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Influence of organic acids on organoleptic and structural and mechanical properties of freshwater hydrobiont meat

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Abstract. Research on the use of organic acids in freshwater fish conservation in aquaculture farms is gaining importance due to the growing interest in the quality and safety of fish products. The purpose of the study was to create and substantiate a new technology for preserving freshwater fish with the addition of spicy root vegetables, and in pretreatment of raw materials with organic acids. During the study, carp were treated with salt and various concentrations of organic acids, stored under certain temperature conditions. Sensory assessment, pH, and shear boundary stress were used to evaluate the results, and chemical analysis was performed using a penetrometer and potentiometric method. Based on the findings, a scientifically based technology for preserving freshwater fish with the addition of spicy root vegetables with a fundamentally new method of treatment of raw materials with organic acids has been developed to expand the scope of application of fish products that will have a higher biological value. It was found that at the initial stages of processing, no noticeable organoleptic changes were observed in the samples under study. Comparative analysis of the results confirmed the positive effect of acetic acid at a concentration of 1.0%, for tartaric acid, the best effect was determined with the addition of 0.5-1.0%, and for citric acid, the study indicates that its use does not lead to softening of muscle tissue, indicating the feasibility of its use. Studies have confirmed the use of pretreatment of semi-finished products of salted carp treated with 1.0-1.5% malic acid for 60 minutes to obtain a tender, juicy, and soft consistency, which corresponds to 5 points in the sensory assessment. According to the results of the conducted studies, the positive effect of using organic acids to soften carp meat in the production of preserves from freshwater fish was established. The practical significance of the study is to improve the technologies of meat processing and food production, and to determine the optimal conditions for preserving the taste and texture qualities of meat products

Keywords: carp; shear limit stress; hydrobionts; organoleptic parameters; under-ripening fish

Introduction

Providing the population with high-quality food products of increased biological and nutritional value is quite relevant today. The reason lies in the violation of the diet, that is, the lack of many necessary food elements. In this regard, the concept of creating new, balanced food products rich in functional ingredients to ensure their sustainability and safety after processing and during storage is becoming increasingly relevant.

In Ukraine, the current state of human nutrition requires the development and implementation of technology for products that would be made from raw materials of natural origin and predictive composition. Currently, these products do not yet meet the human needs for basic food elements. Freshwater fish farming and fishing are developing in Ukraine, but the product range is limited to live and chilled fish. The sensory and nutritional value of freshwater fish is necessary to improve processing technology by combining it with vegetable raw materials. Changes in the structure of the raw material base of Ukraine towards increasing the catch of freshwater aquaculture species necessitate the expansion of the range of food products based on these hydrobionts. Unlike marine fish, freshwater fish has lower nutritional and biological value. In this regard, in recent years, much research has focused on the development of technologies for the production of aquatic products from freshwater fish with plant ingredients and materials of animal origin in order to increase the nutritional value, maintain and regulate functional properties (Gao *et al.*, 2023).

Carp is one of the most common fish in the water farms of Ukraine. This fish contains a lot of protein with a full set of essential amino acids, biologically effective fatty acids, but fish meat has a low percentage or does not contain fibre, some trace elements necessary for nutrition according to the needs of modern ideas about nutrition. In particular, freshwater fish do not have high taste properties, which requires their improvement. Spicy root vegetables are grown in Ukraine, which are little used in fish production. Previous studies have shown that the use of raw materials of plant origin, including root vegetables that are spicy-aromatic, in the technology of processing hydrobionts, contributes to the improvement of sensory indicators and the creation of food products containing functional ingredients (Holembovska *et al*., 2021).

N.V. Novikova *et al.* (2023) investigated the biological and nutritional value of newly developed preserves that were enriched with biologically valuable plant-based ingredients. A special feature of the newly developed preserves is that the fruit filling used is fresh juice obtained from cranberry and elderberry fruits. The use of natural preservatives that can increase the shelf life of fresh fish has been investigated by many researchers, in particular S. Pedrós-Garrido *et al*. (2020). Their studies identified 8 essential oils (lemon, lemongrass, lime, garlic, onion, oregano, thyme, and rosemary) and 3 organic acids (ascorbic, citric, and lactic).

