese and cheese analogues with combined protein and fat phases were produced according to the technology of hard cheese with a low temperature of the second heating (DSTU 4421:2005 (2007). Basic technical parameters of cheese production:

Fat content in dry matter (by weight) – 45%.

Water content (by weight):

➤ after pressing – 44-46%;

➤ ripe cheese – 40-41%.

NaCl content (by weight) – 1.5-1.9%.

the pH of cheese:

➤ after pressing – 5.4-5.5;

➤ ripe cheese – 5.3-5.4.

Second heating temperature – 38-41°C.

Ripening time – 45 days.

Ripening temperature – 10-12°C.

The cheese was shaped like a low cylinder with a slightly convex side surface and rounded edges. The top and bottom surfaces may be slightly convex, the height of the curd head was 8-9 cm, diameter 24-25 cm, weight 3.5-3.7. The cheese had a moderately mild taste and aroma with a slightly spicy aftertaste and slight acidity. The dough is soft and plastic. On the cut, the cheese had a pattern consisting of holes of a round or slightly flattened shape. The skin of the cheese was thin, even, without damage and a thick subcortical layer, the coating was paraffin wax, and the colour of the dough was from white to slightly yellow.

When developing analogue products with a combined protein phase, soy isolate, which by mass fraction was 30% of the total protein content, was dissolved in cow milk and the mixture was pasteurized for 10 minutes at a temperature of (75±3)°C.

For the products with a combined fat phase, the milk mixture was prepared as follows: whole cow's milk was pasteurized for 10 minutes at a temperature of (75±3)°C. Part of the milk was separated to extract the cream. Cream and MFS were used to normalize the initial milk formula for fat (up to 3%), while MFS was added in an amount of 30% of the total fat content in milk.

The resulting milk mixture was subjected to homogenization under pressure (12.5±2.5) MPa at a temperature of (60±3)°C.

The control cheeses were made from whole cow's milk. The ripening duration of cheese analogues and control cheeses was 45 days at a temperature of 10-12°C. The following research methods were used in the work:

➤ determination of active acidity (pH) – potentiometrically according to (DSTU 8550:2015 (2017);

➤ determination of solids content (by weight) – according to (DSTU 8552:2015 (2017); ISO 5534:2004 (2004) and on a water content analyser MA 30 Sartorius;

➤ determination of non-casein nitrogen content (by weight) – by the Kjeldahl method according to (ISO 17997-1: 2004 (2004);

➤ determination of total protein (by weight) – by the Kjeldahl method according to (ISO 8968-1:2014.(2014);ISO 8968-5:2001(2001).

➤ determination of casein content (by weight) – according to (ISO 17997-1: 2004 (2004);

➤ determination of the fractional composition of proteins – by the Polyacrylamide Gel Electrophoresis (PAAG) (Laemmli, 1970).

For the correct analysis of the data obtained by splitting the proteins the value of the sum of caseins (α_s -casein+ β -casein) was taken in the products at the post-pressing stage as 100% and further calculations were performed based on the said provision.

Analysis of the fractional composition of the proteins of the studied samples was fulfilled by denaturing electrophoresis in 12.5% polyacrylamide gel in the presence of sodium dodecyl sulfate using an electrophoretic chamber, at a constant current strength and a voltage of 55 V and 130 V, for 2 hours. A marker consisting of 11 preparations-standards with molecular weights of 250, 150, 100, 70, 50, 40, 30, 20, 15, 10, and 5 kDa (Thremo, USA) was used as a standard solution. Qualitative and quantitative determination of the protein composition of the samples was carried out after densitometry

of the obtained electropherograms with their subsequent processing using the specialized computer software ImagePro v3.

Characterization of the quantitative composition of free amino acids was carried out on the amino acid composition of the samples and was studied on the analyser LC-2000 (Biotronik). A sample of the product (20 g) was placed in 50 cm³ beakers, and 10 cm³ of a trichloroacetic acid solution with a mass fraction of 60% was added to precipitate protein fractions. After 20 min, the precipitated proteins were filtered off on a paper filter and washed with 10 cm³ of a trichloroacetic acid solution with a mass fraction of 5%. 1 cm³ of concentrated sulfuric acid and 6 cm³ of a solution of phosphotungstic acid with a mass fraction of 25% were added to the filtrate. The precipitate formed after 24 h was filtered off on a paper filter and washed with 15 cm³ of H₂SO₄ solution with a mass fraction of 5%. The filtrate was used to determine the content of free amino acids.

The obtained results and graphical representation of the experimental data were carried out using standard Microsoft Excel 2010 statistical processing programs. The accuracy of the obtained results was ensured by three or five repetitions of the studies. Graphical dependencies were built, and the table shows the arithmetic results of parallel measurements with a value of $p=0.05$.

Results

Commercially available products were used to ensure the practical significance of the study. The bacterial concentrate chosen is a mesophilic starter used in the production of cheeses with a low heating temperature. It is suitable for all soft cheeses, semi-hard cheeses as well as hard cheeses such as Swiss cheese and Parmesan. The manufacturers guarantee a high yield of the finished product, good water-holding capacity, and facilitate easy separation of whey. The bacterial concentrate gives the cheese a classic sour-milk taste. Another important trait of the bacterial concentrate chosen was the possibility to apply it not only to whole milk cheeses but also to the cheese analogues with the milk fat replaced by substitutes. All the products used in the formulations of cheese analogues produced in the study were specialized milk fat substitutes manufactured from plant raw materials and guaranteeing proper trans isomers content as well as proper nutritional value and reliable technological characteristics.

Even though the basic conditions for the manufacture of cheese analogues were the same (temperature regimes, bacterial concentrate), the experimental variants of cheese analogues differed in terms of active acidity and moisture content (Table 2).

Table 2. Physical and chemical indicators in the production of cheese analogues

Variant	Active acidity, pH units		Water content (by weight), %	
	after pressing	ripened for 45 days	after pressing	ripened for 45 days
Control	5.58 ± 0.05	5.15 ± 0.03	44.39 ± 0.12	38.21 ± 0.62
Cheese analogues				
with MFS1	5.16 ± 0.04*	5.10 ± 0.04	48.15 ± 0.67*	41.81 ± 0.75*
with MFS2	5.26 ± 0.01	5.03 ± 0.01*	55.09 ± 1.06*	47.83 ± 0.17*
with MFS3	5.17 ± 0.01*	5.18 ± 0.02	46.01 ± 0.75	41.90 ± 0.34*
with MFS4	5.26 ± 0.02	5.17 ± 0.01	45.03 ± 0.17	39.86 ± 0.25
with soy isolate	5.64 ± 0.02	5.13 ± 0.01	48.84 ± 0.05*	39.89 ± 0.13

Note: * – the difference with the control is significant at $P \leq 0.05$

Source: author's development

After pressing the cheese dough of the experimental variants with MFS a lower active acidity and higher moisture content in comparison to the control cheeses was highlighted. The variant of the cheese analogue with soy isolate was similar in terms of active acidity to the control, but its moisture content was also higher.

During ripening, the active acidity of the curd dough in all experimental variants, except for the variant with MFS2, became similar to the control cheese, however, the water content remained higher. Figures 1 and 2 present the fractional composition of proteins determined according to the method specified above.

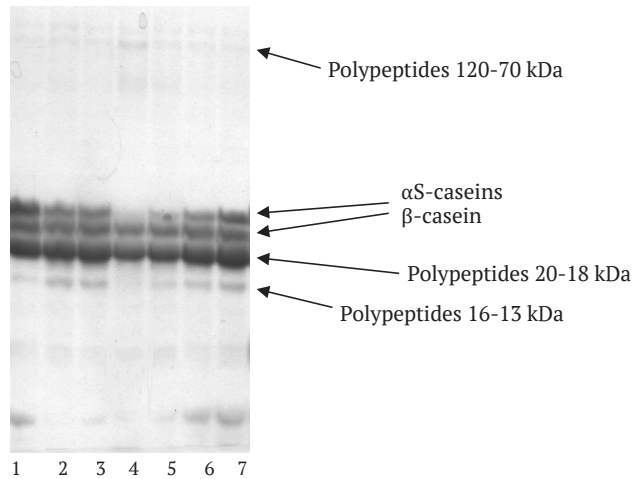


Figure 1. Electrophoretic separation of proteins of analogue cheeses with MFS. 1 – cheese after pressing; 2,3 – ripened control cheeses; ripened analogue cheese products: 4 – with MFS1; 5 – with MFS2; 6 – with MFS3; 7 – with MFS4

Source: author's development

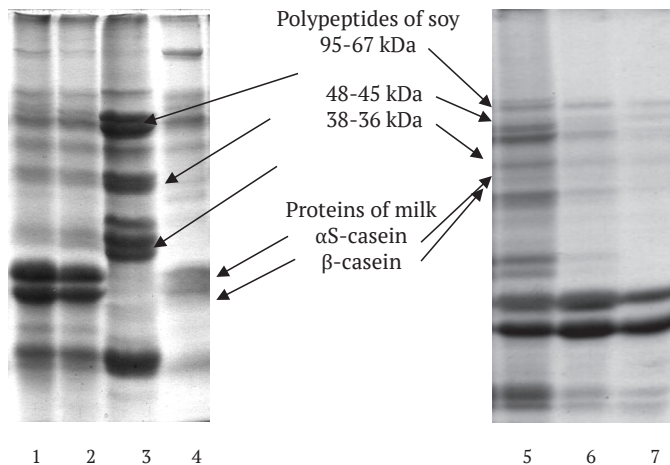


Figure 2. Typical protein electropherograms: 1,2 – cheese analogues with soy isolate after pressing; 3 – soy isolate; 4 – cheese after pressing from cow's milk; 5 – ripe cheese analogue with soy isolate, partially washed from caseins; 6 – cheese analogue with soy isolates on the 45th day of ripening; 7 – ripe cheese (control)

Source: author's development

After pressing, the content of individual fractions of proteins and polypeptides was similar in all variants of cheese analogues MFS and the control, in the variant with soy isolate, the analysis of the protein composition showed the presence of clear bands of characteristic soy proteins with molecular masses of 48 – 45 kDa and 38 – 3 kDa (Fig. 2). During further ripening, proteolytic processes proceeded with different intensity and had different directions in experimental versions of cheese analogues and controls. The α_{s1} -casein fraction, especially in the version with MFS2, was subjected to the strongest splitting during the ripening of the cheese dough, α_{s2} -casein was much weaker, and β -casein was hydrolysed to the least degree. A decrease in the level of caseins was accompanied by the formation of polypeptides of different molecular weights.

In experimental cheese analogues with soy isolate after pressing, the total content of caseins was 52.89% of the total protein content, and soy proteins – were 29.71%. The weakening or almost complete absence of characteristic bands of soy proteins in the protein spectrum of ripe cheese analogues with soy isolate can be explained by active proteolytic processes that occurred during ripening, proteins of soy isolate accounted for less than 5% of the total protein content in the ripe product.

The total content of caseins in control cheeses and cheese analogues pressing was (83.53±0.20) % of the total protein content. Table 3 shows the relative content of α_s -casein, β -casein and their sum in experimental variants and control cheeses after pressing and after 45 days of ripening.

Table 3. Decomposition of caseins during ripening of cheese analogues

Variant	The relative content of caseins (%)		
	Sum of caseins	α_s -caseins	β -casein
Cheese analogues and control cheeses after pressing	100	56.36	43.64
Control, ripening for 45 days	84.75	43.31	41.44
Ripe cheese analogues			
with MFS1	79.53	37.88	41.65
with MFS2	69.84	30.94	38.90
with MFS3	80.96	38.11	42.85
with MFS4	80.63	39.09	41.54
Cheese analogue with soy isolate after pressing	100	55.85	44.15
Ripe cheese analogue with soy isolate	85.11	42.87	42.24

Source: author's development

During ripening, the content of caseins in the control cheeses decreased by 15.25%, mainly due to the cleavage of α_s -casein.

On the 45th day of ripening, the decomposition of caseins in all variants of cheese analogues with MFS was higher than in the control. If the total level of caseins in control ripe cheese decreased by 15.25%, then in the variants with MFS1 – by 20.47%, with MFS3 – by 19.04%, with

MFS4 – by 19.37%. To the greatest extent, the total level of caseins decreased in the variant with MFS2 – by 30.16%, and, compared to other experimental variants, the decomposition of β -casein was also higher.

Changes in the spectrum of free amino acids at the beginning (after pressing) and at the end of ripening (after 45 days) of the studied cheese analogues with soy isolate are given in Table. 4.

Table 4. Qualitative and quantitative composition of free amino acids in cheese analogues and control cheeses (mg/100 g of product)

Amino acid	Control		Cheese analogue	
	after pressing	ripe	after pressing	ripe
Threonine	-*	27.168**	-	14.085
Valine	-	6.189	0.159	2.883
Methionine	-	0.954	-	0.306
Leucine	0.297	42.063	0.696	20.067
Phenylalanine	0.426	50.619	0.738	28.218
Lysine	8.4	22.932	15.615	16.446
Glutamic acid	20.124	51.156	31.428	35.385
Proline	9.444	16.587	12.327	11.163
Alanine	-	8.43	-	4.581
Aspartic acid	0.945	3.105	0.387	0.411
Tyrosine	6.429	37.281	9.075	28.551
Histidine	2.409	32.217	3.78	16.14
The total amount of free amino acids:	48.474	298.701	74.205	178.236
of which are essential:	9.123	149.925	17.208	82.005

Note: * No compound; ** Measurement error does not exceed 5%

Table 5. Qualitative and quantitative composition of free amino acids in ripe cheese analogues with MFS (mg/100 g of a product) *

Amino acids	MFS1	MFS2	MFS3	MFS4	Control
Threonine	-	-	10.29	8.69	-
Valine	11.13	8.49	10.23	7.21	11.59
Methionine	1.70	1.76	3.02	1.60	3.01
Ileucine	1.51	1.43	0.77	0.54	1.31
Leucine	29.83	21.03	16.88	15.59	22.99
Lysine	29.54	22.54	21.35	23.95	26.18
Phenylalanine	31.44	24.90	23.95	24.51	31.85
Serene	7.65	4.80	-	-	9.67
Glutamic acid	59.09	46.41	42.89	40.08	50.70
Proline	25.40	59.48	43.80	40.16	50.81
Alanine	7.28	6.18	7.54	6.49	8.76
Tyrosine	39.71	24.54	29.67	26.05	30.72
Histidine	6.67	17.83	17.92	17.39	20.94
Arginine	1.30	1.18	0.69	0.66	-
Glycine	1.64	1.68	1.62	1.47	2.38
Aspartic acid	12.45	9.35	9.42	8.80	-

Note: * Measurement error does not exceed 5%

Source: author's development

From the data presented in Table 5, the content of free amino acids, which is low at the beginning of ripening, significantly increased in ripe products. In the experimental variant with soy isolate by 2.4 times, and in the control variant by 6.2 times compared with the stage after pressing. At the same time, in ripe cheese analogues, the level of free amino acids was 1.7 times lower, and free essential amino acids – were 1.8 times lower compared to control cheeses. Such values of the above said parameters confirm a lower nutritional value of cheese analogues which can be a benefit within special diet plans.

Discussion

Carious cheese analogues were analysed and studied by researchers around the world. Full or partial replacement of milk fat with vegetable analogues provides cost reduction and stabilizes product quality since the production and composition of substitutes are not subject to seasonal fluctuations (Kamath *et al.*, 2022). The possibility of making an analogous cheese product by incorporating and/or substituting, partially, milk fat with olive oil, as well as determining the optimal parameters for its production was studied. The addition of olive oil generated a beneficial effect on the level of cholesterol in the blood, therefore, although the fat content of the product and its caloric level is increased, the detrimental effect of saturated fatty acids is not increased, but it decreases. The lipid content of the whey of the respective preparations was analysed to calculate the amount of fat that passed to the whey (Alonso Degeneffe, 2019). The role of polyunsaturated fatty acids and phospholipids in the prevention and treatment of lipid metabolism disorders, in particular atherosclerosis, has been proven (Sokoła-Wysoczańska *et al.*, 2018). The most important characteristic of fat is the composition of the fatty acids that form it. It should be balanced in terms of the ratio of saturated, monounsaturated, and polyunsaturated fatty acids,

especially in terms of the ratio of polyunsaturated fatty acids of the omega-3 and omega-6 families. At the same time, the undesirability of the use of products containing the trans isomers of fatty acids, and the consumption of products containing a significant amount of saturated fatty acids, has been proven (Dhaka *et al.*, 2011).

According to A. Badem & G. Uçar (2016), the agent starting casein hydrolysis is plasmin. During storage proteolysis increases, but such texture parameters as firmness, viscosity and chewiness tend to decline. No efficiency is shown by non-starter lactic acid bacteria compared to plasmin during casein hydrolysis. However, the said bacteria do form casein-peptide derivatives. Peptidase, dipeptidase, tripeptidase, carboxypeptidase, aminopeptidase and endopeptidase and proteinase are the enzymes *Lactobacillus* has the said enzymes being utilized and the necessary amino acids are thus obtained. The formation of the above-listed enzymes is determined by isolating peptides as well as free amino acids from the casein having undertaken the hydrolysis process. During storage plasmin and microbial enzymes behave in a contradicting way thus maximizing free amino acids content (Mulvihill & McCarthy, 1994).

In the study C.R. Cunha *et al.* (2010), analogues of cheese “Requeijão cremoso” were studied, which were made by replacing 25% and 50% of milk fat with vegetable fat. Replacing part of the milk cream with vegetable fat led to an increase in hardness. Traditional cheese was a homogeneous protein mass, in which numerous small fat particles were dispersed, while in analogues fat globules were present of a large diameter, such changes were observed in samples with an enlarged vegetable fat particle.

The manufacturing process of a cheese analogue with the second heating is characterized by (Mogutova *et al.*, 2021). The said process consists of three successive stages, which are carried out at a speed of not more than 10...20 revolutions per minute with intensive

kneading. Stage 1 – heating is carried out at a temperature of 38 ... 42°C for 10 ... 15 minutes. Stage 2 – vegetable flour and salt are added to the curd (no more than 2 ... 2.5%). The curd is intensively mixed with an increase in temperature to 50 ... 55°C. Stage 3 – the curd is kept at a temperature of 60 ... 65°C for 10 ... 15 minutes. The product is formed in the form of bars, cylinders, spheres, and other shapes and pressed at a load of 2...3 kg/cm² for 1...3 hours until the cheese product reaches a mass fraction of moisture of 40...60%. Ripening of the curd product is within 12...18 days at a temperature of 5...15°C and a relative air humidity of about 85%. After the ripening period, the cheese analogue has a curd, slightly sour taste, and smell characteristic of prescription components; homogeneous, tender, slightly fragile or brittle, as dense as possible; colour – from white to yellow, uneven colour is allowed. Dough without holes; single cells of irregular shape are allowed. For a cheese product, the shelf life is 14 days from the end date of maturation.

A new type of semi-hard cheese product has been developed using a milk fat substitute. The said cheese product is made from a normalized vegetable-milk mixture, by acid-rennet coagulation of proteins, followed by moulding, self-pressing, pressing, salting, and ageing. Physical and chemical parameters of the raw materials for the cheese product were fat content in dry matter (by mass) – 45.2%, water content – 48%, and pH – 5.5. Compared to typical cheeses with low second heating temperature new type of cheese analogue had higher moisture content. This has been achieved by norming milk during the formation of the mixture, adjusting the temperature of the second heating and the duration of cheese grain processing, as well as reducing the duration of pressing and ripening (Savchenko *et al.*, 2018).

The use of corn oil in the production of light cheese instead of milk fat significantly affected the amount of dry matter, fat and salt in the dry matter, protein and titrated acidity and

pH value of the samples. During the ripening of cheeses, the content of water-soluble nitrogen increased. However, no differences were found between the peptide profiles of all cheese samples (Arslan *et al.*, 2014).

The replacement of milk fat with emulsified olive oil and the production of the Gouda cheese analogue resulted in a lower solids content in cheeses containing fat substitutes than in full and low-fat control cheeses due to the higher water-binding capacity of fat substitutes. The values of free fatty acids were highest in the case of cheese products with reduced fat content (Felfoul *et al.*, 2015).

Reduced-fat cheese analogues are made from partially skimmed cow's milk with the addition of soy protein concentrate. It was found that cheese analogues had higher protein content with an increase in soy protein content, which confirms that part of the soy proteins remained in the casein matrix, and the fat content increased to 22% relative to the control sample. However, foods were classified as low-fat foods and considered functional as an additional source of plant-based protein with a high biological value due to the high content of amino acids and essential fatty acids (Rinaldoni *et al.*, 2014).

Thus, the obtained values of the physical and chemical indicators of cheese analogues do not correspond to the results obtained by (O'Malley *et al.*, 2000): results of pH values measured were from pH 5.03 to pH 5.18 when authors showed pH 7.29 – 7.33 for rennet caseins; pH 6.40 – 6.50 for pilot scale cheese analogue; pH 6.46 – 6.48 for industrial scale cheese analogue. For our samples water content (by weight) was 39.86% – 41.90%, while (O'Malley *et al.*, 2000) showed 50.30% – 50.88% for sample scale cheese analogue and 47.42% – 47.76% for industrial scale cheese analogue. Such a discrepancy seems to originate from the differences in plant raw materials used in the studies compared. However, the electrophoretograms of proteins are the same in both studies.

Comparing our results of pH and water content (by weight) shown in the paragraph above and the data of other scholars: pH 6.10 and water content 48.80% for analogue pizza cheese (Fox *et al.*, 2000; Chavan & Jana, 2007; Gao *et al.*, 2022); pH 5.66 and water content 53.35% for analogue mozzarella cheese (Jana, 1998; Chavan & Jana, 2007) it can be stated that the said results are consistently close.

The results of pH and water content (by weight), according to D.M. Mulvihill & A. McCarthy (1994) were pH 6.17 and 47.21%. The electrophoretograms of proteins do not contradict those made during our studies.

The comparison of the data obtained during the research and the results published by the scholars mentioned shows that results of the determination of physical and chemical traits as well as the described peculiarities of the technological processes applied are not controversial and originate mostly in the great diversity of raw material used.

Conclusions

If in the cheese dough after pressing the differences in the spectrum of free amino acids between the variants were insignificant, in ripe cheese analogues they accumulated significantly less than in the control cheeses: from MFS1 and MFS4 – by 24.4%, from MFS3 – by 26.7%. Only in ripe cheese analogue with MFS1 the total amount of free amino acids was almost equal to the control product. During ripening, the content of these compounds increased in the variants with MFS from 2.2 times (MFS3 and MFS4) to 3.0 (MFS1) and 3.3 times (MFS2), in the control the content of free amino acids increased by 2.5 times.

During ripening, individual free amino acids accumulated from 3-7 to 30-40 times. The ratio between essential and non-essential free amino acids slightly increased in all variants.