M.A. Ezzat *et al.* (2023) developed traditional fermented foods from freshwater fish such as Mozambique tilapia (*Oreochromis mossambicus*) and carp with the addition of tamarind pulp (*Tamarindus indica*) and dried slices of *Garcinia atroviridis* to promote the fermentation process. N. Hananiah & A.A. Rahim (2022) were concerned with preserving food quality by eliminating or controlling the proliferation of pathogens, which could be achieved by adding natural ingredients such as lime juice (*Citrus aurantifolia*) to the main recipe of preserves. The presence of organic acids in lime acted as an acidifier that provides a low pH environment for microbes to slow their growth. However, no systematic studies have been conducted in the field of activating the maturation of freshwater fish when introduced into preserves. Therefore, the development of technology for preserving freshwater fish and spicy root vegetables is an urgent problem and the solution of which will contribute to the creation of high-quality, safe, affordable, and biologically valuable fish products from domestic raw materials.

The purpose of the study was to determine the patterns of change in indicator complexes of carp meat under the influence of various acid concentrations during cold storage at temperatures from 0 to 5°C within 90 minutes of treatment.

Literature Review

Analysis of current trends in the development of fish production technology allows identifying the following key areas related to the technology of multicomponent food products, improvement of canning technology from hydrobionts that have the ability to ripen slowly, enrichment of recipe ingredients, pretreatment of raw materials by various methods, using physico-chemical technologies, biotechnologies for the production of traditional fish products: canned food, preserves, pates, dry, salted, dried culinary products, and products made from washed minced meat for the production of surimi (Holembovska *et al*., 2021).

It is known that the addition of enzyme preparations when salting fish helps to obtain a good quality product from unripe fish species. In the production of fish preserves, expensive enzyme preparations from plant and microbial raw materials can be used, such as *H. Mediteranei* fermentation broth for softening pollock meat or the proteolytic enzyme preparation *Sal Intersor EC* for the production of fish fillet preserves (Rodak & Fil, 2016). These researchers

demonstrated that some marine hydrobionts contain highly active complexes of proteolytic enzymes that can be widely used. The muscle tissue of freshwater fish has a low level of activity of its own proteinases, which would not allow obtaining the necessary degree of maturity without the addition of proteolytic enzyme preparations to enhance the process.

M. Pobedash developed the technology of enzymatic processing of zebra mussels with proteolytic action, the effect of using the proteolytic enzyme preparation "Protofermol" from bivalve zebra mussels without separating the leaf in advance to enhance the maturation process of preserves from dissected herring (Pobedash, 2013; Pobedash & Sydorenko, 2014). A.J. Reid *et al.* (2019) substantiated the mechanism of action of antibacterial drugs on microorganisms, their proteolytic and lipolytic enzymes, which reduces microbial contamination of semi-finished products and finished fish products. Considerable interest was aroused by the technology of production of preserves from non-fish aquatic products, in particular, squids and sea snails, which are under-ripening raw materials for the production of preservatives. The technology of preserves from these shellfish involves pretreatment of raw materials by blanching or using progressive peak wave processing to soften the consistency of meat. The effectiveness of treatment of fish preserves with orange juice, which promotes their maturation, has been proven. The use of orange juice as a food additive gives the product the following functional properties: saturates preserves with biologically active substances, vitamins C, PP, B vitamins, beta-carotene, and potassium, calcium, iron, and selenium (Bytiutskaia, 2010).