The specific cheese taste is known to be determined by significant amounts of free proline, methionine, as well as aspartic and glutamic acids. These amino acids accumulated during ripening in all variants, both experimental and control. Active proteolytic processes led to the appearance of all ripe products of such essential amino acids as methionine and isoleucine, there was also an increase in the level of glycine. The presence of free arginine was characteristic of ripe products with MFS, which in significant quantities can give the product an unpleasant aftertaste of spoilage. In the control cheeses, the presence of this amino acid in the unbound state was not found.

The results obtained and the literature sources reviewed substantiate the possible directions of research the most promising of these being: embracing a wider range of cheese analogues and the ingredients used for their manufacturing, mostly milk fat substitutes as well as complex bacterial concentrates. Series of research on the texture of the said cheese products should be fulfilled embracing the recognized sensorial methods as well as Texture Profile Analysis employing a relevant testing machine.

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None.

Conflict of Interest

None.

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Перебіг протеолітичних процесів при визріванні комбінованих сирних продуктів

Світлана Григорівна Даниленко

Доктор технічних наук, старший науковий співробітник
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
Інститут продовольчих ресурсів Національної академії аграрних наук України
02002, вул. Євгена Сверстюка, 4а, м. Київ, Україна
<https://orcid.org/0000-0003-4470-4643>

Лариса Вацлавівна Баль-Прилипко

Доктор технічних наук, професор
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0002-9489-8610>

Таїсія Миколаївна Рижкова

Доктор технічних наук, професор
Державний біотехнологічний університет
62341, вул. Академічна, 1, смт. Мала Данилівка, Україна
<https://orcid.org/0000-0002-1029-8838>

Сергій Борисович Вербицький

Кандидат технічних наук
Інститут продовольчих ресурсів Національної академії аграрних наук України
02002, вул. Євгена Сверстюка, 4а, м. Київ, Україна
<https://orcid.org/0000-0002-4211-3789>

Олександр Григорович Панасюк

Аспірант
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0001-6439-6604>

Анотація. Незважаючи на широке виробництво аналогів сирів, до складу яких входять рослинні речовини, теоретичні основи процесів їх виробництва, у тому числі протеоліз, ще недостатньо вивчені, тому тема даної роботи є актуальною. Метою даної роботи є визначення впливу вмісту аналогів сиру, а саме заміників молочного жиру, ізолятів соєвого білка, лактобацил виду *Lactococcus lactis ssp. Lactis*, *L. lactis ssp. cremoris*, *L. lactis ssp. lactis* *bv. diacetilactis*, а також *Leuconostoc mesenteroides* на фізичні, хімічні та органолептичні характеристики, а також на вміст амінокислот в аналогах сиру. Визначення фізико-хімічних показників проводили за нормативами: активної кислотності (рН) – ДСТУ 8550:2015; вміст сухих речовин (за масою) – ДСТУ 8552:2015 та ISO 5534:2004; вміст неказеїнового азоту (за масою) – ISO 17997-1:2004; загальний білок (за масою) – ISO 8968-1:2014 та ISO 8968-5:2001; зміст казеїну (за масою) – ISO 17997-1:2004. Визначення фракційного складу білків проводили методом електрофорезу поліакриламідному гелі. Якщо в сирній масі після пресування відмінності в спектрі вільних амінокислот між варіантами були незначними, то у зрілих аналогах сиру їх накопичувалося менше, ніж у контрольних сирах, тільки в одному аналогу

сиру загальна кількість вільних амінокислот було менше, майже зрівнявся із контролем. У процесі дозрівання вміст цих сполук збільшився в аналогах сиру від 2,2 до 3,3 рази, у контролі вміст вільних амінокислот збільшився у 2,5 рази. При дозріванні накопичення окремих вільних амінокислот становило від 3-7 до 30-40 разів. Активні протеолітичні процеси призвели до появи у всіх стиглих продуктах таких незамінних амінокислот, як метіонін та ізолейцин, також відбулося підвищення рівня гліцину. Практичне значення роботи полягає в обґрунтуванні раціональних протеолітичних процесів при виготовленні аналогів сиру з метою забезпечення споживчої якості, наближеної до якості сирів з молока

Ключові слова: молочна промисловість; молоковмісний замінник молочного жиру; фізико-хімічні властивості; амінокислоти



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Analysis of the state and prospects of milk production and dairy products in Ukraine in the post-war period

Tetiana Gutsul*

PhD in Economics

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

<https://orcid.org/0000-0002-1826-240X>

Nataliia Sulima

PhD in Economics

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

<https://orcid.org/0000-0002-3852-7989>

Boryslav Kuderskyi

Master's Degree

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

<https://orcid.org/0009-0004-1118-1393>

Abstract. The indicators of recent years demonstrate stability and high results in milk production at agricultural enterprises. The relevance is confirmed by the fact that milk production and its optimization are key aspects of the economic development of the agricultural sector of Ukraine in the post-war period, because dairy farming is a system-forming branch of agriculture that opens up new opportunities for the formation of promising business development vectors. The purpose of the article is to analyse the problems and prospects of economic aspects of the efficiency of resource use and optimization of milk production in agricultural enterprises of Ukraine in the post-war period. The research used the following methods: induction and deduction, synthesis, theoretical generalization, statistical analysis, comparative analysis, modelling. The links of

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*Corresponding author



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inconsistency between resources, production of dairy products and the capabilities of enterprises, state policy, the level, and norms of consumption of milk and dairy products by the population in the post-war period are studied. Accordingly, the dynamics of production, export, sales, stages of production, peculiarities of cooperation and import of basic dairy products were considered. A direct connection between the production and consumption of milk, the dependence of the demand and consumption of milk and dairy products on the level of total income of households has been established. Based on this, the work presents a full description of the dairy industry in Ukraine, the volume of milk production in the pre-war period and prospects for development in the post-war period. A systematic study of the market properties of various types of dairy products of Ukraine using SWOT and PEST analysis is given in general. In order to improve the state of the market of milk and dairy products of Ukraine, appropriate proposals have been formulated. The practical value of the work lies in the fact that its results can be used as a basis for the development of technologies for the use of milk production resources in the post-war period, as well as in the work of specialists in the agro-industrial and economic sectors

Keywords: economic development; perspectives and innovations; competitiveness; investment

Introduction

Milk production is a powerful business direction in Ukraine. It is important to investigate the conditions for the production of products in accordance with the state food safety standards. Milk is a necessary component of a healthy human diet and belongs to the basic food products.

Many researchers paid attention to the economic problems and prospects of the efficiency of resource use and optimization of milk production in agricultural enterprises of Ukraine in the post-war period in their studies. M. Rakhman & D. Hryzo (2021) speak of the dairy industry as one of the most powerful. The authors analysed the state of the dairy market from 2017 to 2020, and noted that in January-April 2021, 2,519.3 thousand tons of milk were produced in Ukraine. In addition to it, agricultural enterprises produced 901.9 thousand tons of milk, and households – almost twice as much (1617.4 thousand tons). An analysis of threats and opportunities for dairy enterprises was carried out, which include: changes in legislation that open up favourable opportunities in the supply and demand market, economic instability, development, and improvement of

technologies at the enterprise, demographic changes, and the state of the environment.

N.M. Prysiashnyuk *et al.* (2023) focused on the evaluation of innovative approaches to milk production in Ukraine in the context of food security of the country's population. Milk production in agricultural enterprises of Ukraine is considered as a type of business that involves increasing economic potential and meets high requirements for production technologies and quality. Yu. Kindzerskyi (2022) outlines the post-war arrangement of Ukraine's industry, namely the development of economic and other industries, which before the war were the causes of the country's economic, technological and military backwardness. T. Pouch & A. Trouve (2018) analysed the consequences of the milk market crisis in Europe, their features. A.M. Moskalenko & V.P. Kolosha (2020) investigated the economic efficiency of dairy production that meets the necessary product requirements in world markets. M. Gladiy & O. Prosovykh (2022) indicate further prospects in the development and improvement of the dairy industry and highlight the main factors affecting them. The main ones are the state of

the production base, the solvency of consumers, market infrastructure, etc.

T. Gutsul (2022) examined the determinants of trends in the development of the milk market in wartime conditions and gave an assessment of further development in the postwar period. Promising directions are the functioning of the milk market, problems of agricultural development, solving the problems of providing dairy farms with fodder and exporting milk to world food markets. Z. Dvulit & Z. Komarenska (2022) characterized the peculiarities of exporting high-quality dairy products to foreign countries in conditions of full-scale war. The main problems that arose in the conditions of the war regarding the sale of dairy products were identified: the destruction of infrastructure, the reduction of livestock, the destruction of documentation, the impossibility of using pastures, the reduction of qualified personnel and many others.

In the world, milk production has its own standards of quality and volume of processing. Because of this, the international market will demand high-quality dairy products. Only countries with a high level of milk self-sufficiency can become suppliers of such products. Taste, purity, health benefits, awareness of the production process, convenience in using new trends in the demand for vegetable milk (soy, rice, coconut, etc.) and lactose-free products have a significant influence on the buyer's choice (Kozak, 2020).

The development of the domestic milk market directly depends on the strengthening of food security and the development of one's own agro-industrial production. For Ukraine, the basis of dairy provision should be its own agricultural and processing production with a raw material base, the food market, and reserve funds. In general, the situation in the world is of direct importance for the state of milk production in Ukraine, and production volumes in the EU show constant growth. The largest producers of milk in the EU are Germany,

France, Great Britain, the Netherlands, Poland, and Italy (Borawski *et al.*, 2020).

To study the development of the dairy industry in the pre-war (2017-2021) and war (2022-2023) periods, current problems and ways to solve them, scientific research methods were used: theoretical generalization for studying scientific works and research, forming conclusions; statistical analysis to study the impact of military actions on the territory of Ukraine, respectively, the dynamics of the number of cattle and dairy production; comparative analysis to determine the trends of the main changes in indicators of the development of milk production at agricultural enterprises; method of SWOT and PEST analysis; modelling to interpret the results of investment of this production in the post-war period.

The purpose of the article is to study the indicators and prospects of milk production in Ukraine according to the efficiency of resource use and milk production in agricultural enterprises. To achieve the definition of the goal and in accordance with the content of the scientific research, the following tasks were set:

- analysing the indicators of dairy production in the pre-war and post-war periods;
- describing the real state of milk production enterprises;
- identifying the characteristic features of production improvement and determining the ways of selling products in the EU after the war.

The informational and theoretical base of research is made up of the works of modern Ukrainian researchers (Gladiy & Prosovych, 2022; Komlichenko & Rotan, 2023; Sobkevich *et al.*, 2023), analytical reviews of foreign experts (Borawski *et al.*, 2019; Borawski *et al.*, 2020; Rakhman & Hryzo, 2021), as well as statistical information on the dynamics of crucial indicators of the dairy market in Ukraine (Union of dairy enterprises of Ukraine, 2021; Association of milk producers, 2021; Ministry of Agrarian Policy and Food of Ukraine, n. d.).

In the post-war period, the agricultural sector, which includes milk production, will

receive significant, highly effective resources to achieve its competitiveness and compliance with quality requirements and standards. The development of dairy production, including the use of resources and optimization of production, with a focus on export in the post-war period, will become one of the promising directions for the Ukrainian economy, as it is in demand in the world.

Dynamics of dairy production in the periods of pre-war and wartime transformations

Milk production is one of the constituent elements of the agro-industrial complex. It includes a number of groups of industrial and agricultural enterprises focused on the production, processing, and sale of milk. The dairy industry is socially oriented and should provide nutritious and high-quality food products to Ukrainian consumers. The complex of milk production at agricultural enterprises is the basis of food security (Korman *et al.*, 2022).

In recent years, Ukraine's economy has suffered the devastating impact of the Covid-19 pandemic and the military-political threats that harm it. In particular, the development and functioning of enterprises producing milk and milk products in Ukraine is changing, which is an extremely negative factor. Milk is a necessary food product in the diet of adults and children. In addition, the dairy industry stimulates the development of the Ukrainian countryside. The indicators of milk production from 2017 to 2021 changed significantly, showing its reduction from 20.7% of the total volume to 15.1% (Gutsul, 2022).

The production of milk and dairy products constitutes a whole system of state commodity-monetary relations between subjects and objects of management: producers of raw materials, processing enterprises and consumers of final products. In general, there is a process of reproduction, which consists of the following processes: production, distribution, exchange,

consumption, and receipt of expected income by all market operators. But the main task is to solve the food problem, which is one of the main conditions of the socio-political activity of the state (Rossokha & Petrychenko, 2018).

Speaking about the dynamics of production in dairy cattle breeding of Ukraine over the past 33 years, a sharp reduction is observed here. The reason for this is a reduction in the production of raw milk and a decrease in the number of cows – from 8.53 million heads in 1990 to 1.79 million heads at the beginning of 2020. And over the past three years, production has changed radically and is undergoing certain transformations in connection with political events (Dovhal, 2020). In such conditions, milk production enterprises cannot work in the usual mode, atypical signs appear in their activity: unstable conditions of supply and demand on the market, economic fluctuations, changes in the operation of the enterprise and in the main priorities. Of course, such factors ultimately affect the efficiency, optimization, and development of enterprises in modern conditions and complicate the process of planning and forecasting. In addition, these difficulties lead to a struggle for consumers, the process of setting up the product sales system is complicated (Korman *et al.*, 2022).

The Law of Ukraine “On Milk and Dairy Products” contains contradictory and conflicting norms, which leads to the emergence of additional barriers for business entities, restrains the development of cooperation in the countryside and causes the unprofitability of agricultural enterprises, deprives the business of dairy production of sufficient support (Gruzinska *et al.*, 2018). After all, the issue of ways to optimize costs for the sale of dairy products should also be resolved at the state level. Therefore, in order to improve the dairy market, its resources and their functioning, it is necessary not only to increase the production of dairy products, but also to optimize the costs of establishing a clear marketing system (Melnyk & Stetsenko, 2018).

As of March 2023, according to the Ministry of Agrarian Policy and Food of Ukraine, the number of cows was about 1,400,000, which is 13% less than last year (Ministry of Agrarian Policy and Food of Ukraine, n.d.). Agricultural enterprises have 389,900 heads, which is 7.9% less. In all regions of Ukraine, there is a decrease in livestock. Only in the Khmelnytskyi region, the number of cattle increased by 1.9% during the year. Experts in the agricultural industry indicate that the number of livestock in Ukraine will continue to decrease due to problems with fodder, the destruction of farms in the context of continued hostilities (Barylovich, 2023).

Milk production in 2022 amounted to 7.7 million tons, which is 12.1% less than in 2021. In regions where hostilities are not taking place, the situation with milk production is better: 7.3 million tons, compared to 0.4 million tons in the affected regions. The production of dairy products decreased by 15% (Barylovich, 2023). Despite the difficulties and military operations, milk production at agricultural enterprises of Ukraine continues. There is a certain reduction in the range of milk, however, despite the losses of factories by

producers, most enterprises successfully overcome difficulties and ensure the supply of the product to the market (Svynous et al., 2022). The current state of the domestic market of milk and dairy products is characterized by a moderate level of consolidation. Almost 80% of the market is controlled by 50 enterprises, a large part of which is part of large holdings (Association of milk producers, 2021).

The formation of a chain link between the producer of milk and the consumer of this product is important in the activity of agricultural enterprises. It is necessary to form a sales market in adjacent and related spheres and sectors with the help of the institute of state order and public procurement. Simplified procedures and high export prices for Ukrainian dairy products support sales on foreign markets. In 2022, milk exports reached 344 million US dollars, which is 39% more than in 2021. The results of the research are shown in Figure 1.

The study of the properties of the objects of the market of milk and dairy products of Ukraine and the connections between them is presented with the help of SWOT analysis. His results are shown in Table 1.

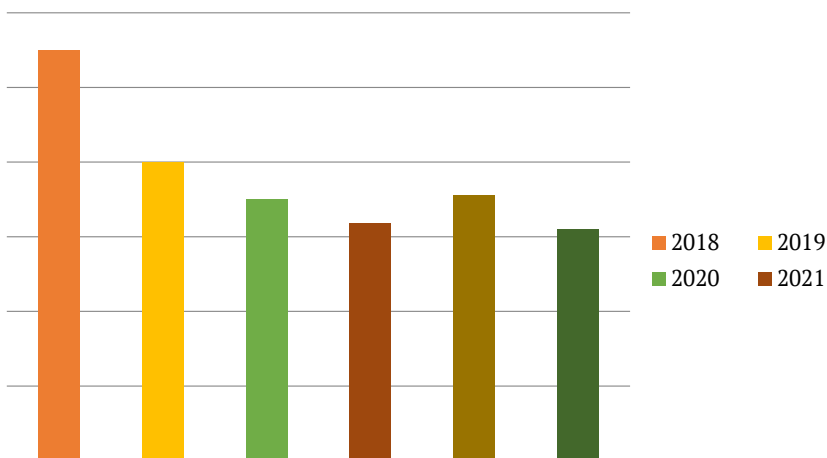


Figure 1. Dynamics of the export of dairy products in terms of milk 2018-2023, thousand tons
Source: Milk export is a powerful support for the dairy industry (2023)

Table 1. SWOT analysis of milk production in agricultural enterprises of Ukraine for 2021

Internal environment		Environment	
Strengths		Opportunities	
<ul style="list-style-type: none"> ➤ Application of innovative technologies. ➤ Effective organization of structure and management style. ➤ Activities in accordance with international quality standards. ➤ A diverse selection of products and its constant updating. ➤ Information resources are developed at a sufficient level (websites, social networks). 		<ul style="list-style-type: none"> ➤ Due to the difficult situation of companies and enterprises in the dairy market, increasing competitiveness. ➤ Increasing the volumes and markets of export products. ➤ In connection with continuous changes and innovations in the technical process, the emergence of new and modern markets and their segments. 	
Weaknesses		Threats	
<ul style="list-style-type: none"> ➤ Consumption is affected by seasonality. ➤ Not all representatives of milk production enterprises have active advertising on television and in social networks. ➤ Insufficiently formalized comprehensive sales strategy. 		<ul style="list-style-type: none"> ➤ Gradual decrease in the number of cows and, accordingly, milk production, and increase in prices for dairy products. ➤ Increase in prices for keeping cattle. ➤ Emergence and growth of new requirements of suppliers and buyers. ➤ The variability of customer tastes (constant updating of the product line by competitors). 	

Source: Compiled by the author based on Union data of dairy enterprises of Ukraine (2021) Association of milk producers (2021)

The analysis of the economic, political, technological and social aspects of the external environment, which significantly affect the production

and market of dairy products of Ukraine in general and its subjects, is illustrated with the help of a PEST analysis. These results are shown in Table 2.

Table 2. PEST analysis of Ukrainian dairy enterprises for 2021

Political Aspects (P)		Economic aspects (E)	
<ul style="list-style-type: none"> ➤ State regulation of competition dairy industry. ➤ Political reformation. ➤ Active changes in tax legislation in favour of milk producers. ➤ Update of legislation on dairy industry. 		<ul style="list-style-type: none"> ➤ Economic situation in Ukraine. ➤ Inflation rate. ➤ Changes in the exchange rate of the national currency. ➤ Dynamics of tax policy. ➤ The impact of changes in utility tariffs (transportation, water supply) on the costs of an agricultural enterprise. ➤ Changes in the income of the population. 	
Social Aspects (S)		Technological aspects (T)	
<ul style="list-style-type: none"> ➤ Basic social values are undergoing noticeable changes. ➤ Attitude of the population of life and change of style. ➤ Situation and environmental changes in the country. ➤ Spreading the trend of consumption of vegetable milk ➤ Demographic changes (structure and rates of population growth or decline). 		<ul style="list-style-type: none"> ➤ New technologies are emerging. ➤ Transformations in the mechanization of milk production. ➤ Adaptations and speed of changes of the latest technologies in production. ➤ Production of dairy products based on innovations 	

Source: Compiled by the author based on Union data of dairy enterprises of Ukraine (2021); Association of milk producers (2021)

The development of the dairy industry of Ukraine in the post-war period remains an important issue. Ukraine may face a number of problems after the war, which will need to be solved immediately, to find ways to avoid an acute crisis in the dairy industry. These include: lack of funds for payments; most of the enterprises are in a ruined state; low investment attractiveness due to the long investment payback period; the spread of various diseases between animals and people; an increase in the cost of products and, accordingly, a decrease in purchases, etc. Ukrainian specialists should already think about the post-war period, look for ways to preserve the production and sale of milk. As a result of the fruitful work of all parties, it should become a reliable, global and competitive partner that meets the needs of the world market, can offer a wide range of high-quality products of the processing and food industry at an appropriate price, as well as interesting authentic taste properties.

Cooperation and innovation strategies in the dairy industry: A review of foreign and domestic experience

It is useful to pay attention to the experience of foreign countries in which the main producers and sellers of dairy products in the world are cooperatives represented by medium and small producers. This enables the representatives, by uniting, to enter the international market, sell their products and work on favourable terms. Thanks to this, they achieve significant success and are designed for a larger number of participants. S. Shupyk (2020) highlights the experience of foreign dairy producing countries and indicates that the leading suppliers to the world market of milk and dairy products are the USA, which ranks first in milk production. 91.3 million tons. The largest commodity producers of milk in the world are also India – 60.6 million tons, China – 35.7, Brazil – 34.3, Germany – 31.1, France – 21.7, New Zealand – 18.9, Turkey – 16.7, Great Britain – 13.9 million tons.

In addition, it was established that the main factor that increases the profitability of dairy production in developed countries is the improvement of the quality and differentiation of the assortment. The main factors contributing to the successful development of dairy farming include increased state support and economical use of resources. They also use a set of financial incentives, including reducing the tax burden (Shupyk, 2020).

The production of dairy products in foreign countries is characterized by the special efficiency of the product market, which ensures the high development of innovative equipment and facilities. This, in turn, promotes cooperation and integration, the introduction of innovations in the processing sphere and trade infrastructure. Speaking about the borrowing of experience by Ukraine in the post-war period, this is possible only if certain circumstances are taken into account. In particular, local natural and economic factors. In addition, the state should take into account the use in domestic conditions of achievements in the field of production, storage, processing, formation of the infrastructure of the researched market, as well as the system of state regulation tools, improvement of the sales system based on the development of cooperatives.

For many years, Germany was the largest importer and exporter of milk in the EU domestic market. This country is also the largest producer of milk in the EU, despite the fact that in 2017 it had a deficit in the trade of milk and dairy products. Belgium, Italy, the Netherlands, France, etc. have a significant influence on trade. Analysing the export and import of milk products within the EU, it is worth noting that Germany also dominates with 4826.10 thousand tons in 2017, France is the second country – 2262.76 thousand tons. In Belgium, the level was 1827.64 thousand tons, which was equal to about 38% of the level of the largest exporter (Borawski *et al.*, 2019).

It is worth considering steps to improve the state of the milk market on the basis of forecasts

and assessment of the real state of dairy production by researchers M. Rakhman & D. Hryzo (2021). These include: compliance with international food safety standards and successful international agricultural practices; changing marketing strategies and correct positioning of one's product in foreign markets; stimulation of cooperation between enterprises; support for the export of dairy products; strengthening the competitiveness of enterprises through product improvement and diversification; financial assistance to agricultural enterprises that lost their business due to military actions; modernization of production technology at enterprises; professional development of personnel; investing in product line development; creation of the Fund for Support and Preservation of Animal Husbandry; introduction of state subsidies to encourage export of products; exemption from paying value added tax (VAT) on imported equipment for the processing industry and dairy farming.

O.O. Komlichenko, & N.V. Rotan (2023) note that the financial and economic development of the region is a complex concept and includes the totality of general production opportunities and the financial side. Taking into account the introduction of hostilities on the territories of Ukraine, the economic sector of the region can be restored and modernized only under the condition of the cessation of hostilities, the deoccupation of the captured territories on the basis of the growth of its investment potential, which is the combined possibility of own and attracted financial resources that are able to provide investment activities with the aim of obtaining profit, in the goals and scope determined by the economic policy of the region. Investment policy is one of the most important steps in the economic development of the region. It should be carried out by coordinated efforts of national, regional and local authorities. The key task of this policy is to increase the investment attractiveness of the region, the formation of attractive investment

products and effective promotion of investment markets.

A.M. Moskalenko, & V.P. Kolosha (2020) argue that the post-war concentration of milk production should be based on a real economic mechanism, which allows the size of enterprises to have a higher level of competitiveness. This means that in reality this group of enterprises may have advantages in terms of the level of production organization, its technical positioning on the market, product quality, and only then will it be able to increase milk production in the post-war period. In particular, Yu. Kindzerskyi (2022) indicates that the successful experience of the post-war recovery of the dairy industry should take place on the basis of a mobilization model of development with an increase in the coordinating and entrepreneurial role and share of the state in economic processes, clear and comprehensive planning and design of balanced economic, social and spatial development, and not further deregulation in the conditions of destroyed markets, which in Ukraine even before the war led to weakness and backwardness in economic and military terms.

O.V. Sobkevich *et al.* (2023) propose steps to preserve the chain "production – processing – storage – supply of food products to the population": rehabilitation of the agricultural economy in the territories affected by hostilities; strengthening the decentralization of the agrarian economy; strengthening of communities' own food system; restoring the integrity of the agro-industrial complex and strengthening the national food system; improvement of state support for the dairy industry; repositioning of Ukraine in the global food system; use of bio-energy potential, which will make it possible to partially replace traditional types of energy with alternative ones both at the national and local levels of consumption, to strengthen the country's energy sustainability.

In the post-war period, state policy should form positive trends, promote the development

of investment attractiveness programs for the sector, create support vectors for Ukrainian dairy products business in accordance with high-tech production and standards on a cooperative basis. A successful solution to the task of effective use of resources, optimization of milk production and provision of milk and dairy products to the population of the country is possible only in the case of close cooperation of all branches and sub-branches of the industry of the state and enterprises where direct production, transportation, storage, processing, and sale of milk is carried out. All these links should form a single, balanced system. In the post-war period, this will raise the level of industry specialization and determine the effectiveness of all participants in the dairy chain.

Conclusions

The production of milk is an important aspect of the economic development of the agricultural sector of Ukraine in the post-war period. It is necessary to develop anti-crisis measures to preserve the functioning of dairy farms, develop dairy farming, stop the decrease in the number of cows at enterprises and improve the quality of dairy products. Analyzing the indicators of dairy production in the pre-war and post-war periods, significant changes are observed. The indicators of milk production and export for 2017-2021 showed its reduction from 20.7% of the total volume to 15.1%. Such changes are also observed during the war period (2022-2023 years). Since the beginning of the full-scale war, numerous dairy farms and processing enterprises have suffered or found themselves in temporarily occupied territory. Statistical data, in general, for 2022, milk production in Ukraine decreased by 12.1%, which amounted to 7.6 million tons.

The real state of milk production enterprises reflects the decline in resource efficiency and the optimization of milk production. And thanks to the effective activity of dairy farms in safe regions and individual regions, high-quality production and sales of products are carried

out in compliance with all requirements, as well as the relocation of livestock from affected areas. This makes it possible to minimize the losses of dairy enterprises. Problems in the Ukrainian legislation are highlighted, which hold back projects to support the successful development of the dairy industry. To change this situation at the legislative level, it is necessary to make appropriate changes and develop a policy aimed at overcoming crisis phenomena. On the basis of their own research and the opinions of other scientists, the ways of successful activity and production of milk products in the post-war period have been determined, namely, the effective use of resources and their optimization of opportunities, full support of the dairy business, improvement of innovative activities, the use of achievements of scientific and technical progress and the experience of foreign countries. the use of various forms of management, the contribution of investments to the development of the industry.

Further scientific research should consider the issue of the development of the application of the proposed prospects for the production of milk and milk products in Ukraine in the post-war period, in particular, the analysis of their impact on the state and development of the country's economy.

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Conflict of Interest

None.

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Аналіз стану та перспектив виробництва молока і молокопродукції в Україні в повоєнний період

Тетяна Анатоліївна Гуцул

Кандидат економічних наук

Національний університет біоресурсів і природокористування України

03041, вул. Героїв Оборони, 15, м. Київ, Україна

<https://orcid.org/0000-0002-1826-240X>

Наталія Миколаївна Суліма

Кандидат економічних наук

Національний університет біоресурсів і природокористування України

03041, вул. Героїв Оборони, 15, м. Київ, Україна

<https://orcid.org/0000-0002-3852-7989>

Борислав Костянтинівич Кудерський

Магістр

Національний університет біоресурсів і природокористування України

03041, вул. Героїв Оборони, 15, м. Київ, Україна

<https://orcid.org/0009-0004-1118-1393>

Анотація. Показники останніх років демонструють стійкість і високі результати щодо виробництва молока в сільськогосподарських підприємствах. Актуальність підтверджена тим, що виробництво молока і його оптимізація є ключовими аспектами економічного розвитку сільськогосподарського сектора України в повоєнний період, адже молочне тваринництво є системоутворюючою галуззю сільського господарства, яка відкриває нові можливості для становлення перспективних векторів розвитку бізнесу. Метою статті є аналіз проблем та перспектив економічних аспектів ефективності використання ресурсів та оптимізації виробництва молока в сільськогосподарських підприємствах України в повоєнний період. У дослідженні було використано наступні методи: індукція та дедукція, синтез, теоретичне узагальнення, статистичний аналіз, порівняльний аналіз, моделювання. Досліджено зв'язки невідповідності між ресурсами, виробництвом молочної продукції і можливостями підприємств, політикою держави, рівнем і нормами споживання молока й молочних продуктів населення в повоєнний період. Відповідно до цього, розглянуто динаміку виробництва, експорту, збуту, етапів виробництва, особливості кооперації та імпорту основних молочних продуктів. Встановлено безпосередній зв'язок між виробництвом і споживанням молока, залежність попиту і споживання молока й молочних продуктів від рівня сукупних доходів домогосподарств. На основі цього в роботі подано повну характеристику молочної галузі в Україні, обсяг виробництва молока в довоєнний період і перспективи розвитку в повоєнний час. Наведені в систематизованому вигляді дослідження властивостей ринку різних типів молочної продукції України за допомогою SWOT- та PEST-аналізу загалом. Для покращення стану ринку молока та молочної продукції України сформульовано відповідні пропозиції. Практична цінність роботи полягає в тому, що її результати можуть бути використані як підстава для розробки технологій використання ресурсів виробництва молока в повоєнний період, а також у роботі спеціалістів агропромислового й економічного секторів

Ключові слова: економічний розвиток; перспективи та інновації; конкурентоспроможність; інвестиції



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Increasing the productivity of young pigs in the context of overcoming technological stress

Marina Koroban*

Postgraduate Student

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

<https://orcid.org/0009-0003-1763-2629>

Vadym Lykhach

Doctor of Agricultural Sciences

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

<https://orcid.org/0000-0002-9150-6730>

Anna Lykhach

Doctor of Agricultural Sciences

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

<https://orcid.org/0000-0002-0472-6162>

Yevhen Barkar

PhD in Agricultural Sciences

Mykolaiv National Agrarian University

54008, 9 Georgiy Gongadze Str., Mykolaiv, Ukraine

<https://orcid.org/0000-0002-0692-5392>

Sergiy Chernysh

Commercial Director

Limited Liability Company “Agrotradehim”

03022, 34 Vasylykivska Str., Kyiv, Ukraine

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*Corresponding author



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Abstract. Production activities are mandatory elements of the technological cycle and cause various types of animal stress. The use of feed additives based on plant extracts in a liquid composition for pigs is an important strategy for developing their stress resistance and increasing productivity. The purpose of the experiment is to investigate the optimal duration of drinking a liquid feed additive in different periods of technological stress on pig productivity parameters. In farm conditions, 90 crossbred pigs were randomly divided into three groups according to generally accepted zootechnical methods. Animals of the I group (control) were fattened according to the basic technology; animals of II experimental group – BT+ administration of liquid feed additive three days before and three days after the technological action, and pigs of III experimental group – BT+ administration of liquid feed additive seven days before and seven days after. Swine of the III research group at the age of 11-26 weeks probably exceeded their counterparts in live weight by 1.70-5.43 kg, in average daily gains in the period from 14-22 weeks – by 20.0-82.5 g. According to the precocity indicator, pigs of the III group reached a live weight of 100 kg 6.1 days earlier and 120 kg 7.3 days earlier. The values of average daily gains in pigs of the III group were probably higher by 51.9 g – for a live weight of 100 kg and 38.1 g – for 120 kg. Pigs of III group had the lowest level of feed conversion – 0.11 kg at 100 kg and 0.14 kg at 120 kg. It has been established that in order to increase the productivity of pigs, it is worth drinking LFA 7 days before and 7 days after the period of technological stress during the entire growing period. The practical significance of the experiment is to identify the optimal duration of the use of liquid feed additive to increase the stress resistance and productivity of pigs

Keywords: fattening; weight condition; liquid feed additive; stress corrector; technology

Introduction

The production of pork on an industrial basis is aimed at obtaining the maximum benefit in the shortest possible time, and is also based on the principle of a technological conveyor, which does not sufficiently take into account the biological needs and capabilities of pigs (Povod *et al.*, 2021). The body of pigs is affected by numerous factors: the physiological state of sows, the presence of various diseases, the process of weaning piglets from the sow (Ramirez *et al.*, 2022), the process of transportation to the point of slaughter, sharp changes in temperature regimes in the room for keeping animals (Yang *et al.*, 2021), inappropriate behaviour, aggressive behaviour (Nielsen *et al.*, 2022), which cause psychological and physical distress (Nazarenko, 2023) and, as a result, maladaptive behaviour of pigs and a decrease in productive characteristics. And therefore, without proper support of the pig body with stress correctors, a number of biomarkers are

produced, in particular: reactive oxygen species, malonic acid dialdehyde, cortisol and immunoglobulins causing diarrhoea and growth retardation in pigs (Upadhaya & Kim, 2021), reduce the reproductive characteristics of middle-aged pigs, and due to accelerated muscle glycogenolysis, the moisture-retaining capacity of meat decreases (Gonzalez-Rivas *et al.*, 2020) as a result of which there are economic losses in the pig industry.

Feed “interventions” are effective strategies in combating the consequences of stress factors in pig farming, such as: deterioration of health, loss of live weight and growth parameters, deterioration of meat quality, etc. (Lauridsen, 2020). Feed additives based on plant extracts are of increased interest in the production of animal feed. There are 250,000 to 500,000 species of plants on earth; however, only a small fraction (1-10%) is used for human consumption

or for the production of animal feed. Recently, more attention has been paid in pig farming to stress-correcting supplements, functional amino acids, low-protein feed, plant extracts, organic acids, prebiotics, probiotics, minerals, and vitamins. These measures demonstrate the potential and vector of changes in the composition of the intestinal microbiota, improvement of its barrier function, assimilation of nutrients and, as a result, an increase in pig productivity (Bomko *et al.*, 2023).

As noted by a number of researchers, the intestine is the main organ of digestion and absorption of feed (Hao *et al.*, 2021). The physical, chemical and immune layer of the intestinal mucosa acts as a defence against the invasion of bacteria, endotoxins, and antigens in response to stress (Li *et al.*, 2018). In this regard, in practical pig farming, two main methods of delivering target components (stress correctors) to the body are used – with feed (added during the production of compound feed in the form of premixes) and with water (through medicators to the watering system). The way through feeding was the most common and tested in many experiments (Povod *et al.*, 2021), and various methods and functional feeds with increased concentrations of various antioxidants were developed for use in stress conditions. However, it was later found out that using such functional feeds and additives in production conditions is technologically quite difficult. And most importantly, in a state of stress, the animal consumes much less feed.

Therefore, in such production conditions, the need for vitamins, minerals, and a number of other substances increases, and their supply with feed decreases. And as a result, even more strengthening of the negative effects of stress. The dosing devices at watering system has firmly entered the technology of pork production. Currently, medicinal products, probiotics, vitamins and other drugs are administered through the system of dosing devices. Therefore, when developing technological maps of production in pig farming and combating stresses of various

origins, practitioners proceed from the fact that the inclusion of the drug in the drinking system through the medicator is the most effective way to achieve the goal. And this approach makes it possible to quickly respond to a stressful situation as a whole (Lykhach *et al.*, 2022).

Therefore, there is an important scientific and practical task of researching the optimal duration of the influence of the liquid fraction of the feed additive when it is introduced into the feeding system with the help of a medicator on the fattening characteristics of pigs. Therefore, the purpose of the work is to study the duration of the effect of a liquid feed additive on the productive qualities of fattening young pigs of different weight conditions for use in periods of significant technological stress (weaning, transfer of young animals to rearing and fattening, change of rations, etc.).

Materials and Methods

Experimental studies were carried out during 2023 in the conditions of the agricultural production cooperative “Agrofirma” “Mig-Service-Agro” of the Mykolaiv region. The production of pig products in the conditions of the enterprise met the conditions of industrial technology with the appropriate organization of technological processes. As part of the scientific and economic experiment, 90 heads of fattening young pigs of the combination ♀ (VB×L)×♂ were used Maxter, which were kept in the conditions of the specified farm.

The experimental young were randomly divided into three groups: I – control group, II and III – experimental groups, according to the scheme of the experiment (Table 1). Liquid feed additive “LIPTOTRAN L” (*Lipidos Toledo S.A., Liptosa, Spain* (registration certificate AA-08631-04-19 dated 06.08.2019) (Product registration certificate Liptotran L) was introduced into the water supply system using the medicator “Dozatron” (France) at a dose of 1 l per 1000 l of drinking water in the corresponding fattening periods.

Table 1. Scheme of a scientific and economic experiment

No.	Group	Elements of the technological map of raising young pigs
I	control	BT*
II	experimental	<ul style="list-style-type: none"> ➤ BT + introduction of LFA** three days before weaning and three days after weaning (week 4); ➤ BT + introduction of LFA** three days before transfer to fattening (11 weeks) and three days after; ➤ BT + introduction of LFA** three days before transfer to final fattening (16 weeks) and three days after.
III	research	<ul style="list-style-type: none"> ➤ BT + administration of LFA** seven days before weaning and seven days after weaning; ➤ BT + introduction of LFA** seven days before transfer to fattening and seven days after; ➤ BT + introduction of LFA** seven days before transfer to final fattening and seven days after.

Notes: *BT – the basic technology for growing young pigs, which is adopted and approved in this experimental farm; ** – LFA – liquid feed additive “LIPTOTRAN L” manufactured by Lipidos Toledo S.A., Liptosa, Spain

Source: author’s development

The composition of 1 kg of liquid feed additive “LIPTOTRAN L” contains the following active components (%): magnesium chloride – 1.00; sodium chloride – 0.50; propylene glycol – 1.50; propionic acid – 1.60; ammonium propionate – 0.32; flavourings (Esholtia Californian, valerian and lemon balm) – 5.0; excipient (water) – up to 100% (Liptotran L – product information). The components of the specified feed additive, in particular: magnesium chloride – the main intracellular cation and important K-factor for many enzymes, participates in several biochemical and physiological processes that are important for the functioning of muscles and the formation of bone tissue. Propionic acid and ammonium propionate suppress the development of pathogenic microorganisms in the intestine, prevent the development of digestive tract diseases and have an antifungal effect, reduce the *pH value* of the intestinal contents, create optimal conditions for the development of propionic and lactic acid bacteria and inhibit the reproduction of pathogenic microflora. Sodium chloride is the main source for the formation of hydrochloric acid in gastric juice, it is well absorbed from the gastrointestinal tract when taken internally and has a selective effect on tissues and organs. Extract of California eschscholzia (*e schscholzia*

californica), has a calming effect on the entire nervous system, is a gentle and mild tonic for the nervous system when it is under stress, providing soothing, analgesic and antispasmodic activity, promotes sleep and inhibits motor activity, contains alkaloids berberine, protopine, californidin, allocryptopine, which belong to the group of benzyloisoquinoleins, flavonoids, cyanogenetic heterosides and carotenoids. Melissa extract (*m elissa officinalis*), due to the presence of maltol and ethyl maltol, has a sedative and tranquillizing effect on the central nervous system, has anticonvulsant, analgesic and anxiolytic effects. Valerian extract (*valeriana officinalis*) exhibits antispasmodic properties, which affects the relaxation of smooth muscles (related to the content of valenol and valeric acid) – hypnotic and anticonvulsant properties. Its activity is due to the synergism between serkiterpenes (valeric acid), GABA (gamma- aminobutyric acid), glutamine and flavonoids. This reduces the degradation of GABA, increasing its release into synaptic spaces and reducing its reuptake, causing a sedative effect. It contains glutamine, which can be transformed into GABA in the body (Chudak *et al.*, 2021).

Conditions for keeping experimental animals are organized according to Departmental

norms of technological design Pig enterprises (2005) and recommendations of genetic companies on maintenance. Superstarter feed and protein-mineral-vitamin supplements and pre-mixes produced by Cowdais Ukraine LLC were used to feed suckling piglets and balance the rations of young animals during rearing and fattening.

In experimental animals using liquid feed additive “LIPTOTRANL”, the following were studied: live weight (kg), average daily gain (g) according to methods generally accepted in pig breeding (Ibatulin & Zhukorskyi, 2017). The study of fattening characteristics (age of reaching live weight of 100 and 120 kg (days), average daily gain during fattening (g), feed conversion (kg) of experimental animals when they reach pre-slaughter live weight of 100 and 120 kg was carried out according to appropriate methods (Povod et al., 2021; Ladyka & Khmelnychiy, 2023). The conditions of feeding, watering, keeping, care and prevention of the animals in the experiment took place in accordance with the European legislation on the protection of animals and their comfort (Council Directive 91/630/EU, 2008/120/EU, 2008; Council Directive 98/58/EU, 2008; Council Directive 2010/63/EU, 2010).

Experimental data were processed by the method of variational statistics using computer equipment and MS Excel 2000 and Statistica V.5.5 application software packages.

Results and Discussion

In the conditions of an experimental farm a four-phase system of growing young pigs was implemented, according to which piglets after weaning (4 weeks) are transferred to the rearing shop, where they remain until they reach a weight of 30 kg, and then move to the fattening shop (11 weeks) with a division into fattening periods: I period (30-60 kg) and II period (60-120 kg), in which they are kept until delivery to the meat processing plant. According to literary data (Lykhach et al., 2022) this method of cultivation is physiologically difficult for animals and causes stress during two regroupings, which reduce the productivity of animals, but from an economic point of view, it is the most justified.

Thus, taking into account the active composition of the liquid feed additive in the production conditions of the basic pig complex, its influence on indicators of live weight and average daily growth of young pigs was investigated (Table 2).

Table 2. Productive characteristics of experimental groups of pigs (n=30), $\bar{X} \pm S_{\bar{x}}$

Sign	Group/Age		
	I – control	II – experimental	III – experimental
4 weeks			
Live weight, kg	8.00±0.111	8.14±0.112	8.23±0.119
11 weeks			
Live weight, kg	32.63±0.273	33.70±0.359°	34.33±0.241***
Average daily gain, g	502.8±4.60	521.6±5.82**	532.7±3.89***
14 weeks			
Live weight, kg	46.67±0.344	48.13±0.328**	50.10±0.308***s
Average daily gain, g	668.3±14.35	687.3±10.76	750.8±5.43***s
17 weeks			
Live weight, kg	63.40±0.382	65.90±0.391***	68.03±0.394***s
Average daily gain, g	796.8±10.79	846.0±11.34**	854.0±12.69***
22 weeks			
Live weight, kg	94.00±0.432	97.10±0.385***	99.33±0.471***s

Table 2. Continued

Sign	Group/Age		
	I – control	II – experimental	III – experimental
Average daily gain, g	874.3±8.18	891.4±6.03	894.3±5.62 ^a
26 weeks			
Live weight, kg	115.17±0.356	118.87±0.287 ^{***}	120.60±0.228 ^{***s}
Average daily gain, g	755.9±13.16	777.4±11.95	759.5±15.12

Notes: ^a – $p < 0.05$; ^{**} – $p < 0.01$; ^{***} – $p < 0.001$ (in comparison with animals of the control group – Group I); ^a – $p < 0.05$; ^b – $p < 0.01$; ^s – $p < 0.001$ (comparing animals of experimental group III with analogues of experimental group II)

Source: author's calculations

It was established that during the period of fattening from weaning (4 weeks) to a live weight of 120 kg (26 weeks), the young animals of the experimental groups probably outperformed the control counterparts in terms of productive characteristics. Thus, at the age of 4 weeks, young pigs of the II and III experimental groups exceeded the control animals by 0.14 kg and 0.23 kg, respectively, according to the live weight indicator, but the difference is statistically not significant. At the age of 11 weeks, young animals of the III experimental group, which already received a feed supplement twice during the growing period (Table 1), significant exceeded the control group (I) in terms of live weight by 1.7 kg ($p < 0.001$) (Table 2). The animals of the II experimental group during the shorter period of use also exceeded the control peers by 1.07 kg in live weight ($p < 0.05$). In this age period, not significant difference was found between the experimental groups. Regarding the index of average daily gains, a similar trend is noted, their values were higher in the animals of the experimental groups. The fact that the live weight and average daily growth rates of the experimental groups were higher than those of the control group was due to the presence in the feed supplement of functional components with a calming effect, which allowed the animals of the experimental groups to resiliently overcome the manifestations of technological stress: veterinary preventive vaccinations, weaning, transition from pre-starter

compound feed to starters, not reducing, but, on the contrary, increasing indicators of productive signs.