N.B. Rathod *et al.* (2021) considered technologies of fish ingredients that are promising for improving preservation technologies, since products in which the natural properties of raw materials are better preserved due to the absence of heat treatment. Therefore, changes in the structure of fisheries, general trends to reduce the volume of marine fish caught and the growth of freshwater fish used for aquaculture, leads to the need to improve raw material processing technologies and increase the scale of production of food products based on freshwater fish. The most promising way to solve these problems is to develop a technology for preserving freshwater fish, based on the principle of combining food products by enriching them with plant raw materials. The technology of preserves from under-ripening hydrobionts is associated with the use of factors that activate the own enzyme system of muscle tissue and contribute to the formation of a "bouquet" of food products. However, in the production of many products, including preserves, in order to increase the safety of products during storage, organic acids (acetic, tartaric, citric, malic, etc.) are more often used in the technology, the positive effect of which is proved by the researchers (Derkach *et al.*, 2022; Wang *et al.*, 2023).

However, in order to determine the pattern of exposure to different acid concentrations both to ensure safety and to stimulate maturation, it is necessary to conduct additional studies that are conditioned by the characteristics of the type of raw material. The concept of creating new food products that are balanced in chemical composition, enriching functional ingredients, ensuring their safety after and during processing, is becoming increasingly relevant. Based on the generalisation of theoretical literature, the study highlights the expediency of using organic acids in the technology of freshwater fish canning, which contribute to the maturation of semi-finished products with a salty taste and form food products with high sensory properties and increased biological value.

Materials and Methods

The research was conducted in 2022 at the laboratories of the National University of Life and Environmental Sciences of Ukraine, the Faculty of Food technologies and quality management

of agricultural products at the Department of meat, fish, and seafood technology. Carp were cut into fillets, salted with brine to a salt concentration of 5% at room temperature for 24 hours. The fillets were then treated with organic acids of various concentrations. The control samples consisted of carp meat only, which was salted without the addition of organic acids. Canned samples were stored in plastic containers with a capacity of 200 cm^3 at a temperature of 0 to +4°C. The average sample was then taken from each unit, which was characterised by sensory assessment, pH, and shear limit stress after grinding.

The main raw materials used in the research were live fish (carp) according to DSTU 2284:2010 (2012), grown in the reservoirs of Cherkasy fish farms; table salt according to DSTU 3583:2015 (2017); table vinegar according to DSTU 2450:2006 (2006); apple cider vinegar according to DSTU 2450:2006 (2007), citric acid according to DSTU 908:2006 (2006), wine vinegar according to DSTU 2450:2006 (2007), and laboratory equipment: pH meter (LLC Chemlaborreactive, Ukraine) and penetrometer PCE-PTR 200N (LLC Chemlaborreactive, Ukraine). To determine the value of the ultimate shear stress (USS), a PCE-PTR 200n penetrometer was used using a room temperature exposure of 5 s, placing the pieces in identical metal measuring containers when using a measuring cone with a vertex angle of $2\alpha=60°$. For research, the muscle tissue of raw fish was crushed in an electric meat grinder with a grid hole diameter of 3 mm. After grinding, the water index (pH, active acidity) was determined in the obtained samples using the potentiometric method (Slobodianiuk *et al.,* 2018).

Evaluation of organoleptic characteristics included analysis of the appearance, taste, aroma, colour, and consistency of the product. An expert panel set up to assess the sensory quality of the fillets used a 5-point scale developed by the authors throughout the processing. Each of the parameters was evaluated according to a five – point system, where 5 points corresponded to an excellent level of quality, 4 points – good quality, 3 points – satisfactory, 2 points – unsatisfactory, and 1 point – low quality. The results of sensory evaluation were reflected in profile graphs that detail changes in fish consistency after acid exposure compared to the control group. 5 repetitions were performed for the study, and the analysis of experimental data was carried out using mathematical and statistical methods.

All data was processed using mathematical statistics methods, using Microsoft Excel and STATISTICA editors. The accuracy of the experimental results was determined using the Student's t-test with a confidence level of $\leqslant 0.05$, provided that the number of parallel measurements was at least 5. To solve linear programming problems, the MS Excel table processor (Excel Solver) was used.