Similar studies were obtained by R. Faustov *et al.* (2022) on fattening pigs, which were fed the complex drug “Hepasorbex” together with the main diet. The scientists found that pigs in the third experimental group for 14 weeks, consuming this feed additive, probably exceeded the animals of the control group in terms of live weight by 1.93 kg ($p < 0.05$), and in terms of average daily gain, the advantage was observed relative to control animals group by 114.3 g ($p < 0.001$), as well as 2 experimental groups, a similar trend was observed in the following age periods. According to the researchers, this advantage of the experimental groups over the control group in terms of live weight and average daily gains is due to the presence of the following active components in the complex feed additive: silicon dioxide; aluminum oxide; magnesium carbonate; titanium dioxide; selenium; clinoptilolite; dry brewer's yeast, milk thistle. The obtained practical results are consistent with the results of research, which indicates the objectivity and scientific reliability of the conducted experiments.

It is worth noting that stress during the weaning of piglets from sows is often accompanied by intestinal, microbial and immunological changes that cause diarrhoea and other diseases and, as a result, negatively affects the realization of the productive characteristics of

fattening pigs (Fairbrother *et al.*, 2005). M. Peng *et al.* (2019) in their experiments on piglets after weaning proved that dietary supplements with *Eucommia leaf extract ulmoides* improve the growth indicators of piglets (the average daily gain of experimental animals increased by 65 g ($p<0.05$) compared to control counterparts), feed conversion (by 0.06 kg ($p<0.05$)) and the morphology of the small intestine (an increase in the depth of microvilli crypts by 1.25 times) with a simultaneous decrease (by 2.8 times) in the frequency of diarrhoea. *Eucommia oleracea* is a traditional Chinese medicinal herb, and therefore has a composition rich in biologically active compounds and exhibits anti-inflammatory, antioxidant, antiviral and hepatoprotective functions. In the same case, under the influence of technological stress, a liquid feed additive containing propionic acid prevented the development of gastrointestinal disorders in piglets, and therefore diarrhoea was not observed in the experimental groups.

In the course of the experiment, it was observed that pigs that used a feed additive, which includes an extract of *Eschscholzia californica*, showed less toxic oral manipulations in relation to each other, the consequences of oxidative stress were easier due to the presence of a number of useful phytochemical additive compounds. Similar results were obtained by M. Han *et al.* (2016), using grape seed proanthocyanidins at a dose of 250 mg/kg in the feed of pigs, which contributed to a 1.8-fold reduction in their aggressive behaviour, an increase in average daily growth from 467 g to 499 g, eased the course of diarrhoea from 34 to 23 cases due to reduction of intestinal permeability and improvement of its morphology, respectively, and also increased the average assimilation of feed of experimental pigs from 862 g to 893 g per day. As noted by G. Chen *et al.* (2015; 2020), due to the high therapeutic and nutritional effects of alkaloids and flavonoids of the extract of leaves of paper mulberry (*Broussonetia papyrifera*) in the amount of 150-300 g/t in thirty piglets during the 42 days

of the experiment, the live weight increased by 12.8%, their average daily gains increased by 9.8%, the frequency of diarrhoea decreased by 62.9%, and the feed conversion ratio increased by 1.09 times, which indicates the mental and physical resilience of animals.

The analysis of the productivity of young pigs in the following age periods makes it possible to state that it is at this time (14-22 weeks of fattening) that the positive effect of the liquid feed additive (II and III research groups), which was used at the stages of weaning and transfer to fattening, is more effectively manifested, therefore, in periods of significant technological stress. Thus, at the age of 14 weeks, young pigs that received a stress corrector during periods of technological stress in accordance with the scheme of the experiment (Table 2) with prolonged exposure, of the III group probably exceeded in terms of live weight and average daily growth of peers of the control group and analogues of the II experimental group groups that received a stress corrector for schemes: three days before and three days after changing the technology of fattening and growing. We note that the animals of the III experimental group exceeded the analogues from the I and II groups by 3.43 kg and 82.5 g in terms of live weight and average daily gains ($p<0.001$); 1.97 kg and 63.5 g ($p<0.001$), respectively.

At the age of 17 weeks, a similar trend was observed, the animals that received a liquid feed additive during periods of technological stress (II and III experimental groups) probably outperformed the control (I group) in terms of live weight and average daily gains. In the final phase of fattening (22-26 weeks), which is equivalent to the age of reaching a live weight of 100 and 120 kg, it is possible to note somewhat equalized indicators of average daily gains in the section of groups, which is confirmed by the fact that during these age periods the animals were almost not subjected to technological stress, had already stably formed groups according to behavioural characteristics (stable

hierarchical relations were established) and therefore the indicators of growth energy were equalized. But, taking into account the significant advantage of the experimental groups in terms of average daily growth rates over the control group in the early periods (significant technological stress), in the age period of 22-26 weeks there is a probable advantage of young animals of the II and III groups over the analogues of the I group in terms of live weight – 3.1 kg ($p<0.001$) and 5.33 kg ($p<0.001$) and 3.7 kg ($p<0.001$) and 5.43 kg ($p<0.001$), respectively.

Based on the comparison of indicators of live weight and average daily gains in the control and experimental groups, it can be stated that longer exposure to the use of the stress corrector is more effective, because the animals of the III experimental group in all age periods had higher indicators than both the control pigs and the analogues of the II research group. The expediency of using a liquid feed additive during the period of fattening of young pigs in order to overcome the negative consequences of technological stress in the conditions of industrial technology due to the increase of productive characteristics in all age periods has also been proven.

The obtained results of the study are consistent with a number of experiments conducted in order to overcome the effects of technological stress. Thus, a group of researchers from Wuhan (People's Republic of China) conducted an experiment on 170 fattening pigs with a random distribution of animals into two groups, where the first group – the control group – received

the basic diet, and the pigs of the second group (experimental) received the same basic diet with the addition of quercetin in a dose of 25 mg per 1 kg of feed. After the experiment, the scientists recorded that the second group of experimental pigs had natural flavonoids quercetin increased feed conversion by 1.95 times, average daily gains increased by 20 g, pigs better overcame the effects of technological stress in fattening conditions (Zou *et al.*, 2016). In the conditions of the cooperative innovation centre of sustainable pig farming in China, researchers together with scientists of the nutrition laboratory of the Faculty of Veterinary Medicine of Aristotle University (Greece) randomly divided 180 domestic pigs into two groups: the animals of the first control group consumed the basic diet, and the peers of the second experimental group together with the basic diet consumed essential oil of oregano with carvacrol and thymol in a dose of 25 mg per 1 kg of feed. Based on the research results, the scientists noted that the pigs of the second research group reduced the level of aggressive behaviour and quickly overcame the effects of technological stress, in particular transport stress, and the pig population increased by average daily gains from 16.2 to 20.6 g (Zhang *et al.*, 2015).

As a result of the study of the fattening qualities of the young pigs of the experimental groups, depending on the use during significant technological stress of liquid feed additive (Table 3), it was established that the animals that had the opportunity to receive the stress corrector were more precocious.

Table 3. Performance responses of fattening pigs, ($n=30$), $\bar{X} \pm S_{\bar{X}}$

Group	Age a live weight, days	Average daily gain, g	Feed conversion, kg
	live weight 100 kg		
I – control	161.0±0.57	876.7±5.99	2.91
II – experimental	157.4±0.47	905.7±6.19	2.82
III – experimental	154.9±0.53	928.6±6.20	2.80
+/- II to I	-3.6***	+29.0***	-0.09
+/- III to I	-6.1***	+51.9***	-0.11

Table 3. Continued

Group	Age a live weight, days	Average daily gain, g	Feed conversion, kg
+/- III to II	-2.5 ^s	+22.9 ^b	-0.02
live weight 120 kg			
I – control	188.5±0.55	842.2±4.58	3.32
II – experimental	183.5±0.39	869.1±4.24	3.22
III – experimental	181.2±0.30	880.3±3.13	3.18
+/- II to I	-5.0 ^{***}	+26.9 ^{***}	-0.10
+/- III to I	-7.3 ^{***}	+38.1 ^{***}	-0.14
+/- III to II	-2.3 ^s	+11.2 ^a	-0.04

Notes: * – p<0.05; ** – p<0.01; *** – p<0.001 (in comparison with animals of the control group – Group I); a – p<0.05; b – p<0.01; s – p<0.001 (comparing animals of experimental group III with analogues of experimental group II)

Source: author’s calculations

Thus, the young pigs of the III experimental group reached a live weight of 100 and 120 kg 6.1 and 7.3 days earlier, compared to animals of the control group, and 2.5 and 2.3 days earlier, compared to the analogues of the II experimental group, (p<0.001). The value of indicators of average daily growth closely correlates with precociousness. So, the more animals reach their final live weight, the higher their growth. Values of average daily gains in pigs of the II and III experimental groups were probably higher (p<0.001) than the control, by 29.0 g and 51.9 g, respectively; 26.9 and 38.1 g at weight conditions of 100 and 120 kg.

With regard to the conversion of feed for the II experimental group, the indicator reached 2.82 kg, and for the III experimental group – 2.80 kg, which is lower than the similar indicator of the animals of the I control group by 0.09 and 0.11 kg, where the feed conversion is 2, 91 kg when fattening up to a live weight of 100 kg. A similar trend is observed in the next weight category – 120 kg. Similar results were obtained by researchers in Germany and France on 120 heads of crossbred piglets of breed combinations, where the maternal basis was animals (German Landrace × Big White), and the paternal basis was Pietren, which received low-protein diets with different contents of crystalline essential amino acids, such as leucine and histidine. During the six weeks of

the experiment, experimental pigs significantly (p<0.001) reduced feed consumption per unit of gain, and their average daily gains increased to 8.7% (Wessels *et al.*, 2016). In addition, in this experiment, the optimal amount of amino acid introduction was determined to optimize the ratio of growth to feed, which is a distinguishing feature of our experiment (liquid feed additive was added to water and the time of its drinking was taken into account). In turn, studies of L. Wang *et al.* (2023) established that drinking organic acids together with water to pigs with the help of a medicator through a water system contributes to excellent digestibility of feed in the diet, ensures a high degree of conversion of consumed feed, reduces the colonization of pathogenic microorganisms, increases appetite in animals, stimulates growth parameters and increases motor activity pigs, as they are used as an energy source.

In the section of experimental groups (II and III), there is an advantage of the animals of the III experimental group in terms of fattening qualities, which received the stress corrector for a longer period during periods of technological stress (7 days before and 7 days after) over the animals of the II experimental group, which received the specified supplement with a shorter duration (3 days before and 3 days after) in weight conditions of 100 and 120 kg, which is consistent with the results of experiment

L. Wang *et al.* (2023). Scientists from a number of laboratories and institutes in the Hunan province of the People's Republic of China have developed various nutritional strategies for pigs to combat stress and replace antibiotics, including phytogetic feed additives, plant extracts, organic acids, functional amino acids, low-content of protein, prebiotics, probiotics, vitamins, minerals, etc. Scientists have established that the addition of plant extracts to the main diet of pigs, which have been used for a long time in traditional medicine for the purpose of therapeutic and preventive functions, positively affects the increase of stress resistance of animals, the growth of live weight from 3.2-4.6%, average daily gains from 10.2 g to 12.4 g, reducing the age of reaching live weight to slaughter conditions from 1.6 to 2.4 days.

It is worth noting that as a result of the research, the experimental young of the III research group reached a live weight of 100 and 120 kg earlier than the analogues of the II research group by 2.5 days ($p<0.001$) and 2.3 days ($p<0.001$), respectively. Average daily gains of animals of the III group were higher by 22.9 g ($p<0.01$) when fattening up to a live weight of 100 kg and 11.2 g ($p<0.05$) when fattening up to 120 kg compared to analogues from the II experimental group. Thus, increasing the stress resistance of pigs to overcome technological stress by adding appropriate functional components of plant origin, optimizing their quantity and duration of use, is currently a useful practice to increase the alternative of key signal searches in pig farming.

Conclusions

On the basis of the obtained research results, it was established that the liquid feed additive, as an additional component of the diet, prevents, to a certain extent, the manifestation of technological stress during the critical periods of fattening of young pigs, and due to the innovative composition, it stimulates their internal body reserves and has a calming effect without

reducing productive qualities, in contrast to traditional technology. It was found that the II and III experimental groups, which received a liquid feed supplement along with water in the critical periods of fattening according to the research scheme, had a significantly: increase in live body weight by 0.14-3.1 kg and 0.23-5, 43 kg; average daily gains – 17.1-49.2 g and 3.6-57.2 g. Pigs of the III experimental group reached a live weight of 100 kg 6.1 days earlier and a slaughter weight of 120 kg 7.3 days earlier than peers of the control group. Relative to the analogues of the II research group, the probable advantage was recorded again in the animals of the III group, since they exceeded the age of reaching a live weight of 100 kg by 2.5 days, and 120 kg by 2.3 days, the difference is statistically significant ($p<0.001$). Indicators of average daily gains in pigs of both II and III experimental groups were significantly higher ($p<0.001$) compared to control analogues, by 29.0 g and 51.9 g, respectively; 26.9 and 38.1 g at weight conditions of 100 kg and 120 kg. Pigs of the III experimental group had the lowest level of feed conversion when they reached the slaughter weight of both 100 kg and 120 kg, which is 0.11 kg and 0.14 kg, respectively, lower than the animals of the I control group. It was established that the animals of the III experimental group, which received the stress corrector for a longer period during the periods of technological stress (7 days before and 7 days after), had an advantage in terms of productive parameters over the animals of the II experimental group, which consumed the specified supplement with a shorter duration (3 days before and 3 days after) during the entire research period.

Prospects for further experiments are to study the effectiveness of using a liquid feed additive on other technological groups of pigs.

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None.

Conflict of Interest

None.

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Підвищення продуктивності молодняку свиней в контексті подолання технологічного стресу

Марина Петрівна Коробань

Аспірант

Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0009-0003-1763-2629>

Вадим Ярославович Лихач

Доктор сільськогосподарських наук

Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0002-9150-6730>

Анна Василівна Лихач

Доктор сільськогосподарських наук

Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0002-0472-6162>

Євген Володимирович Баркарь

Кандидат сільськогосподарських наук

Миколаївський національний аграрний університет
54008, вул. Георгія Гонгадзе, 9, м. Миколаїв, Україна
<https://orcid.org/0000-0002-0692-5392>

Сергій Володимирович Черниш

Комерційний директор

Товариство з обмеженою відповідальністю «Компанія «Агротрейдхім»
03022, вул. Васильківська, 34, м. Київ, Україна

Анотація. Виробничі дії є обов'язковими елементами технологічного циклу і викликають різні види стресу тварин. Використання кормових добавок на основі рослинних екстрактів у рідкій композиції для свиней є важливою стратегією для випрацювання їх стресостійкості та підвищення продуктивності. Мета експерименту – дослідити оптимальну тривалість випоювання рідкої кормової добавки у різні періоди технологічного стресу на параметри продуктивності свиней. В умовах ферми, помісних свиней чисельністю 90 голів рандомно розділили на три групи згідно з загальноприйнятими зоотехнічними методами. Тварини I

групи (контрольна) відгодовувалися за базовою технологією; тварини II дослідної групи – БТ + введення рідкої кормової добавки за три доби до технологічної дії і три доби після, а свині III дослідної групи – БТ + введення рідкої кормової добавки за сім діб до і сім діб після. Свині III дослідної групи у віці 11-26 тижнів вірогідно перевищували за живою масою на 1,70-5,43 кг, за середньодобовими приростами у період з 14-22 тижня – на 20,0-82,5 г своїх аналогів. За показником скороспілості свині III групи на 6,1 добу раніше досягали живої маси 100 кг і на 7,3 доби – 120 кг. Значення середньодобових приростів у свиней III групи були вірогідно вищими на 51,9 г – за живої маси 100 кг і 38,1 г – 120 кг. Свині III групи мали найнижчий рівень конверсії корму – 0,11 кг при вазі 100 кг і 0,14 кг – 120 кг. Встановлено, що для підвищення продуктивності свиней варто їх випоювати РКД 7 діб до і 7 діб після періоду технологічного стресу впродовж всього періоду вирощування. Практичне значення експерименту полягає у виявленні оптимальної тривалості застосування рідкої кормової добавки задля підвищення стресостійкості і продуктивності свиней

Ключові слова: відгодівля; вагова кондиція; рідка кормова добавка; стрес-коректор; технологія



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Morphological variability of individual colour forms of *Cyprinus carpio haematopterus* of different origin

Oleksandr Lysak*

Graduate Student

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

<https://orcid.org/0009-0007-4238-905X>

Petro Shevchenko

PhD in Biological Sciences, Associate Professor

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

<https://orcid.org/0000-0002-5996-4328>

Mykola Ratushny

Graduate Student

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

<https://orcid.org/0009-0008-2540-1317>

Dmytro Khrystenko

PhD in Biological Sciences

Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine

03164, 135 Obukhivska Str., Kyiv, Ukraine

<https://orcid.org/0000-0002-1432-7093>

Ganna Kotovska

PhD in Biological Sciences

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

<https://orcid.org/0000-0001-7155-1086>

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*Corresponding author



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Abstract. The relevance of the work on the study of morphological indicators of koi lies in the increased attention at international koi exhibitions to such external indicators as colour, shape, and body proportions of fish that occupy high prize places and receive monetary rewards of hundreds of thousands of US dollars. The purpose of the work was to study the morphological features of various coloured forms of various origins. Such indicators are not taken into account in traditional fish breeding, which also adds novelty to the conducted research. 280 koi samples were selected for analysis. The work evaluated the variability of plastic features of fish of individual colour forms (*magoi*, *kumonryu*, *utsurimono*, *kohaku*, *kawarimono*, *bekko*, *doitsu bekko*, *doitsu kawarimono*) of different origins (Japanese, Turkish, Israeli, and Ukrainian). As a result of the work, it was established that there was not found a statistically significant difference between different colour forms of the same origin. At the same time, a reliable difference was established in fish of Israeli origin in terms of features characterizing the shape and proportions of the head in relation to the proportions of the body. The distribution of morphological indices of the studied individuals of different samples in the space of the main components also confirms the reliable differences of the Israeli morphotype from fish of Japanese, Turkish, and Ukrainian origin according to the characteristics related to the ratios of the head parts and body proportions. The obtained data is the basis for the work of breeders and breeders of koi carp who wish to grow valuable specimens – potential winners of international koi carp competitions

Keywords: koi carp; who; nishiki-go; method of principal components

Introduction

Common carp was traditionally raised in ponds of various sizes and used for human consumption. It is known that in the isolated mountainous area of Japan between the cities of Nagaoka and Ojiya, about 180 years later, selection and crossing of different colour variations of carp began. The selection and formation of coloured forms began approximately in the 50s of the last century, when decorative fish farming spread massively in Japan. The carp that is known now is a colour variation of common carp (*Cyprinus carpio* L.), which is confirmed by modern genetic studies X. Li *et al.* (2023). In modern conditions, about 80 breeds of carp are known. For convenience, they are divided into main groups based on the characteristics of their colour, the number of which does not have a single point of view. Most researchers distinguish 15 such groups, which are combined by one or more characteristics. Most of the studies characterizing various aspects of koi carp relate to the study of colour inheritance, the study of the genetics of colour forms, thanks to which the common carp was differentiated from the coloured forms of koi

(Dong *et al.*, 2020, Luo *et al.*, 2023). At the same time, the individual lines themselves are not differentiated from each other (Jawad *et al.*, 2022; Du *et al.*, 2022). In the process of painstaking selection work, the bulk of the fish is consumed as food, and only single individuals with the most promising and “standard” colour are selected for further work and receive the name “nishiki-go” or koi (Kaur *et al.*, 2022). In general, the larval period of the life of these fishes is quite well studied (Hajibeglou *et al.*, 2022), as well as their size-age characteristics in adulthood (Nazari *et al.*, 2023). There are a number of studies on the characteristics of survival under the influence of stress factors and diseases (Grynevych *et al.*, 2022; Hasnidar *et al.*, 2023).

Exhibitions and championships are held periodically among carp breeding specialists. When evaluating the “quality” of koi, special attention is paid to the structure of the fish’s body. There are only general ideas about the principles of assessing the structure of a koi’s body (Nazari *et al.*, 2023). The research of previous years established that koi carp from artificial ponds in

Materials and Methods

Japan with different abiotic conditions of existence reliably differ in indicators of the external structure of the body, which also affect the quality of standard forms (Huan Yang *et al.*, 2022).

At the same time, the question of the peculiarities of the morphological structure of coloured carp is rather poorly studied. In particular, comparisons of koi carp from wild and domesticated populations indicate an increase in phenotype deviations in the latter. If in the domesticated populations of Japan, the necessary specimens are constantly selected, then in the populations of Israel, Turkey, Iraq, and other countries, there is an opportunity to consolidate the obtained deviations from the standard due to less control (Jawad *et al.*, 2022). The relevance of the conducted research is explained by the fact that the obtained data will help specialists in the breeding of decorative koi carp in achieving better indicators of the morphology of future koi carp. In this regard, the purpose of the work was to study the morphological indicators of different colour forms of carp of various origins, which are not measured during traditional fish boning.

Taking into account many years of human economic and breeding activities and different approaches to assessing the quality of koi breeders in different countries under the condition of the constant complex action of abiotic and biotic factors of the habitat, the authors of the study assumed the presence of certain differences in the body structure of fish, which the authors tried to highlight in this study.

280 specimens of koi of different colour forms and different origin were selected for analysis. All of them were taken from the corresponding decorative ponds with similar abiotic conditions and feeding regimes. The work was carried out in strict accordance with the existing norms of bioethics (Directive 2010/63/EU), and the research design itself complied with the recommendations of “Animal Research: Reporting of In Vivo Experiments” – ARRIVE (Percie du Sert *et al.*, 2020). No fish were harmed or exposed to toxic chemicals during the research.

13 groups of fish were formed (Table 1) according to external features and origin by random selection from the corresponding group of fish. The analysis of the morphological variability of koi was carried out according to a set of plastic features: body length to the end of the scaly cover (*l*), head length (*c*), eye diameter (*do*), snout length (*lr*), extraneous space (*po*), height (*ho*) and width forehead (*io*), length of the upper (*mx*) and lower (*mn*) jaws, height of the head near the back of the head (*hc*) and through the middle of the eye (*hc₂*), the largest (*H*) and smallest (*h*) body heights, the length of the tail stem (*pl*); from a distance – andedorsal (*aD*), postdorsal (*pD*), antepectral *aP*), anteventral (*aV*), anteanal (*aA*), pectroventral (*PV*), ventroanal (*VA*); length of the bases of the fins – pectoral (*lP*), dorsal (*lD*), anal (*lA*); the greatest heights of fins – pectoral (*hP*), dorsal (*hD*), anal (*hA*); lengths of the upper (*lC₁*) and lower (*lC₂*) blades of the caudal fin (Romanenko, 2006).

Table 1. Groups of koi of individual colour forms and different origins

Group	Markings in the drawings	Colour form	Origin	Place of detention
I	★	<i>Magoi</i>	Japanese	Decorative pond of the Nemyshaiv Agrotechnical College
II	◆	<i>Kumonryu</i>	+	
III	⊗	<i>Utsurimono</i>	+	
IV	▲	<i>Kohaku</i>	+	
V	✱	<i>Kawarimono</i>	Ukrainian	Decorative reservoir of the Arboretum “Olexandria”
VII	+	<i>Bekko</i>	+	
VI	⊗	<i>Doitsu Bekko</i>	Turkish	
VIII	⊗	<i>Doitsu Kawarimono</i>	+	

Table 1. Continued

Group	Markings in the drawings	Colour form	Origin	Place of detention
IX	★	<i>Bekko</i>	Ukrainian	Decorative pond of the Nemyshaiv Agrotechnical College
X	▲	<i>Kawarimono</i>	+	
XI	✦	<i>Doitsu Bekko</i>	Turkish	
XII	★	<i>Doitsu Kawarimono</i>	+	
XIII	★	<i>Kawarimono</i>	Israeli	Decorative reservoir of the Novokakhovsk fish breeding plant of partial fish

Source: author's data

Results and Discussion

Plastic features were measured with an electronic caliper with an accuracy of 0.1 mm. Minimization of the influence of age variability on the variation of signs was achieved by analysing a separate size-age group for each sample by one operator. A total of 280 specimens of koi with average linear dimensions of 61.44 ± 0.20 mm (from 52.89 to 70.34 mm) aged 0+ were analysed. In the calculations, indices of plastic features were used (Plypyenko *et al.*, 2017). To reduce the dimensionality of the data and obtain a smaller number of significant indicators, the analysis of the distribution of the average values of the indices in the individuals of individual samples was carried out in the space of principal components (PCA) (English *Principal component analysis*). The above-mentioned analysis, as well as the calculation of the mean, error of the mean, squared deviation and others were carried out using the *Statistica data analysis system 8.0* for Windows.

The analysis of indices of morphological variability of koi carp of the studied samples using head (Table 2) and body (Table 3) features, using their comparison according to the Student's *t-test* (criterion), showed that there are no statistically significant differences in fish of individual colour forms of the same origin. In addition, for most of the main indices of the body of fish of different origins, namely, the largest (*H*) and smallest (*h*) body height; more distantly: antedorsal (*aD*), postdorsal (*pD*), antepectral (*aP*), anteventral (*aV*), anteanal (*aA*), pectroventral (*PV*), ventroanal (*VA*); the length of the bases of the fins: pectoral (*IP*), dorsal (*ID*), anal (*IA*); the greatest heights of fins: pectoral (*hP*), dorsal (*hD*), anal (*hA*); there are no statistically significant differences between the lengths of the upper (*IC₁*) and lower (*IC₂*) blades of the caudal fin. Statistically significant differences are present in tail stem length (*pl*) from samples of Israeli and Japanese origin.

Table 2. Plastic signs of koi according to head indices, $M \pm m / \min - \max$

	Koi 1.	Koi 2.	Koi 3.	Koi 4.	Koi 5.	Koi 6.	Koi 7.	Koi 8.	Koi 9.	Koi 10.	Koi 11.	Koi 12.	Koi 13.
	sta	sta	sta	sta	sta	sta	sta	sta	sta	sta	sta	sta	sta
<i>c</i>	26.97±0.12 25.90-28.24	27.46±0.67 13.86-52.57	27.30±0.25 25.66-32.00	26.88±0.17 25.81-29.10	25.95±0.27 23.08-27.96	26.21±0.36 24.97-27.49	25.79±0.27 24.57-27.90	25.95±0.36 24.57-27.90	27.54±0.30 25.12-32.57	27.69±0.17 25.83-29.15	26.96±0.21 25.21-29.07	27.22±0.22 24.56-28.93	27.11±0.30 24.67-30.46
	<i>In % of p</i>												
<i>lr</i>	35.77±0.38 31.84-39.91	38.09±1.72 29.66-77.14	34.52±0.51 28.92-39.09	34.61±0.45 31.94-41.31	36.52±0.65 30.67-42.54	38.84±0.82 36.53-43.20	37.38±0.43 34.13-39.63	36.98±0.60 35.55-40.69	37.81±0.57 32.77-43.25	38.94±0.44 35.27-44.20	37.47±0.54 31.94-41.80	36.39±0.53 32.38-42.17	38.52±0.45 34.89-43.32
<i>do</i>	25.74±0.50 21.01-34.32	28.19±1.33 22.41-57.14	25.94±0.34 22.69-28.97	24.75±0.31 21.60-27.35	24.90±0.37 22.65-29.14	24.58±0.75 20.66-26.88	24.38±0.69 19.29-28.71	24.26±0.69 19.71-26.43	22.74±0.42 18.82-26.50	23.37±0.33 20.15-26.64	22.69±0.44 19.11-27.07	22.06±0.40 19.34-26.61	20.75±0.34 17.12-24.54
<i>po</i>	42.18±0.31 40.09-45.58	45.39±2.09 36.21-90.00	42.28±0.36 37.50-45.09	43.27±0.41 37.97-48.09	43.91±0.45 40.36-48.56	43.58±0.67 40.27-46.00	43.58±0.46 40.50-46.89	43.98±0.68 40.53-46.91	44.02±0.56 40.35-50.05	44.20±0.43 39.03-48.51	44.30±0.36 40.38-47.19	43.42±0.55 39.10-48.46	44.01±0.39 39.68-47.51
<i>ho</i>	6.85±0.10 6.22-7.78	8.99±0.79 6.90-27.14	8.34±0.20 6.48-11.17	9.06±0.20 7.62-11.39	5.34±0.23 4.05-8.67	5.12±0.17 4.43-5.91	5.41±0.14 4.64-6.45	5.43±0.16 4.30-6.03	4.67±0.11 3.57-5.50	4.74±0.08 4.20-5.56	4.46±0.08 3.82-5.52	4.52±0.20 4.00-5.28	6.40±0.12 5.41-7.98
<i>io</i>	34.41±0.34 31.32-38.96	33.72±1.38 27.03-64.29	34.46±0.47 30.00-40.69	36.16±0.36 33.54-41.88	34.86±0.42 30.30-38.00	34.18±0.38 32.30-35.44	33.79±0.59 29.69-36.94	34.79±0.92 29.39-38.14	35.49±0.44 31.23-39.36	36.95±0.32 33.65-40.54	36.46±0.41 32.81-40.66	34.95±0.43 31.99-39.57	4.02±0.06 3.46-4.82
<i>mk</i>	24.16±0.30 21.20-26.51	27.01±1.06 20.26-50.00	25.53±0.32 22.08-29.19	26.62±0.34 23.98-30.95	25.87±0.50 21.19-31.31	26.02±0.67 22.51-28.00	26.00±0.59 20.78-29.28	25.42±0.79 21.08-29.52	24.65±0.35 21.05-28.13	24.75±0.53 18.84-28.73	23.71±0.18 21.74-25.32	23.63±0.77 15.23-28.36	27.34±0.32 23.34-29.47

Table 2. Continued

	Koi 1. sta	Koi 2. sta	Koi 3. sta	Koi 4. sta	Koi 5. sta	Koi 6. sta	Koi 7. sta	Koi 8. sta	Koi 9. sta	Koi 10. sta	Koi 11. sta	Koi 12. sta	Koi 13. sta
<i>mm</i>	20.50±0.32 17.10-22.79	22.70±1.02 15.05-42.86	21.81±0.28 18.75-24.84	23.17±0.33 19.62-26.19	22.64±0.44 18.54-26.64	22.54±0.67 19.19-25.00	22.81±0.68 17.65-26.71	21.39±0.86 16.67-24.67	21.18±0.36 17.19-25.00	21.59±0.45 16.83-24.79	20.08±0.33 16.73-23.15	18.93±0.67 12.19-23.26	22.74±0.35 19.69-26.47
<i>hc</i>	72.31±0.42 69.01-77.50	78.35±3.30 69.44-154.29	74.69±0.69 25.81-22.66	75.12±0.58 62.25-81.22	72.41±0.57 69.48-80.21	73.93±1.06 67.61-78.67	74.26±1.05 70.85-80.11	73.74±1.00 69.02-81.53	72.76±0.85 67.74-76.65	68.80±2.00 65.35-82.21	76.26±0.80 25.23-80.50	74.10±0.75 69.58-84.74	69.28±0.59 64.73-75.83
<i>hc2</i>	62.60±0.42 58.72-67.82	66.52±3.09 58.15-137.14	63.25±0.60 57.50-69.08	61.89±0.71 54.55-68.06	60.68±0.49 56.51-64.50	60.59±0.94 54.61-64.16	60.57±0.94 53.73-66.50	60.91±0.57 57.79-63.23	59.08±0.58 52.89-66.35	59.70±0.53 55.26-65.66	62.06±0.71 55.57-68.77	64.04±0.65 58.56-70.34	59.34±0.76 53.85-67.25

Source: author's development

Table 3. Plastic signs of koi according to the indices of the body, $M \pm m/\min\text{-max}$

	Koi 1. sta	Koi 2. sta	Koi 3. sta	Koi 4. sta	Koi 5. sta	Koi 6. sta	Koi 7. sta	Koi 8. sta	Koi 9. sta	Koi 10. sta	Koi 11. sta	Koi 12. sta	Koi 13. sta
<i>l</i>	61.78±1.50 50.00-79.50	46.96±1.17 36.00-57.50	53.20±1.02 44.50-65.50	57.49±1.48 44.00-74.10	68.75±2.20 47.00-88.50	67.44±3.09 59.50-85.50	73.25±2.54 59.80-92.50	65.98±2.12 60.50-81.30	72.18±1.91 56.15-90.98	69.25±1.29 56.40-79.90	71.50±1.97 53.31-85.99	69.44±1.46 54.78-81.19	86.36±1.92 69.58-100.47
<i>In % of l</i>													
<i>lcor</i>	54.45±0.38 48.91-59.46	53.74±0.73 49.15-67.79	53.84±0.33 49.17-56.18	54.06±0.36 50.98-57.41	54.83±0.56 49.74-63.78	54.92±0.26 54.04-55.96	55.13±0.59 51.09-58.26	54.72±0.64 51.70-58.89	54.76±0.50 49.15-62.63	55.11±0.40 51.14-58.42	56.50±0.37 52.12-59.42	56.87±0.35 53.69-60.33	55.25±0.56 49.24-60.45
<i>H</i>	27.94±0.16 26.43-29.73	26.89±0.44 22.40-33.63	27.48±0.23 25.54-30.27	27.70±0.30 23.61-30.00	25.87±0.55 23.12-28.27	23.75±1.42 14.44-27.95	26.01±0.34 24.07-27.66	25.39±0.37 24.26-27.60	27.87±1.08 4.92-37.62	29.03±0.19 26.79-30.48	29.03±0.19 24.79-28.11	27.10±0.55 22.84-29.81	25.88±0.28 23.95-28.60
<i>h</i>	9.99±0.11 8.92-10.73	9.72±0.15 8.51-12.04	10.08±0.09 9.16-11.26	10.23±0.10 8.89-10.98	9.76±0.16 8.15-11.34	9.66±0.22 8.76-10.46	9.37±0.19 8.19-10.51	9.51±0.22 8.52-10.86	10.31±0.12 9.39-11.77	10.73±0.10 9.86-11.76	9.77±0.15 8.64-11.29	10.05±0.15 8.57-11.41	10.20±0.15 8.90-11.78
<i>ad</i>	41.04±0.22 39.51-43.17	41.38±0.38 38.72-48.50	41.51±0.19 40.50-43.84	41.15±0.21 38.82-44.03	41.97±0.42 39.33-48.78	43.00±0.41 40.88-44.50	41.58±0.42 39.61-43.78	41.29±0.51 38.45-43.43	44.13±0.48 40.34-53.14	43.19±0.26 25.35±0.22	43.49±0.24 25.22±0.30	43.99±0.29 25.76±0.27	42.61±0.45 27.01±0.31
<i>pd</i>	39.80±0.31 37.25-43.51	39.78±0.56 35.41-51.35	38.28±0.61 35.20-42.84	39.40±0.21 32.35-42.84	40.25±0.49 29.67-41.94	38.76±1.43 29.67-43.26	39.88±0.41 37.67-42.95	39.82±0.51 37.11-41.97	39.55±0.39 34.01-44.00	39.74±0.40 37.77-44.24	40.56±0.25 37.89-42.82	40.75±0.31 38.15-43.49	41.32±0.35 37.18-44.68
<i>pl</i>	11.31±0.20 9.10-12.72	13.62±0.31 10.87-16.81	12.63±0.20 11.14-15.97	14.54±0.46 11.37-19.51	11.88±0.25 9.71-14.53	13.10±0.29 12.19-14.27	12.22±0.31 9.16-14.36	11.09±0.35 9.69-12.42	13.39±0.21 11.19-15.20	13.15±0.23 10.74-15.41	13.76±0.20 12.57-16.55	12.17±0.21 10.19-13.91	14.56±0.24 12.71-16.34
<i>ap</i>	26.87±0.15 25.81-28.31	27.13±0.27 24.20-31.15	27.24±0.14 25.86-28.57	26.21±0.18 24.59-27.91	26.00±0.28 23.55-28.80	26.04±0.44 24.31-27.81	25.81±0.25 24.14-27.43	25.89±0.25 24.71-27.20	25.43±0.25 22.37-27.93	25.35±0.22 23.47-27.57	25.22±0.30 22.83-28.27	25.76±0.27 23.53-28.12	27.01±0.31 23.05-29.85
<i>av</i>	43.70±0.56 39.42-56.13	43.19±0.38 40.20-50.09	43.12±0.17 41.55-44.80	43.15±0.28 40.34-45.82	41.55±0.30 38.46-44.29	41.99±0.51 39.33-43.97	42.29±0.43 40.39-45.13	42.19±0.33 40.76-43.79	42.86±0.38 37.06-48.00	42.90±0.23 40.11-44.91	43.26±0.27 40.90-46.05	43.46±0.32 40.55-47.04	43.57±0.41 39.94-47.00
<i>aa</i>	60.67±0.17 58.65-61.94	60.19±0.55 57.10-70.97	60.29±0.26 57.74-62.40	60.31±0.31 57.56-63.75	60.09±0.27 56.62-62.14	61.11±0.44 59.43-63.18	61.98±0.35 59.22-61.85	60.54±0.27 58.48-64.92	62.32±0.59 54.58-71.09	62.09±0.31 58.84-64.04	61.43±0.28 59.62-65.65	62.87±0.31 59.62-65.65	60.94±0.45 56.78-65.42
<i>ld</i>	24.49±0.24 22.31-26.23	25.37±0.32 21.06-29.20	25.11±0.26 22.56-28.76	24.62±0.23 22.56-26.72	27.49±0.37 23.80-30.85	26.15±0.75 23.76-29.47	26.41±0.53 22.61-29.82	27.16±0.36 25.44-28.59	25.52±0.49 21.56-31.12	26.12±0.39 20.76-29.39	25.10±0.23 22.12-27.01	26.04±0.25 23.41-28.88	25.03±0.47 21.17-28.68
<i>hd</i>	16.14±0.19 13.76-17.92	16.33±0.19 13.75-18.51	16.31±0.25 13.44-18.67	15.96±0.18 14.38-17.56	15.49±0.36 10.00-16.50	12.79±0.59 9.82-14.83	12.78±0.38 9.72-15.09	13.78±0.76 9.70-16.21	13.33±0.24 9.34-15.07	13.72±0.19 12.16-15.63	13.13±0.26 11.09-15.25	12.50±0.30 9.91-15.22	14.89±0.26 12.74-17.39
<i>la</i>	6.36±0.10 5.44-7.56	6.83±0.19 4.60-8.70	6.58±0.12 5.56-7.65	7.18±0.14 5.45-8.41	6.71±0.17 6.03-8.76	6.43±0.10 6.03-6.85	6.27±0.10 5.49-6.82	6.88±0.18 6.27-8.11	5.62±0.11 4.26-6.60	5.53±0.12 4.39-6.38	5.65±0.14 3.95-6.42	5.95±0.10 5.27-6.90	7.22±0.10 6.21-8.09
<i>ha</i>	13.70±0.13 12.14-14.76	14.21±0.21 12.68-17.88	13.68±0.18 12.18-15.33	13.37±0.13 11.45-14.26	13.27±0.25 9.31-14.88	12.31±0.26 11.13-13.33	12.83±0.29 11.10-14.97	13.51±0.27 12.24-14.64	12.48±0.15 10.81-13.90	13.21±0.29 11.90-15.87	12.58±0.28 9.13-14.84	13.91±0.25 11.55-16.01	15.15±0.23 13.54-18.08
<i>lp</i>	14.19±0.18 11.97-15.37	15.05±0.21 13.00-16.99	15.00±0.20 12.93-17.07	15.27±0.18 13.11-17.25	14.46±0.17 12.87-16.22	13.84±0.43 11.54-15.15	14.08±0.28 12.28-15.63	14.11±0.27 12.60-15.16	14.43±0.21 12.51-17.54	14.57±0.17 11.94-15.90	14.06±0.22 12.19-15.84	13.74±0.23 1.55-15.93	14.74±0.23 12.77-16.60
<i>lv</i>	13.23±0.10 12.58-14.04	13.59±0.18 12.16-16.46	13.19±0.16 11.61-14.71	13.46±0.17 12.17-15.14	12.88±0.14 11.66-14.00	12.30±0.31 10.87-13.25	12.46±0.25 10.84-14.04	12.56±0.33 10.64-13.85	13.62±0.18 12.44-16.57	13.62±0.18 10.96-15.08	13.81±0.15 12.15-15.11	12.98±0.20 11.37-15.21	14.27±0.25 12.01-16.98
<i>pv</i>	17.46±0.16 15.68-18.91	17.76±0.28 15.20-20.17	17.66±0.14 16.35-19.12	18.06±0.22 16.49-19.86	16.33±0.21 13.74-18.23	16.63±0.39 15.03-18.82	16.53±0.30 14.17-19.08	16.89±0.26 15.72-17.75	17.37±0.18 15.37-19.36	17.72±0.18 15.65-18.81	17.58±0.24 15.41-20.08	18.00±0.24 16.12-20.23	16.85±0.20 14.55-18.25
<i>va</i>	18.06±0.15 16.27-19.14	17.75±0.23 16.00-21.24	18.43±0.15 17.12-20.31	18.56±0.17 16.75-20.63	18.54±0.18 17.32-20.69	18.37±0.29 17.13-19.47	19.41±0.27 17.36-19.42	18.46±0.21 17.18-23.31	19.72±0.24 17.18-23.31	19.90±0.20 18.27-22.06	18.92±0.26 15.72-20.98	19.85±0.20 18.01-21.69	18.14±0.20 16.15-19.89
<i>lci</i>	18.88±0.22 17.31-20.83	19.64±0.22 17.34-21.28	19.24±0.24 16.61-21.98	20.10±0.30 16.79-22.66	19.23±0.33 16.34-21.74	19.44±0.35 17.75-21.19	18.41±0.36 17.14-22.22	18.95±0.32 17.35-20.50	19.36±0.28 14.80-22.63	20.49±0.21 17.94-22.74	18.79±0.27 16.29-20.98	19.36±0.28 14.80-22.63	19.41±0.33 16.62-22.13
<i>lc2</i>	20.11±0.16 18.21-21.64	20.71±0.30 18.59-25.49	20.25±0.20 18.22-22.48	20.90±0.31 18.29-23.91	20.02±0.44 12.87-22.45	20.10±0.40 18.09-21.99	19.14±0.42 16.10-22.48	19.52±0.30 18.41-21.00	19.81±0.30 15.03-24.11	21.20±0.19 19.27-23.30	19.71±0.20 18.21-22.17	19.81±0.30 15.03-24.11	21.06±0.34 18.08-24.98

Source: author's development

However, according to the indices of the head, a rather interesting picture is observed: there are statistically significant systematic differences between fish of Israeli and Japanese, as well as Ukrainian and Turkish origin in terms of the length of the snout (*lr*), the diameter of the eye (*do*), the absent space (*po*), the height of the forehead (*ho*), width of the forehead (*io*), length

of the upper jaw (*mx*), the height of the head near the back of the head (*hc*) and through the middle of the eye (*hc₂*). At the same time, fish of Japanese origin differ slightly from fish of Ukrainian and Turkish origin only in the height of the forehead (*ho*) and the length of the snout (*lr*).

The analysis of the variation of the obtained samples using the quadratic deviation (σ) also

showed that according to the features of the shape and proportions of the body of the fish, the studied groups were characterized by low indicators. At the same time, the indicators of the shape and proportions of the head had the maximum values. Therefore, in order to evaluate the quality of young koi carp, which are planned to be used for ornamental fish farming and international exhibitions, in addition to traditional normative indicators, special attention should be paid to the study and analysis of indicators characterizing the shape and proportions of the head.

For a more complete and clear understanding of the trends in the variability of plastic features of carp, the obtained samples were examined using the method of principal components. The distribution of studied koi individuals of different origin in the space of the main components demonstrated the reliability of the differentiation of the samples according to the set of studied morphological features according to the first (*Factor 1*) and third (*Factor 3*) main components, which provide about 30% of their morphological variability (Fig. 1).

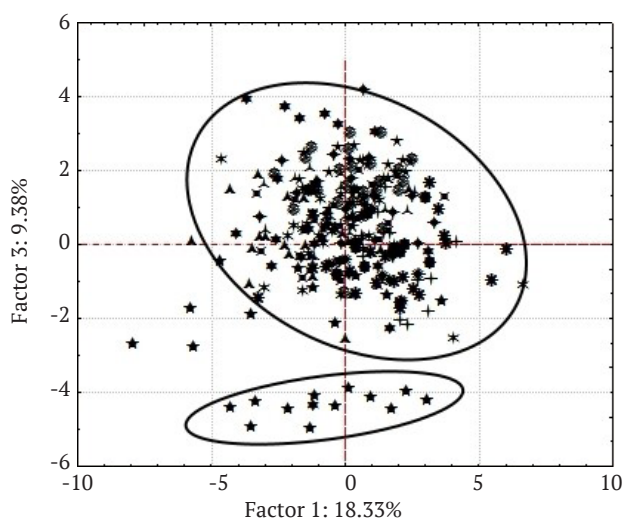


Figure 1. Distribution of individual groups of koi in the space of the first and third main components according to the set of plastic features

Note: designation of groups of which see in Table 1

In the space of the main components according to the set of indices of external signs, two scatterers are formed, clearly differentiated portion with a statistically reliable hiatus according to the first and third components (*Factor 1* and *Factor 3*). The first scatterer is formed by fish of Japanese, Turkish and Ukrainian origin, regardless of individual colour forms. The second scatterer included fish of Israeli origin, which indicates the presence of some adaptive changes in the morphotype of the above-mentioned groups. The conducted analysis allows

us to accurately state the presence of morphological differentiation of fish of Israeli origin from the fish of all other koi carp studied by us.

The analysis of the eigenvalues of the components shows that the greatest contribution to differentiation according to the first component is the largest (*H*) and smallest (*h*) body height, antedorsal (*aD*), anteventral (*aV*) and anteanal (*aA*) distances, as well as the length of the base of the pectoral fin, and according to the third – the diameter of the eye (*do*), the width of the forehead (*io*) and the length of the upper jaw (*mx*) (Table 4).

Table 4. Eigenvalues of the main components

Indicators	Factor 1	Factor 2	Factor 3
<i>lcor</i>	-0.445715	-0.323915	-0.143927
<i>lr</i>	-0.201176	-0.313997	-0.393066
<i>do</i>	0.187197	0.405049	0.539620
<i>po</i>	-0.191437	-0.195516	-0.199854
<i>ho</i>	0.089693	0.800161	0.255138
<i>io</i>	0.164883	-0.297427	0.597280
<i>mx</i>	0.062909	0.467734	-0.517222
<i>mn</i>	0.136142	0.435543	-0.472400
<i>lc</i>	-0.774133	0.106925	0.207097
<i>hc</i>	0.093956	0.046720	0.468378
<i>hc₂</i>	0.112898	0.099871	0.532598
<i>H</i>	-0.553657	-0.006104	0.343649
<i>h</i>	-0.636626	0.031700	0.056675
<i>aD</i>	-0.676122	-0.475133	-0.025441
<i>pD</i>	-0.456830	-0.160428	-0.285199
<i>pl</i>	-0.264125	0.186603	-0.338132
<i>aP</i>	-0.512868	0.425999	0.085375
<i>aV</i>	-0.721078	0.066149	0.200807
<i>aA</i>	-0.751439	-0.421773	0.046978
<i>ID</i>	-0.116583	-0.162999	-0.205824
<i>hD</i>	-0.147218	0.755906	0.150866
<i>IA</i>	-0.054958	0.592956	-0.262066
<i>hA</i>	-0.368351	0.493726	-0.245714
<i>IP</i>	-0.417155	0.470516	0.071925
<i>IV</i>	-0.577681	0.303097	-0.152358
<i>PV</i>	-0.443651	-0.027143	0.395253
<i>VA</i>	-0.450057	-0.569926	-0.007821
<i>IC₁</i>	-0.442051	0.301600	0.088646
<i>IC₂</i>	-0.505441	0.398568	-0.017637

Source: author's development

Thus, the differentiation of the studied fish in the space of the main components is provided by the features characterizing the ratio of the proportions of the head and body. Long-term selection work, various approaches to assessing the quality of breeders and the predominant orientation of breeders to obtain offspring of a certain colour range contributed to the consolidation of certain "morphological deviations" in koi carp. These indicators are practically insignificant for fish breeding, and in traditional commercial fish farming they were not paid attention

at all. In ornamental aquaculture, these aspects are of key importance and significantly affect the class, quality, and prospects of individual fish at international exhibitions, and, accordingly, the price of individual fish. The issue is that during international exhibitions, experts analyse such indicators as body shape, body proportions, swimmers' proportions, etc. So, for example, the shape of the head should be symmetrical and without any deviations, the length of the fins should be average, not long and not short (Kuroki, 1990; Axelrod *et al.*, 1996). That

is, such indicators are not measured on farms during traditional credit rating (Grynevych *et al.*, 2022; Nazari *et al.*, 2023), and these little things distinguish an ordinary fish from an exhibition winner. Thus, the farm, having good industrial herds and carrying out simple measurements, can receive significant additional income, which can even cover the entire product revenue of the enterprise for a year.

The issue is complicated by the fact that various housing conditions, feeding regimes and stresses during long-term transportation (including air travel) can induce directed adaptive changes in phenotypes, which determine intrabreed differentiation, which is manifested externally at the level of plastic (measured) body features. That is, there is an opinion that depending on the conditions of keeping, the same fish will have a different morphotype if it is kept in various conditions. In our research, this factor was negated by the fact that was chosen almost the same artificial decorative reservoirs in the same climatic zone with similar living conditions. At the same time, it is evident that if they are grown in different conditions, they can give a different result. The author's research differs in that the use of the ichthyological research method (biological sciences) was combined to achieve fishery goals (agricultural sciences). For the sake of fairness, it should be noted that fish farmers perform certain measurements during the grading of brooders, but they aim to determine the quality of the brooder and its size and weight characteristics in the fish farming sense of this term, which does not correspond to the values of decorative aquaculture (Balon, 2004; Ghosh *et al.*, 2012; Jawad *et al.*, 2022). For example, fish breeders do not study purely meristic (counting) ichthyological signs, namely, wine is a mathematical reflection of the shape and proportion of the body of fish, which play a key role in the evaluation of ornamental objects of aquaculture.

Artificial breeding of ornamental koi carp imposes a great responsibility on breeders and

fish farmers to preserve the authentic body shape and colour of the fish. At the same time, there are quite a few real specialists and connoisseurs of koi. The prestige factor plays here: it is nice to have carp swimming in a fountain in the front yard of a wealthy house or in the office of a large company. This approach created the demand for koi carp. In the pursuit of obtaining koi carp of the required colour, there was a loss of preservation of their traditional morphological parameters of the external structure, which was confirmed in our work. Different approaches to the targeted selection of breeders for crossings also lead to certain changes in koi phenotypes of different origins, which determine the morphological differentiation of fish of individual branches, which is most evident at the level of plastic (measurement) body features, which are evaluated at international competitions. Established changes in head proportions in koi of Israeli origin are provided by stabilizing selection, which makes fish of this group more viable in certain abiotic conditions of existence due to increased hydrodynamic and trophological capabilities, which is necessary for life in a warmer climate. Thus, the increase of the head and gill covers increases the volume of water used for breathing, which is better in conditions of increased water temperature. An increase in the head has also been noted for Iraqi reservoirs (Kataoka, 1989; Jackson *et al.*, 2000; Jawad *et al.*, 2022).

Established differences in the appearance of Israeli koi carp confirm the hypothesis voiced by E.K. Balon (2004) about the possible fixation of individual deviations in appearance in koi populations, which may be connected with a less strict selection of broodstock morphotypes. The author's studies specify and confirm the above opinion, indicating that the existing changes are related to the features of the shape and proportions of the head and their relationship with the proportions of the body.

However, obtaining high-class, competitive prize-winning fish of international exhibitions

will be quite difficult even for a smaller number of individuals. Our research has shown that Israeli koi morphologically differ from fish of Japanese, Turkish and Ukrainian origin. The established differences are related to features of the shape and proportions of the head, as well as their relationship with the proportions of the body. Specialists should take this feature into account when selecting objects for ornamental fish farming, one of which is the variegated koi carp.

Conclusions

Research on assessing the quality of young koi carp, which are intended for ornamental fish farming and participation in international exhibitions, revealed that in addition to traditional normative indicators, special attention should be paid to the analysis of body shape and head proportions. These indicators are of key importance for achieving a positive result at international exhibitions. To study the external structure of various forms of koi, traditional ichthyological meristic morphological measurements are used. These measurements are a numerical representation of the body shape and proportions of the fish, which is an important aspect in ornamental aquarism. The analysis showed that no statistically significant differences were found in fish of different colour forms, but of the same origin. However, statistically significant differences were found between the appearance of Israeli carp, related to the shape and proportions of the head, as well as their relationship with the body proportions. This is confirmed by the analysis of the spatial distribution of the main components, which indicates systematic differences in appearance. Taking into account these results, it is recommended that nurseries and koi carp

fans pay attention to morphological characteristics in addition to evaluating the colour range during the evaluation of breeders. This will help select individuals with the most appropriate body shapes and proportions that have the greatest potential for successful participation in international competitions. Summarizing, the research provides important guidelines for improving the breeding of koi carp in order to obtain the highest quality and aesthetically attractive specimens for decorative fish farming and participation in international exhibitions.

One of the promising future directions of research is the establishment of clear genetic markers that would indicate fish with body proportions close to the ideal standards of international exhibitions. Such markers would help breeders to more effectively select breeders for further breeding and improvement of the genetic pool. In addition, the question of imitation of colour in carp remains open. Given the fact that only a small part of the fish has the correct colour, there are prospects to move from random breeding to the purposeful selection of only those individuals that meet all the necessary parameters. This task is possible in the future if there is more detailed knowledge about the genes responsible for colouring. The novelty of the conducted research is that, for the first time, ichthyological methods of studying the morphology of fish species were applied for the digital expression of the morphological structure of the body of fish in ornamental aquaculture.

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Conflict of Interest

None.

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Морфологічна мінливість окремих кольорових форм *Cyprinus carpio haematopterus* різного походження

Олександр Олександрович Лисак

Аспірант

Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0009-0007-4238-905X>

Петро Григорович Шевченко

Кандидат біологічних наук, доцент

Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0002-5996-4328>

Микола Данилович Ратушний

Аспірант

Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0009-0008-2540-1317>

Дмитро Сергійович Христенко

Кандидат біологічних наук

Інститут рибного господарства Національної академії аграрних наук України
03164, вул. Обухівська, 135, м. Київ, Україна
<https://orcid.org/0000-0002-1432-7093>

Ганна Олександрівна Котовська

Кандидат біологічних наук

Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0001-7155-1086>

Анотація. Актуальність роботи з вивчення морфологічних показників кої полягає у підвищеній увазі на міжнародних виставках кої до таких зовнішніх показників як забарвлення, форми і пропорціям тіла риб, що займають високі призові місця і отримують грошові винагороди у сотні тисяч американських доларів. Метою проведених робіт

було вивчити морфологічні особливості різних кольорових форм різного походження. Подібні показники не враховуються у традиційному риборозведенні, що також додає новизни проведеним дослідженням. Для аналізу було відібрано 280 зразків кої. В роботі оцінювалась мінливість пластичних ознак риб окремих кольорових форм (*magoi, kumonryu, utsurimono, kohaku, kawarimono, bekko, doitsu bekko, doitsu kawarimono*) різного походження (японського, турецького, ізраїльського і українського). У результаті роботи було встановлено що статистично достовірної різниці між різними кольоровими формами однакового походження нами не виявлено. При цьому, встановлено достовірну різницю у риб ізраїльського походження за ознаками, що характеризують форму і пропорції голови у співвідношенні із пропорціями тіла. Розподіл морфологічних індексів вивчених особин різних виборок в просторі головних компонент також підтверджують достовірні відмінності ізраїльського морфотипу від риб японського, турецького і українського походження за ознаками, що пов'язані із співвідношеннями відділів голови та пропорцій тіла. Отримані дані є основою для роботи селекціонерів і розплідників коропів кої, які бажають виростити цінні екземпляри – потенційних переможців міжнародних змагань коропів кої

Ключові слова: короп кої; кой; нішікі-гой; метод головних компонент



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The effect of carboxylates of food acids and substances of neurotropic-metabolic effect on biochemical indicators of blood and reproductive qualities of new-calved cows

Mykola Seba*

PhD in Agricultural Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0001-9696-934X>

Mansur Yahafarov

Postgraduate Student
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0001-5832-0400>

Ayaz Mammadov

PhD in Biology
Western Caspian University
1001, 31 Istiglaliyyat Str., Baku, Azerbaijan
<https://orcid.org/0009-0005-2240-3673>

Dmytro Nosevych

PhD in Agricultural Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0003-2495-2084>

Maryna Khomenko

PhD in Agricultural Sciences
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0001-7023-3676>

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*Corresponding author



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Abstract. After calving, changes occur in the reproductive system of cows, aimed at restoring the ability to reproduce. These processes occur against the background of exhaustion from childbirth, lactation, and a negative energy balance, which often leads to violations of the reproductive function, so the search for methods that contribute to its restoration is urgent. The aim of the work was to investigate the effect of the drug with neurotropic-metabolic action as a stimulator of postpartum recovery in cows and improvement of fertility. The drug included L-arginine, sodium succinate, sodium glutamate and carboxylates of food acids (Fe, Ca, Mn, Cu, Zn). The study was conducted by the method of groups on cows of the Holstein breed. A control and two experimental groups of 15 heads each were formed. Cows of experimental group 1 were given injections in the subcaudal fold on the 5th, 7th and 9th day after calving, dividing the active substance into 3 parts. Cows of experimental group 2 were administered the drug intraparentherally in the first 1.5-2 hours after calving in a full dose once 21st days after calving, the cows of the experimental groups did not show any deviations in blood biochemical parameters compared to the reference values. But there was a tendency to decrease the amount of calcium, phosphorus, protein and carotene, compared to cows of the control group, which indicates the presence of an influence on metabolic processes. The content of total protein in the blood of cows of experimental group 2 was close to the lower limit of the physiological norm. No placental retention was observed in the cows of the experimental groups, while in the control group this disorder was present in 33% of cows. The introduction of the drug made it possible to reduce the frequency of endometritis by 20%, to shorten the period until the beginning of the first oestrus by 7th and 10th days, to increase fertility after the first insemination by 6 and 13 percentage points, and to shorten the service period by 16th and 22nd days. The second experimental group obtained a higher efficiency of using the drug. It is advisable to use the results of research on dairy farms. Intraparentheral administration of the drug 1.5-2 hours after calving will improve the process of restoring the sexual function of cows and their fertility

Keywords: Holstein breed; service period; L-arginine; sodium succinate; sodium glutamate; oestrus

Introduction

Restoring the reproductive function of cows after calving is an important element of ensuring the sustainability of the technological process of milk production and reproduction of herds. The genetic characteristics of cows associated with the rapid growth of milk productivity at the beginning of lactation is one of the reasons for the negative energy balance, which leads to a decrease in the fertility of cows. O.B. Pascottini *et al.* (2022) believe that about half of the cows after calving fail to adapt to the changes of the transit period and within 5 weeks they develop one or more clinical and subclinical diseases, which is accompanied by a violation of reproductive capacity. Rapid recovery of cows after calving is very important to ensure efficient

reproduction. There are a number of genetic and environmental factors that affect the reproductive function of cows, which affects the length of the service period (Ruban & Matvieiev, 2020).

Since it is difficult to fully balance the needs of cows in the postpartum period, there is a need to find additional ways to maintain the energy balance and stimulate the body's resistance and reproductive function. Significant efforts in this direction are aimed at balancing the energy requirements for calving and initiation of lactation in conditions of limited feed intake. In particular, they studied the possibility of additional administration of L-carnitine to cows, which could increase the efficiency of β -oxidation and reduce the level of negative energy

balance (Meyer *et al.*, 2020). Cows treated with L-carnitine had higher milk fat in early lactation and higher plasma triacylglyceride concentrations, indicating increased efficiency of fat oxidation, but this did not affect cow recovery after calving, energy balance and lipomobilization.

Physiological methods of stimulating cows that are safe for production are of great interest. In particular, research was conducted on the effect of biostimulation on the restoration of ovarian activity, improvement of the reproductive function of cows in the postpartum period by ensuring contact of cows with the bull (Damarany, 2019). The results showed that contact with the bull contributed to an increase in the number of cows that recovered ovarian function during the postpartum period, contributed to a reduction in the interval from calving to the first oestrus and ovulation after calving, reduced the proportion of cows with anestrus, the number of inseminations per insemination and the length of the period from calving before fertilization. But keeping bulls with cows does not solve all reproduction problems and is not always technologically possible, so the search for drugs that could improve the reproductive function of cows at various stages of the production cycle continues. In particular, during embryo transplantation, non-steroidal anti-inflammatory drugs, such as flunixin, have proven themselves well meglumine or ibuprofen (Spencer *et al.*, 2020), but prophylactic administration of these drugs to all cows after calving is not considered sufficiently justified. To optimize the involution of genital organs and restore sexual cycles and timely insemination of cows, it is recommended (Nacu *et al.*, 2021) application of cloprostenol in the first 6 hours after calving with repeated administration on day 11. The use of drugs with neurotropic and metabolic action showed good results at the cellular level. In particular, supplementing the culture medium with L-arginine increased the percentage of oocytes at the stage of germinal vesicle disintegration after 7 hours of cultivation and oocytes that

reached metaphase I after 15 hours (Dubeibe *et al.*, 2017). L-arginine plays an important role in nitrogen metabolism and, in particular, protein synthesis, blood flow, nutrient absorption and ruminant health. There is evidence (Wu *et al.*, 2022) that feed supplements with rumen-protected L-arginine can improve performance and fertility traits in cows without negative effects on metabolism or health.

One of the ways to improve the reproduction function of cows is the use of trace elements. M.O. Yazlık *et al.* (2021) found that the injection of certain microelements during the last third of the pregnancy, on the day of calving and 10 days after it contributed to the increase of the phagocytic activity of the blood of cows and increased the probability of fertilization. In the studies of Y.M. Somagond *et al.* (2023) proved that injections of multivitamins and multiminerals to cows before and after calving are effective in improving the immune response and reducing inflammatory processes during the transit period. Considering the fact that the problem of restoring the reproduction function of cows after calving remains widespread, the search for additional ways of stimulating the activity of the reproductive system is urgent. Among the promising directions, the use of complex drugs that affect the body of cows at the cellular level and contain additional supporting biologically active substances, in particular microelements, should be considered. The purpose of the study is to check the effectiveness of a complex drug of neurotropic and metabolic action, supplemented with a complex of carboxylates of food acids, on the course of the postpartum period of cows and the restoration of reproductive function.

Materials and Methods

The experiment was conducted on Holstein cows at the Zhadkivske dairy farm in the Ichnyan district of the Chernihiv region, in the winter period. Research was conducted by the group method in accordance with European

Convention "For the Protection of Vertebrates Animals Used for Research and other Scientific Purposes" (1986). To create the control and experimental groups, physiologically healthy cows were randomly selected immediately after calving. Cows had a live weight ranging from 500 to 550 kg, had a second calving or older, but their age did not exceed 6 years. During the dry period cows were kept tethered. During the experiment, the animals received a mixed diet, which in terms of composition, energy content and nutrients met the needs of cows of the corresponding technological period.

As a stimulating factor for the postpartum period and restoration of reproductive function in cows, a complex of neurotropic and metabolic

substances was used, in particular L-arginine ($C_6H_{14}N_4O_2$), sodium succinate ($Na_2C_4H_4O_4$), glutamate sodium ($C_5H_8NNaO_4$) and carboxylates of food acids: iron, calcium, manganese, copper, and zinc (Fe, Ca, Mn, Cu, Zn). The animals were divided into 3 groups of 15 animals. The complex of substances was administered according to the scheme shown in Table 1. In experimental group 1, the complex of substances was injected into the subcaudal fold at a dose of 20 cm³ on the 5th, 7th, 9th day after calving, in experimental group 2 the complex of substances was administered intraparenterally in the first 1.5...2 hours after calving in a volume of 60 cm³ once. Animals of the control group were not administered the drug.

Table 1. Formation of groups of animals for research

Group	n	Introduction of a complex of substances				the total amount of the active substance during the period of the experiment, g
		period of introduction	injection volume, cm ³ /head	active substance, g	multiplicity of input	
Control	15	—	—	—	—	—
Experimental 1	15	5, 7, 9 days after calving	20	1.3	3	3.9
Experimental 2	15	1.5...2 hours after calving	60	3.9	1	3.9

Source: author's development

In order to establish the general physiological state of cows, before the experiment, blood tests were carried out 1.5-2 hours after calving. The following biochemical indicators of blood were monitored: the content of calcium, phosphorus, total protein, carotene and reserve alkalinity. Repeated blood analysis was performed 21st days after calving. Haematological indicators were studied in 5 randomly selected cows from each group. The results of haematological analysis were compared with reference indicators (Haidenko & Yevtushenko, 2017) and data of blood indicators in Holstein cows (Cozzi *et al.*, 2011).

During the experiment, a clinical examination of the cows of the experimental and control groups was carried out, and all the changes that occurred in the genital organs of

the animals during this period were recorded. Clinical studies of experimental animals were based on the external and internal examination of the genital organs of animals. The internal examination consisted in palpation of the internal genital organs with the hand through the rectum of the research animal. The physiological process of genital involution in the postpartum period was traced by rectal examination of cows. Daily visual observation of cows was also carried out to detect the first signs of the oestrus stage of the sexual cycle after calving. After the cows showed clinical signs of the oestrus stage and showed sexual desire, they were artificially inseminated with an interval of 12 hours (morning and evening). Fertilization was carried out by the cervical method, with rectal fixation of the cervix

by hand. The first insemination was carried out no earlier than 42nd days after the cow calved. Under conditions of manifestation of oestrus in cows earlier than 42nd days, insemination was skipped, waiting for the next oestrus.

Based on the results of observation, the manifestation of signs of reproduction function was evaluated in each of the groups of cows. In particular, the number with retained placenta and postpartum endometritis, the duration of the period from calving to the first coming into sexual desire, fertilization after the first insemination, the number of pregnant animals 90th days after calving, the length of the service period, and the value and index of insemination were determined. Average values, statistical error, and the share of animals in percent were determined by the research indicators.

Results and Discussion

A cow's reproductive organs and hormone activity undergo active changes during the

transition period. After calving, the uterus involutes, restores its mucous membrane, and resumes the sexual cycle. These processes occur against the background of lactation and increasing milk productivity of cows. The clinical condition of the cow and provision of the body's needs for energy and nutrients are of great importance for the renewal of the sexual cycle and the manifestation of high productivity. During the transition period, there is a high risk of metabolic disorders, which can be caused by nutritional deficiencies and postpartum complications. In order to establish the general physiological condition of the animals, the first biochemical tests of the blood of cows were carried out before the experiment (Table 2). Based on the characteristic clinical signs and blood analysis, it is possible to conclude whether the cows have an acidotic state, disorders of protein, mineral, fat and carbohydrate metabolism, osteodystrophy or other disorders.

Table 2. Biochemical indicators of blood before the experiment

Group	Ca, mg%	P, mg%	Total protein, g%	Reserve alkalinity, vol.% CO ₂	Carotene, mg%
Control	10.4±0.7	5.9±0.4	8.2±0.9	45.5±1.2	0.98±0.01
Experimental 1	10.5±0.2	5.7±0.4	7.8±0.7	45.7±0.4	0.96±0.02
Experimental 2	10.5±0.7	4.8±0.4	7.7±0.6	45.0±0.4	0.82±0.05
Physiological norm	9.5-13.5	4.5-6.5	7.25-8.6	46.0-58.0	≥0.45

Source: author's development

The most critical at the very beginning of lactation are indicators of mineral balance, protein content, vitamin nutrition and acid-alkaline balance in the body. The obtained results indicate that the biochemical parameters of the blood of cows in the experimental groups were within the physiological norm. No significant difference between the groups was found, although in the cows of the experimental group 2, the content of mineral phosphorus in the blood was 0.9 and 1.1 mg% less than in the experimental 1 and

control groups and approached the lower limit of the physiological norm. In general, the effect of the conditions of prenatal care of cows did not lead to negative disturbances of metabolic processes, so the study of the effect of the complex drug took place in similar groups. The re-analysis was carried out 21st days after calving, when all experimental cows were treated, and it was possible to analyse the influence of neurotropic-metabolic substances and carboxylates of food acids on biochemical blood parameters (Table 3).

Table 3. Biochemical indicators of blood after administration of drugs

Group	Ca, mg%	P, mg%	Total protein, g%	Reserve alkalinity, vol.%	Carotene, mg%
Control	10.4±1.2	6.0±1.2	8.1±1.1	45.3±0.6	0.98±0.05
Experimental 1	10.2±0.3	5.5±0.3	7.8±0.6	45.0±7.0	0.93±0.09
Experimental 2	10.2±0.8	5.3±0.1	7.3±0.9	45.0±8.0	0.81±0.01
Physiological norm	9.5-13.5	4.5-6.5	7.25-8.6	46.0-58.0	≥0.45

Source: author's development

Three weeks after calving, the general trend of the biochemical parameters of the cows' blood practically did not change. Cows of the control group had slightly higher levels of calcium, phosphorus, protein, and carotene in the blood, but were characterized by greater variability of these characteristics. A higher content of total protein in the blood of cows of the control group may be an indication of inflammatory processes, but no significant difference in biochemical blood parameters was found between the groups. All established average indicators in the control and experimental groups were within the reference values. It should be noted that in experimental group 2, the total protein index was close to the lower limit of the physiological norm, which may indicate an increased level of protein use in the

body, in particular for milk synthesis or deposition in tissues. A decrease in the content of total protein in the blood of cows of experimental group 2 indicates the activation of milk synthesis processes after treatment with a complex drug according to the scheme of single administration in the first 1.5-2 hours after calving.

During the observation of the animals of the experimental groups, a number of postpartum complications were revealed, among which the most common were retained placenta and endometritis. The use of a complex of biologically active substances with a neurotropic-metabolic effect and carboxylates of food acids allow to a certain extent to normalize the metabolism of newly-calved cows and reduce the number of gynaecological diseases (Table 4).

Table 4. Reproductive qualities of newly-calved cows

Indexes	Groups		
	Control	experimental 1	experimental 2
The period from calving to the first onset of sexual desire, days	42±2.0	35±1.0**	32±2.0**
Body weight 90 days after calving, %	40	53	60
Fertilization after the first insemination, %	27	33	40
Service period, days	152±3.0	136±2.0***	130±4.0*
Number of retained placentas, %	33	0	0
Number of endometritis, %	27	7	7

Notes: * – p<0.05; ** – p<0.01; *** – p<0.001

Source: author's development

A 20-percentage point decrease in the frequency of endometritis was noted in the two experimental groups. Retained placenta was

not detected in the experimental groups, while in the control group this postpartum complication was observed in a third of all cows, which

could have caused an increase in the frequency of endometritis. It is likely that the intrapar-enteral administration of a complex of neurotropic-metabolic substances and microele-ments in the form of carboxylates of food acids contributed to the optimization of postpartum processes in the cows of the second research group. The low frequency of postpartum disorders, in particular, placental retention in cows of experimental group 1, which began to be administered the complex drug only on the 5th day after calving, remains not fully understood.

The cows of the experimental groups, compared to the control, had a shorter period from calving to the first oestrus. Cows of the first group came into heat 7th and the second 10th days earlier after calving. This indicates the accelerated involution of the uterus and the restoration of the sexual cycle. The cows of the experimental groups had 6 and 13 percentage points higher fertility after the first insemina-tion than the control ones. The proportion of fat cows on the 90th day of lactation was greater in the experimental groups by 13 and 20 per-centage points, compared to the control. The final indicator for evaluating the reproductive function of cows is the service period. The use of a complex of substances of neurotropic and metabolic action made it possible to reduce the duration of the service period in experimental group 1st by 16th days, and in experimental group 2nd by 22nd days, due to the activation of the reproductive function and increasing the fertility of cows for the first insemination.

Comparing different methods of using the complex drug, the most effective option was the scheme of administration to experimental group 2. In the group of cows that were injected with a complex of substances into the abdom-inal cavity once in the first 1.5...2 hours after calving, the indicators of reproductive function were better. In particular, the period from calving to the recovery of the sexual cycle and the duration of the service period were shorter by 3rd and 6th days, respectively, and the fertility

after the first insemination was higher by 7 percentage points. The obtained difference is probably due to the effect of the drug in the most critical period for the cow, immediately after calving, which allows her to recover faster.

The reproductive function of cows de-pends on a number of factors. As noted by T. Abdisa (2018), among the main reproductive problems of cows, which directly lead to sig-nificant economic losses, are abortions, dysto-cia, placental retention, metritis, uterine and vaginal prolapse, lack of oestrus, low fertility. Most of these disorders are associated with the postpartum period. The most common problem is retention of the placenta, which leads to a number of problems after its removal. Accord-ing to T.T. Tucho & W.M. Ahmed (2017), due to the placental retention, the number of micro-organisms in the uterus increases, inflamma-tion occurs and the interval to fertilization is extended. Placenta retention is recommended to be treated. There are a number of protocols. In particular, S. Imhof *et al.* (2019) recommend the use of intrauterine devices boluses of tetra-cycline or introduction of ozone foam without attempts to remove the placenta manually. It is also considered effective (Dubeibe *et al.*, 2017) feeding for three consecutive days of herbal powder from *Leonurus artemisia*, *Angelica sin-ensis*, *Ligusticum chuanxiong*, *Sparganium sto-loniferum*, *Buch.-Ham. exjuz*, *Curcuma zedoaria*, *Cyperu srotundus* Linn. and *Glycyrrhiza uralen-sis*, which helps to improve the subsequent fertility of cows with retained placenta. In the author's research, it was established that it is possible to significantly reduce the share of placental retention in cows, if in the first 1.5...2 hours after calving, a single administration of a complex drug of neurotropic-metabolic compounds and carboxylates of food acids is provided. Prevention of placental abruption is more appropriate than further treatment. To avoid the risks associated with postpartum complications, other authors also recommend using preventive methods. As a way to reduce

the risk of placental abruption, especially under the conditions of limited consumption of vitamin by cows in dry and postpartum periods, E. Pontes *et al.* (2015) recommend injections of α -tocopherol. According to the authors, the use of α -tocopherol injection reduced the number of retained placentas from 20.1 to 13.5%, and subsequently increased the frequency of pregnancy after insemination by 22%. In the study, the introduction of a complex drug of neurotropic-metabolic compounds and carboxylates of food acids in the first 1.5...2 hours after calving made it possible to completely exclude placental retention in the cows of the experimental group. The method is more relevant for carrying out multiple vitamin injections, as it coincides with the postpartum care of the cow and has a better positive result.

Retained placenta is one of the risk factors for the development of endometritis. Usually, the share of endometritis in cows is high and reaches 20% 50th days after calving (Grimard *et al.*, 2017). According to I.M. Sheldon *et al.* (2019) up to 40% of cows have postpartum uterine diseases. Clinical endometritis has a strong negative effect on the frequency of first insemination and lengthening the duration of the period from calving to insemination. In Holstein cows, cytologically diagnosed endometritis was associated with a violation of reproductive function, which was manifested in the extension of the period from calving to fertilization and a decrease in the percentage of pregnancy after the first insemination (Gilbert *et al.*, 2005). In research conducted by P. Nyabinwa *et al.* (2020), in cows, that had a positive test for endometritis, in addition to the above-mentioned problems, an increase in the frequency of inseminations to obtain a body and an increased duration of anoestrus after calving were observed. Z.A. Mohammed *et al.* (2019) notes that endometritis increases the likelihood of atypical functioning of the ovaries, in particular the onset and duration of luteal activity. due to the use of a drug from a

complex of neurotropic-metabolic substances, it was possible to reduce the proportion of endometritis by 3.8 times, compared to control cows. This is probably related to both the decrease in the percentage of placental retention and the stimulating effect of the drug's components. In particular, O.V. Chernev (2014) proves that the use of sodium succinate, which was also included in the complex drug, affects cellular respiration, and during high physical exertion improves the lability and functional mobility of nervous processes. Researchers I.I. Hevkan *et al.* (2011) revealed the positive effect of the drug, which includes sodium succinate, it helps to increase the level of metabolic processes in the organs of the reproductive system of cows and accelerates the involution of the endometrium after calving.

There are other ways to reduce the risk of metritis and endometritis in cows. A.A. Barragan *et al.* (2021) proved that the administration of boluses with acetylsalicylic acid twice, starting 12 hours after calving and again 24 hours later, allowed reducing the proportion of cows that developed clinical metritis and clinical endometritis, as well as to shorten the period from calving to calving by 18th days fertilization. Acetylsalicylic acid is a nonsteroidal anti-inflammatory agent that has a short half-life and does not, unlike complex preparations with trace elements, have a supporting effect. In this regard, the use of complex drugs, in particular substances of neurotropic and metabolic action and carboxylates, which stimulate the recovery of the functions of the reproductive system after calving, has a certain advantage.

Not only endometritis, but also other infectious diseases and metabolic disorders caused by a negative energy balance at the beginning of lactation delay the first ovulation after childbirth. The manifestation of the first ovulation after calving is a coordinated process that includes the restoration of the somatotropin hormone-insulin-like growth factor 1 connection in the liver, the increase of follicles and

steroidogenesis, as well as the elimination of negative feedback with estradiol in the hypothalamus (Hevkan *et al.*, 2011). Long periods of anovulation after childbirth have a negative effect on reproductive efficiency. Anovulation of cows requires treatment. C.R. Burke *et al.* (2022) have proved the effectiveness of a long-acting synthetic derivative of kisspeptin to stimulate ovulation when a mature dominant follicle is present in the ovaries, but non-hormonal methods of prevention of this functional disorder are of greater interest. In the study, cows treated with the drug had their first oestrus on average at 35th and 32nd days, or 7th and 10th days earlier than untreated cows. This is a fairly early indicator. S.Yu. Ruban & M.A. Matvieiev (2020) note that it is necessary to allow cows to inseminate (voluntary waiting period) no earlier than 6 weeks after calving, therefore, if oestrus appears earlier, cows are not inseminated. But the early onset of the first oestrus after calving is evidence of the effective effect of a complex of substances with neurotropic-metabolic action and carboxylates of food acids of trace elements on the restoration of ovarian functions. Other complex preparations also have a positive result of stimulating the reproductive function of cows. In particular, the increased level of steroidogenesis, which was manifested by the increase in the blood of cows, cholesterol, as well as glucose, triglycerides and total protein, was detected when the reproductive function of cows was stimulated on the 1st-3rd day of the sexual cycle with the drug "Quatronan-Se" and carboxylates Se, Cu, Mn, Cr, and Ge (Seba *et al.*, 2016). This, in addition to the research, confirms the effectiveness of complex drugs for stimulating the functional activity of the reproductive system of cows and preventing disorders that may require treatment in the future.

Conclusions

The use of a complex of neurotropic-metabolic substances, which include L-arginine, sodium

succinate, sodium glutamate and carboxylates of food acids of iron, calcium, manganese, copper, and zinc affect the biochemical parameters of the blood of newborn cows and the recovery of reproductive function after calving. Cows administered the drug 21st days after calving had less variability in blood biochemical parameters and lower calcium, phosphorus, protein, and carotene content. The use of the complex drug in the cows of the experimental groups, compared to the control group, made it possible to avoid the manifestations of placental retention and reduce the frequency of postpartum endometritis by 3.8 times. Treated cows significantly shortened the period of postpartum uterine involution and recovery of ovarian function. The manifestation of the first oestrus in them was observed 7th-10th days earlier. The reproductive capacity of cows in the experimental groups was also better than in the control group. In the experimental groups, there was an increase in fertility by 6-13 percentage points after the first insemination, an increase in the proportion of heifer cows on the 90th day of lactation by 13-20%, and a reduction in the period from calving to conception by 10.5-14.5%.

If comparing different schemes of administration of the complex drug to cows, the best results are obtained with a single intraparentral administration of 60 cm³ (3.9 g of the active substance) in the first 1.5...2 hours after calving. Cows that were treated according to this scheme (experimental group 2) 21st days after calving, according to blood biochemical indicators, showed signs of increased activity of anabolic processes and tended to be superior to cows of the first experimental group in terms of the onset of sexual activity, fertility after the first insemination and average the duration of the period from calving to fertilization. The reason for this advantage is probably the supporting and stimulating effect of the drug from the first day after calving. The obtained results indicate the impracticality of postponing the first treatment of new-calved cows with a

complex drug for 5 days after calving and dividing the active substance into three injections.

In further studies, it is advisable to study the influence of carboxylates of food acids and substances of neurotropic-metabolic action on the peculiarities of milk productivity of cows in the post-calving period, in particular on changes in daily milk yield and chemical

composition of milk, energy balance and manifestation of metabolic disorders.

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Conflict of interest

None.

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Вплив карбоксилатів харчових кислот та речовин нейротропно-метаболічної дії на біохімічні показники крові та відтворні якості новотільних корів

Микола Васильович Себа

Кандидат сільськогосподарських наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0001-9696-934X>

Мансур Ільдусович Ягафаров

Аспірант
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0001-5832-0400>

Аяз Маммадов

Кандидат біологічних наук
Західно-Каспійський університет
1001, вул. Істіглаліят, 31, м. Баку, Азербайджан
<https://orcid.org/0009-0005-2240-3673>

Дмитро Костянтинівич Носевич

Кандидат сільськогосподарських наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0003-2495-2084>

Марина Олександрівна Хоменко

Кандидат сільськогосподарських наук
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0001-7023-3676>

Анотація. Після отелення проходять зміни в статевій системі корів, спрямовані на поновлення здатності до відтворення. Ці процеси відбуваються на фоні виснаження родами, лактації та негативного енергетичного балансу, що часто призводить до порушень відтворювальної функції, тому пошуки методів які сприяють її відновленню, актуальні. Метою роботи було дослідити вплив препарату неротропно-метаболічної дії, як стимулятора післяродового

відновлення корів та поліпшення плодючості. Препарат включав L-аргінін, сукцинат натрію, глутамат натрію та карбоксилати харчових кислот (Fe, Ca, Mn, Cu, Zn). Дослідження проведено методом груп на коровах голштинської породи. Було сформовано контрольну і дві дослідні групи по 15 голів. Коровам дослідної групи 1 робили ін'єкції у підхвостову складку на 5, 7 і 9 добу після отелення, розподіливши активну речовину на 3 частини. Коровам дослідної групи 2 препарат вводили інтрапарентерально в перші 1,5-2 години після отелення в повній дозі одноразово. Через 21 день після отелення у корів дослідних груп не виявлено зміщень біохімічних показників крові, порівняно з референтними значеннями. Але простежувалась тенденція зменшення кількості кальцію, фосфору, білка і каротину, порівняно з коровами контрольної групи, що вказує на наявність впливу на метаболічні процеси. Вміст загального білка в крові корів дослідної групи 2 був наближеним до нижньої межі фізіологічної норми. У корів дослідних груп не спостерігали затримок плаценти, в той час, як у контрольній групі цей розлад був у 33 % корів. Введення препарату дозволило на 20 відсоткових пунктів зменшити частоту виникнення ендометритів, на 7 і 10 днів скоротити період до початку першої тічки, на 6 і 13 відсоткових пункти підвищити заплідненість після першого осіменіння та на 16 і 22 дні скоротити сервіс-період. Вищу ефективність використання препарату отримали в другій дослідній групі. Використовувати результати досліджень доцільно на молочних фермах. Інтрапарентеральне введення препарату через 1,5-2 години після отелення дозволить поліпшити процес відновлення статевої функції корів і їх плодючість

Ключові слова: голштинська порода; сервіс-період; L-аргінін; сукцинат натрію; глутамінат натрію; еструс



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Application of beekeeping products in the technology of raw smoked sausages

Oksana Shtonda*

PhD in Technical Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0002-7085-6133>

Viktoriiia Kulyk

Postgraduate Student
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0002-6900-6967>

Kateryna Semeniuk

Postgraduate Student
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0003-0934-5155>

Abstract. The safety of fermented sausage products is due to the elimination of a number of risks of biological origin, mainly of a microbiological nature. One of the most promising directions for the development of barrier technologies is the use of starter cultures and the biologically active substances produced by them in the production of fermented meat products. The research aims to study the influence of starter cultures and honey on a complex of physico-chemical, structural-mechanical, organoleptic indicators and nutritional value of raw smoked sausages. The article presents changes in the physical and chemical parameters of raw smoked sausages when using starter cultures in combination with different types of honey. When performing the work, 4 types of honey were used: from sunflower, phacelia, honeydew honey and from medicinal herbs. At the first stage of research, the proportion of honey application and the type of starter crops were selected. The

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*Corresponding author



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next stage of the study was to determine the impact of the starter cultures included in the product BIOSTART PLUS 50 (lactic acid bacteria *Lactobacillus Sake* and yeasts *Debaryomyces Hansenii*) and honey on moisture loss during fermentation of raw smoked sausages, physicochemical parameters of finished products. Determination of moisture content in samples was carried out by drying in a drying cabinet at a temperature of $103\pm 2^{\circ}\text{C}$. Conducted studies prove that the use of the product BIOSTART PLUS 50 (lactic acid bacteria *Lactobacillus Sakei* and yeasts *Debaryomyces Hansenii*) and honey allows shortening the ripening and drying technological process. A comparative analysis of manufactured raw-smoked sausages in terms of nutritional value makes it possible to state that manufactured raw-smoked sausages with the addition of honey meet the requirements of the standard. The results of the set of studies carried out in this work showed that the use of a composition of lactic acid bacteria and yeast with the use of honey as a source of carbohydrates will allow intensifying the technological process and improve the quality of finished products

Keywords: starter cultures; honeydew honey; physical and chemical parameters; lactic acid bacteria; fermentation

Introduction

The increase in the standard of living led to a change in the consumer's attitude towards food products. The consumer is becoming increasingly demanding about food, he wants not only to eat well and avoid health risks, but also to receive products that suit his tastes. Quality becomes an important factor that determines the competitiveness of products. Dynamically developing biotechnology opens up new opportunities in food design. Using modern equipment and innovative technical methods, as well as appropriate food ingredients, we not only expand the range of food products useful and healthy for the human body, but also provide a preventive and necessary therapeutic effect.

The group of fermented meat products can be considered in the field of meat products as functional products that preserve the native properties of raw materials during the production process. Domestic fermented products include raw-smoked and raw-cured sausages and meat products. These products belong to the class of meat products that have a high biological value due to the absence of high-temperature heat treatment, which creates conditions for enriching them with various components, including those sensitive to high temperatures.

O.I. Petrova *et al.* (2020) claim in their work that the use of starter cultures in the technology of raw-smoked sausages ensured, under the condition of a gradual decrease in pH, stabilization of microbial activity, inhibition of the growth of unwanted microflora, and improvement of the organoleptic indicators of the product.

Scientists I. Vlasenko & T. Semko (2019) believe, that the presence of lactic acid bacteria and bifidobacteria (probiotics), including those introduced in the form of bacterial preparations (starters), and their metabolites, in the presence of a number of prebiotics, can further increase the biological value. The reduced moisture content makes them unique concentrates with a high level of complete protein and fat. The decrease in pH and water activity makes the product highly resistant to microbial spoilage and extends the shelf life even at normal temperature values. As carbohydrates, they chose honey, of particular interest is honeydew honey, which, due to its characteristics, as noted by the authors of I.K. Karabagias *et al.* (2020), namely the presence of a significant amount of different carbohydrates (more than 80%), has potential prebiotic capacity and is a source of nutrition for starter cultures.

I. Vlasenko & T. Semko (2019) claim that the use of starter cultures in the production of raw smoked and cured sausages and other meat products provides not only a probiotic effect, but also accelerates the process of ripening, structure formation, drying, changes the expressiveness of various sensory indicators (colour, taste, smell, consistency) to improve the level of biosafety and digestibility of the finished product. When using *Bifidobacterium* strains in practice, it is worth considering the optimal conditions for their development.

The use of starter cultures ensures a gradual decrease in pH, stable functioning of microorganisms, inhibition of the development of unwanted microflora and improvement of organoleptic properties. All over the world, the main criterion for choosing microorganisms as microbial inoculants is the degree of their influence on the smell and taste properties of the finished product. The purpose of this work is to intensify the technology of raw smoked sausages by using starter crops and beekeeping products – honey.

Literature Review

Researching the market of sausage products M. Yakymiv (2021), it was found that in recent years in Ukraine there has been a noticeable trend towards the growth of craft production, that is, small-scale production that uses selected raw materials and traditional methods of preparing meat products. Consumers distinguish such products because of their taste, naturalness and are ready to pay a high price.

Usually, such production is small and produces limited batches. Thanks to this, the manufacturer can control the quality of each product unit. Handmade products are prepared according to traditional recipes, which may include natural food additives, which improves the quality of products. In recent years, the volume and range of production of raw-smoked and raw-cured sausages has increased significantly, largely due to the emergence of numerous small craft-type productions.

M.V. Shynkaruk & O.O. Baluk (2021) claim that the most important technical characteristics of starters are: fermentation of carbohydrates with the formation of lactic acid, reduction of ripening time, improvement of the quality of the finished product and extension of the shelf life, denitrification, resistance to salts. Yeast fungi, which are often part of combinations of starter cultures, also use hydrocarbons as nutrients. Similarly to lactobacilli, yeast is used both to participate in the created taste-aromatic properties of the product and to perform protective and stimulating functions.

The life span of lactic acid bacteria in the presence of yeast increases many times. Together, they form a protected environment that prevents the invasion of foreign microorganisms. In this combination, lactic acid bacteria are more resistant to microbial antagonism than other bacteria. In the works of scientists (Hunko *et al.*, 2022) indicated that the main nutrients for starter cultures are carbohydrates contained in meat raw materials or are added additionally. Lactobacilli are very demanding on food sources. From carbohydrates, they mainly ferment hexoses (glucose, fructose, mannose, galactose) and disaccharides (lactose, maltose, sucrose), and only heterofermentative species, for example, some strains of *L. plantarum*, ferment pentoses (ribose, xylose, arabinose).

In the same meat, there are not enough hydrocarbons for complete fermentation of raw-smoked and cured sausages to obtain the desired result. Accordingly, there is a need for additional added sugars at the stage of making minced meat, to ensure an environment that is easily fermented by microorganisms. In modern production technology, along with traditional carbohydrates, lactose, and fructose are used as a component of taste-aromatic compositions and spice mixtures. Domestic and international researchers are working on improving the quality and safety of raw-smoked and raw-cured sausage products. A number of foreign and domestic researchers have paid attention to the

question of the use of sugars in the technology of raw smoked sausages.

A significant contribution to the study of honeydew honeys was made by the scientist L. Adamchuk *et al.* (2021). In her work, she notes that bees get honeydew from honeydew, the sap of plants that appears under the influence of temperature changes in the environment. It is a sugar substance of the vital activity of insects that parasitize plants. It has a dark colour with different shades. Yes, dark green from linden and spruce, brown from willow, brown-black from oak. From some plants, it is bright with a golden hue. In particular, it contains many potassium and iron salts useful for the body, especially in dark varieties. Compared to flower honey, honey contains more phosphorus, copper, manganese, molybdenum, sodium, and magnesium. Its total ash content is on average 0.569%, and the flower ash content is 0.123%. Compared to floral honey, the water content of honeydew honey is slightly lower, the amount of protein and dextrins is 2-3 times higher (Lesovoi *et al.*, 2020).

Previous studies have shown that the antibacterial and antioxidant activity of honeydew honey is higher than that of nectar. The mechanism of the high antibacterial activity of honeydew honey has not been fully elucidated (Ng *et al.*, 2020, Silva *et al.*, 2020). This situation is most likely due to the higher content of bioactive compounds, such as phenols, proteins and amino acids, in honeydew honey compared to flower honey (Seraglio *et al.*, 2019). In addition, the oligosaccharides of honeydew honey have a potential prebiotic capacity and contribute to the increase of bifidobacteria and lactobacilli populations in the human intestine (Karabagias *et al.*, 2020).

Carbohydrates in raw smoked sausages perform various functions, they not only serve as “food” for the fermentation process, but also directly affect the taste of the product, allowing the product to reach a certain degree of hardness. However, the use of sugar in large

quantities above 0.5-1.0% is advantageous as long as it does not lead to excessive oxidation and, accordingly, to significant weight loss. The use of biologically active ingredients, such as bee products, is one of the ways to improve the production of meat products, especially when creating new high-quality products, such as fermented and other sausage products.

The success of this approach depends mainly on the type of starter cultures capable of reducing product production time, improving quality indicators such as taste and aroma, and ensuring product safety for consumers. The number of various recipe additives introduced into the technology of raw-smoked sausages makes it necessary to carry out additional research on the dependence of the structural-mechanical, physico-chemical and sensory indicators of the finished product and to improve the production process.

Materials and Methods

The research was conducted in the laboratory of the Department of Meat, Fish, and Seafood Technology of the National University of Biore-sources and Nature Management of Ukraine in September-December 2022. For the production of trial samples of sausages, the recipe “Cheese-smoked savelat sausage of the highest grade” according to DSTU 4427:2005 (2006) was chosen.

At the first stage of the work, sausage products were made according to the technological scheme built on the basis of the technological scheme for the production of raw smoked sausages, given by V.H. Vynnykova (2006). For the production of fermented sausage products, meat raw materials were used: top grade boneless beef, boneless lean pork, boneless fatty pork in accordance with the requirements of regulatory documentation.

The procedure for preparing minced meat is as follows: first, frozen beef and lean pork were loaded into the cutter and chopped by cutting at low speeds, adding salt in the amount of 3.5% to the mass of raw materials

and a solution of sodium nitrite in the amount provided by the recipe. In the mixing process, pre-dissolved (1:4) starter cultures were introduced at the rate of 25 g per 100 kg of the main raw material mass, honey and spices, fatty pork were added, and cooking was carried out to the desired consistency of the meat system. Injecting the minced meat into the shell was carried out immediately after the minced meat preparation. Stuffing of minced meat was carried out using a manual sausage syringe. Aitzel casing was used as a sausage casing.

After carrying out a complex of technological operations according to the production scheme, the nutritional value of the developed raw-smoked sausages was determined. In order to ensure the fermentation process, starter cultures included in the BIOSTART PLUS 50 product manufactured by RAPS GmbH & Co. KG (Federal Republic of Germany) and med. were introduced into the minced sausage. According to the information provided by the representative of RAPS in Ukraine, BIOSTART PLUS 50 includes *Lactobacillus lactic acid bacteria Sakei*, and *Debaryomyces yeast mushrooms Hansenii*. As a control sample, the same raw-smoked sausage "High-quality raw-smoked servelat sausage" was taken, which included food glucose in the amount of 0.2% as a carbohydrate and BIOSTART PLUS 50 starter culture. The difference between the developed recipes and the control one is mainly the replacement of food glucose for honey of different types: sample No. 1 with sunflower honey; sample No. 2 with phacelia honey; sample No. 3 with honeydew honey; sample No. 4 with honey of medicinal herbs.

To obtain reliable experimental data, all studies were performed at least five times, two repetitions for each experiment. Sampling for sensory and physico-chemical studies and preparation for analysis were carried out in accordance with DSTU 4823.2:2007 (2007). Physicochemical indicators were determined by the following methods: protein content was determined by the Kjeldahl method according to ISO

1871:2009 (2009). The mass fraction of fat was measured by the Soxhlet method according to DSTU 8380:2015 (2015). The method is based on repeated extraction of fat with a solvent from a dried portion of the product, followed by removal of the solvent and drying of the sample to a constant weight. Extraction is carried out in the apparatus Soxhlet. Petroleum ether was used as a solvent. Determination of moisture content was carried out by drying in an oven at a temperature of $103\pm 2^{\circ}\text{C}$ (arbitration method) according to DSTU ISO 1442:2005 (2008). First, the weight of the product was determined, and then by drying the weight to a constant weight, the content of dry substances was determined; by the arithmetic difference of the mass of the raw and dry weight – the moisture content in the product. The content of table salt (sodium chloride) was determined by Mohr's method according to DSTU ISO 9297:2007 (2009). The organoleptic assessment and determination of the physicochemical parameters of honey were studied by standardized methods in accordance with DSTU 4497:2005 (2007).

Results and Discussion

One of the promising directions of intensification of the technological process of production of raw smoked sausages is the introduction of new biotechnological techniques based on the effective use of meat raw materials as their own enzyme systems, and on the purposeful introduction of starter cultures. A variety of technological techniques with the use of starter crops allows the production of finished products of high quality. When introducing starter cultures, it should be taken into account that the speed of their development directly depends on the amount of nutrient medium for them, at the same time, a significant introduction of sugars will speed up the technological process, but this may affect the organoleptic qualities of the finished product. The use of starter cultures and sugars in the production of raw-smoked sausages is due to the fact that lactic, pyruvic,

tartaric, acetic acids, ethanol, and other low-molecular compounds are formed as a result of the carbohydrate metabolism of microorganisms, which give the finished product a specific taste and aroma. Fat breakdown products: fatty acids and carbonyl compounds also play an important role in the formation of aroma.

The possibility of using honeydew honey in the production of fermented products has not been sufficiently studied. This direction requires the development of the technology of the mentioned ingredients, the study of their functional properties and mechanisms of interaction with the meat system, the preparation of recipes for fermented meat products, which include beekeeping products, and the development of technology.

According to numerous studies, it is known that the extract propolis, which was used to treat fermented sausages, shows an inhibitory effect against bacteria of the *Staphylococcus species aureus* and *Pseudomonas aeruginosa*, but more effective against gram-positive species (Safaei & Roosta Azad, 2020). Scientists Y. Sukhenko *et al.* (2021) during research, revealed inhibiting the growth of microbiota, which causes microbiological spoilage of semi-smoked sausages in a natural casing, 4-5 times more compared to control samples, without the formation of mould. Treatment of natural casings of semi-smoked sausage with aqueous extract of propolis in a dilution of 1:10 does not have a negative effect on the sensory and physico-chemical parameters of sausages.

Scientists J. Kocot *et al.* (2018) found that phenolic compounds, one of the main groups of beekeeping products, have an antioxidant effect. The Folin-Chocalto method is considered the main tool for measuring and estimating the amount of antioxidant compounds in extracts from various natural sources. The obtained results showed that propolis extract is an important source of phenolic components, such as phenolic acids and their esters and flavonoids. Hence, the propolis extract was analysed for

phenolic composition and the results showed that flavanones pinocembrin and naringenin, as well as flavonol galangin were the main (80.1%) compounds quantified. According to the work (Vargas-Sánchez *et al.*, 2019) it was reported that flavonoids pinocembrin and galangin are the main phenolic compounds in propolis extracts. The presence of phenolic compounds in natural extracts is highly correlated with antioxidant activity, so the inclusion of natural extracts in meat and meat products may be an important strategy to improve oxidative stability during storage. According to this study, dried pork sausages were treated with some antioxidants (propolis extracted with ethanol, 0.4% by weight; propolis extracted by water, 0.6% by weight; dried residue of propolis extracted by ethanol, 0.8% by weight./wt.) and stored for four weeks at 5, 10 and 20°C. At the end of storage, the results showed that lipid oxidation was reduced in the treated sausage products (50%, 59%, 35% and 91%, respectively) compared to the control. While in another product, ethanol-extracted propolis reduced lipid oxidation (78.5%) in beef patties during storage (8 days at 2°C), which was attributed to the presence of phenolic compounds such as quercetin, pinocembrin, kaempferol and luteolin.

O. Anjos *et al.* (2019) conducted a study on the use of bee pollen and bee pollen extract in the technology of blood sausages. Research data indicates a high antioxidant property of bee pollen and its extract. Which in turn affects the microbiological safety of the product. Another group of authors (Almeida *et al.*, 2017) investigated the effect of lyophilized bee pollen extract on the degree of lipid oxidation in pork sausage. The use of this extract showed a strong antioxidant effect in pork sausage, probably due to the high antioxidant activity and the presence of phenolic compounds in bee pollen, which has potential for use in sausage products.

The inclusion of flower pollen powder in the composition of sausages in the amount of 0.5 to 1.5 g/100 g (Novaković *et al.*, 2021),

resulted in a stable product during two months of refrigerated storage without negative impact on structural and mechanical characteristics. It can be concluded that pollen has excellent emulsifying properties, fat- and water-absorbing capacity, which qualify it for use in meat products, above all in heterogeneous colloidal products, such as sausages, where these properties are desired. Although excellent emulsifying properties in the pollen powder have been obtained, there is a certain limitation regarding sensory properties, especially due to obtaining a specific smell and taste, which can cause certain negative evaluations by consumers. In scientific research, propolis and bee pollen extracts are used instead of raw materials because they contain a greater number of bioactive components. However, the use of solvents of

different polarity affects the composition of the obtained extracts, since the components of beekeeping products have a diverse structure, and if hydrophilic ones dissolve better in polar solvents, such as alcohols, those with hydrophobic properties show a greater affinity for non-polar solvents, such as hydrocarbons. The properties of the extract strongly depend not only on the solvent used, but also on the extraction conditions. Also, readily available sugars are used for the production of raw smoked products, which contribute to the development and activity of microbiota, since glycogen (meat polysaccharide) cannot satisfy the needs of microorganisms. The authors investigated the characteristics of different types of honey, which were used in the composition of fermented products instead of glucose (Table 1).

Table 1. Physico-chemical indicators of honey

The name of the ingredient	Physico-chemical parameters		Organoleptic indicators		
	Mass fraction of moisture, %	Active acidity, units pH	Colour	Taste and aroma	Consistence
Honeydew honey	18.3	3.92	Dark brown	Pronounced fruity; weak honey	Viscous
Honey from medicinal herbs	18.1	3.84	Amber	Tender; saturated and pronounced	Liquid, viscous
Sunflower honey	17.2	3.75	Golden yellow	Astringent; weak honey	Thick, low tensile strength
Honey from phacelia	16.3	3.78	Light yellow	Saturated; tart-sweet	Thick, sticky

Source: author's development

The pH value of honey determines its antibacterial properties. Bioactive substances of natural components not only provide improved organoleptic properties, inhibit the growth of unwanted microorganisms, inhibit lipid oxidation, but also contribute to better functioning of the beneficial microflora of bacterial preparations.

Previous studies show that the antibacterial and antioxidant activity of honeydew honeys exceeds that of nectaries (Silva *et al.*, 2020). The next step was to examine the nutritional value

of the product. Since one of the important indicators of product quality is the value of its nutritional and biological value, and in most cases the change of these indicators depends primarily on the composition of the raw material, its changes in the course of internal biochemical processes, external influences, as well as due to the additives used.

The nutritional value of the product was investigated after the end of fermentation and drying of the product: cold smoking was carried out

at a temperature of 20°C for 48 hours in a smoking chamber, drying was carried out in two stages. The first stage – at a temperature of 11°C and a relative humidity of 85% for 7 days. The second stage at a temperature of 10°C and a relative humidity of 78% in a cabinet for ripening meat

for control samples, No. 1 and No. 2, the duration of drying was 7 days, for samples No. 3 and No. 4-9 days. The duration of drying for the samples was increased by two days in order to reach the moisture content in the products regulated by the DSTU for raw-smoked sausages (Fig. 1).

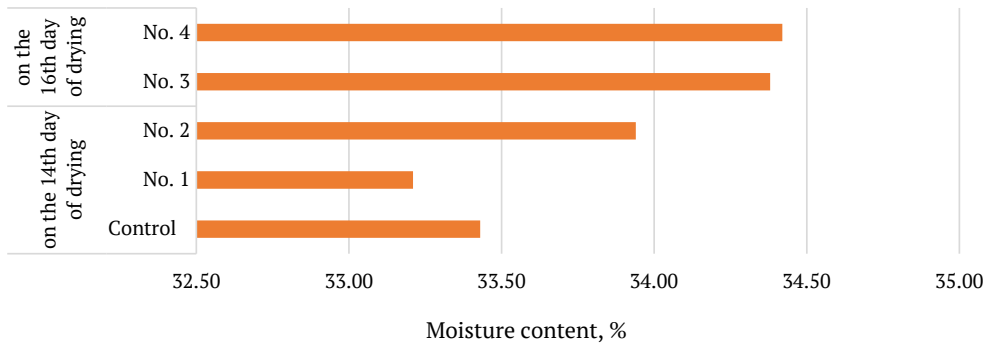


Figure 1. Changes in moisture content in raw smoked sausages during drying

Source: author's development

It was established that during the ripening-drying of loaves of raw-smoked sausages, the distribution of moisture by volume of the product is uneven, and it does not depend on the rate of diffusion of moisture inside the loaves. In general, it is also worth saying that in the samples that lost moisture the slowest (No. 3 and No. 4), lactic acid bacteria had more favourable conditions for increasing their population and producing lactic acid. From the presented data, it can be seen that the moisture content of experimental and control raw-smoked sausages

corresponds to the normative values (no more than 35%).

Probiotic lactobacilli are quite sensitive to salt, so it is necessary to add it to minced meat products according to the norm. For raw-smoked sausages, the mass fraction of kitchen salt is standardized at the level of 5-6%. According to the salt content, all samples at the exit met the requirements of DSTU 4427:2005 (2006), i.e., its fraction was less than 6%. The lowest percentage of salt, 4.12%, was found in the samples with honeydew honey (Fig. 2).

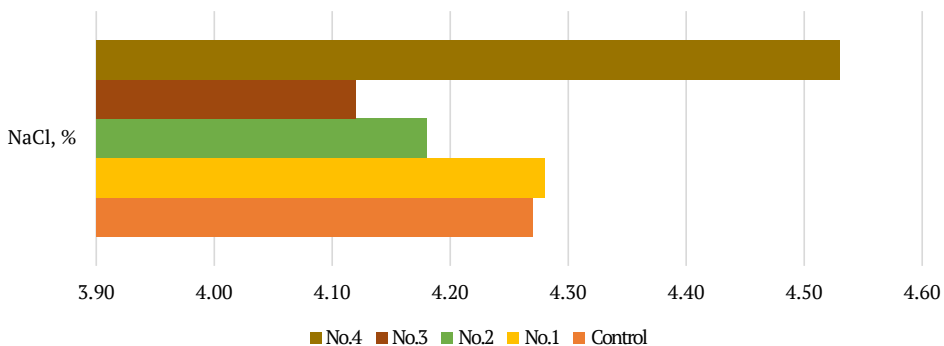


Figure 2. Mass fraction of salt in raw smoked sausages, %

Source: author's development

The main properties of bifidobacteria and lactobacilli are their high acid and aroma-forming properties able, expressed proteolytic activity, thanks to the developed complex of proteinases and peptidases, in relation not only to muscle, but also to connective tissue proteins.

With a decrease in the mass fraction of moisture, there was a redistribution of the mass

fractions of the main components of the product. In particular, in experimental variants, during ripening, the mass fraction of protein increased by 2.7-11.6% compared to the control sample (Table 2). In terms of protein content, sample No. 3 with honeydew honey and No. 4 with medicinal herb honey have the best results.

Table 2. Nutritional value of raw smoked sausages

Characteristic	Sample K with glucose	Sample No. 1 with sunflower honey	Sample No. 2 with phacelia honey	Sample No. 3 with honeydew honey	Sample No. 4 with honey from medicinal herbs
Protein content, g/100g	18.82	19.33	19.01	21.84	21.26
Fat content, g/100g	43.48	42.81	42.88	39.12	39.80
Energy value, kCal (kJ)	466.6 (1952)	462.61 (1935.5)	461.92 (1932.7)	439.44 (1838.6)	443.24 (1854.5)

Source: author's development

According to the results of the protein content study, it can be said that the use of honey in general does not cause a negative impact on the protein component of raw smoked sausage compared to the control sample, and ensures the nutritional value of the product is higher than the control when using its individual varieties. According to the fat content, the indicators of all samples were in the range from 39 to 43.5%, which corresponds to the normative indicator, no more than 65%.

The sample with honeydew honey had the lowest fat percentage of 39.12%, and 10% less than the control. The results of measuring the fat content indicate that the starter cultures most effectively caused the breakdown of fats in the medium with the addition of honeydew, which had a positive effect on the quality of the product. Fat breakdown products, namely free fatty acids and carbonyl compounds, play an important role in forming the aroma of the finished product, thereby influencing its consumer properties. Based on the results of measurements of protein and fat content, it is possible to calculate the energy value (calorie)

of products (Table 1). According to calculations, the samples of raw smoked sausages with phacelia honey and honeydew honey have the lowest caloric values, which is due to the lower fat content after the end of the fermentation period. The comparative analysis of the samples makes it possible to state that the manufactured raw-smoked sausage with the addition of honey generally meets the requirements of the standard in terms of physical and chemical parameters.

Conclusions

Microorganisms included in the composition of the starter cultures cause the breakdown of carbohydrates first, and then protein substances and fats (lipids), especially in the initial period of ripening, and thereby contribute to the formation of the desired aroma of the finished product. For this purpose, the recipes of raw smoked sausages include a significant amount of sucrose. However, based on the quantitative and qualitative composition of starter cultures, the most appropriate is the use of carbohydrates that can cause a synergistic effect in

the formation of functional and technological properties and organoleptic indicators of the product.

The results of the set of studies carried out in this work showed that the use of a composition of lactic acid bacteria and yeast with the use of honey as a source of carbohydrates will allow intensifying the technological process and improve the quality of finished products. This indicates the expediency of using the composition and honey in the technology of raw smoked sausages.

Based on the results of the work, new recipes and technology for raw smoked sausages were developed. The proposed technology makes it possible to reduce the period of production of products from 26 days to 16 days. It has been established that the use of bacterial cultures and honey contributes to the strengthening of the fermentation process, providing a pronounced taste-aromatic bouquet and the formation of a consistency, shortening the duration of the production of the product by 10 days compared to the product without the addition of starter cultures.

The sample with honeydew honey showed the most balanced parameters in the sausage maturation process. The average intensity of the decrease in pH and moisture loss, the lowest fat content, indicates that its composition is close to optimal for use as a carbohydrate for feeding starter cultures in the process of sausage ripening. It has been proven that the use of honey in the amount of 0.3% by weight of raw materials does not inhibit the development of technological microflora, but has a positive effect and stimulates the growth of desirable microorganisms.

In order to ensure high consumer properties of fermented sausages, it is necessary to additionally study the parameters of all technological operations and the mechanisms of influence of the applied additives on the safety of this group of sausages.

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Conflict of Interest

None.

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Застосування продуктів бджільництва у технології сирокочених ковбас

Оксана Анатоліївна Штонда

Кандидат технічних наук, доцент

Національний університет біоресурсів та природокористування України

03041, вул. Героїв Оборони, 15, м. Київ, Україна

<https://orcid.org/0000-0002-7085-6133>

Вікторія Костянтинівна Кулик

Аспірант

Національний університет біоресурсів та природокористування України

03041, вул. Героїв Оборони, 15, м. Київ, Україна

<https://orcid.org/0000-0002-6900-6967>

Катерина Миколаївна Семенюк

Аспірант

Національний університет біоресурсів та природокористування України

03041, вул. Героїв Оборони, 15, м. Київ, Україна

<https://orcid.org/0000-0003-0934-5155>

Анотація. Безпека ферментованих ковбасних виробів обумовлена усуненням низки ризиків біологічного походження, переважно мікробіологічної природи. Одним з найбільш перспективних напрямів розвитку бар'єрних технологій є використання стартових культур і вироблених ними біологічно активних речовин у виробництві ферментованих м'ясних продуктів. Дослідження має на меті вивчення впливу стартових культур та меду на комплекс фізико-хімічних, структурно-механічних, органолептичних показників та харчову цінність сирокочених ковбас. У статті представлено зміни фізико-хімічних показників сирокочених ковбас при використанні стартових культур в поєднанні з різними видами меду. При виконанні роботи використовували 4 види меду: з соняшника, фацелії, падевий мед та з лікарських трав. На першому етапі досліджень підбирали частку внесення меду та вид стартових культур. Подальшим етапом дослідження було визначення впливу стартових культур, що входять до складу продукту BIOSTART PLUS 50 (молочнокислі бактерії *Lactobacillus Sakei* та дріжджові гриби *Debaryomyces Hansenii*) та меду на втрати вологи під час ферментації сирокочених ковбас, фізико-хімічні показники готової продукції. Визначення вмісту вологи в зразках проводили методом висушуванням в сушильній шафі при температурі 103 ± 2 °C. Проведені дослідження

доводять, що застосування продукту BIOSTART PLUS 50 (молочнокислі бактерії *Lactobacillus Sakei* та дріжджові гриби *Debaryomyces Hansenii*) та меду дозволяє скоротити технологічний процес дозрівання і сушіння. Порівняльний аналіз виготовлених сиркопчених ковбас за харчовою цінністю дає змогу стверджувати, що виготовлені сиркопчені ковбаси з додаванням меду задовольняють вимоги стандарту. Результати комплексу досліджень, проведених у даній роботі, показали, що застосування композиції молочнокислих бактерій та дріжджів з використанням меду, як джерела вуглеводів, дозволить інтенсифікувати технологічний процес та підвищити якість готової продукції

Ключові слова: стартові культури; падевий мед; фізико-хімічні показники; молочно-кислі бактерії; ферментація

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E-mail: info@animalscience.com.ua

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