Results and Discussion

The technology of preserves made from under-ripening aquatic organisms is based on the use of factors that activate the muscle tissue's own enzyme system and contribute to the development of a pleasant taste inherent in this food product. Organic acids (acetic, tartaric, citric, malic, etc.) are increasingly used in the production of many products, especially preserves, to improve product safety during storage, as evidenced by the development of research papers. However, to determine the regularity of the effect of different acid concentrations both to ensure safety and to stimulate maturation, it is necessary to conduct additional studies due to the characteristics of the type of raw material. Sensory evaluation of carp meat under the influence of different concentrations of acetic acid showed a positive effect depending on the processing time (Fig. 1).

At the first stage of processing, no significant organoleptic changes were observed in the control and experimental samples. After 30 minutes of treatment, the sensory parameters

of the prototypes improved compared to the control, especially at a concentration of 0.7-1.5%. Carp pieces after treatment with different concentrations of acetic acid differed from the control samples in the best shape indicators. After 30 minutes of acid exposure, there was an improvement in taste, smell, and colour. The consistency of all the tested variants was soft, very tender and juicy, and after 90 minutes of processing, it became less tender and softer with obvious signs of white coating.

Similar technologies were obtained by T. Maevskaia *et al.* (2012), who used organic acids in the washing of minced meat to produce better surimi from freshwater fish, increasing the washing efficiency. The proposed methods improved some indicators of washed minced meat, while worsening others. The proteins of minced meat obtained during the washing process contained almost the same amount of non-replaceable amino acids, and the use of catholyte allowed obtaining a product whose proteins are richer in non-replaceable amino acids, helping to identify the effect of the type of washing liquid on some values of indicators.

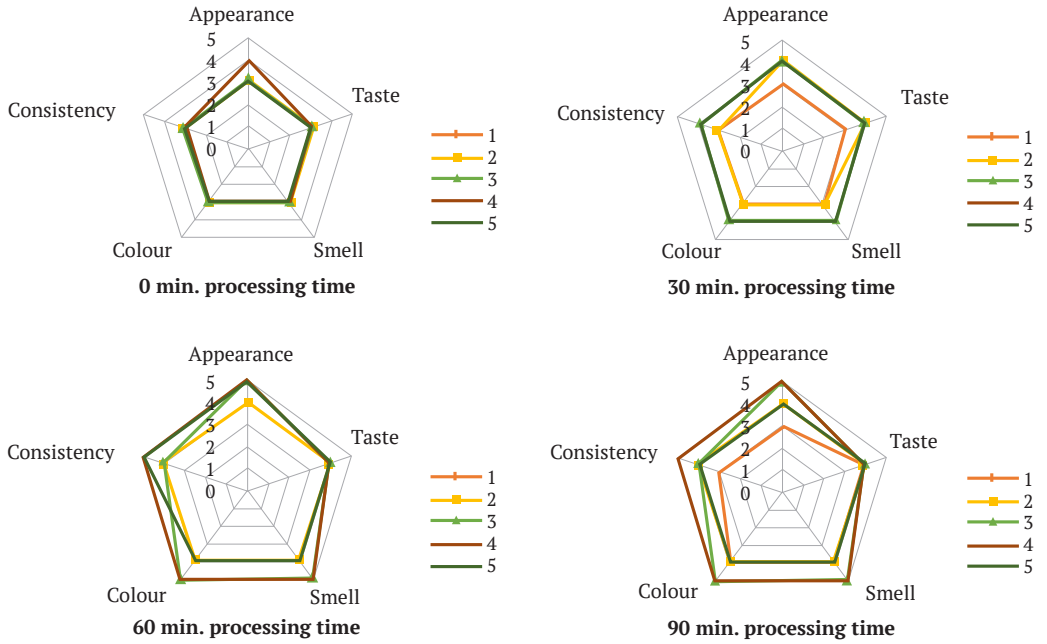

Figure 1. Changes in the organoleptic evaluation of carp meat under the influence of different concentrations of acetic acid and dependence on processing time

Notes: (n=5, p≤0.05): 1 – control; 2 – C=0.5%; 3 – C=0.7%; 4 – C=1.0%; 5 – C=1.5%

Source: developed by the authors

The best results in terms of softening consistency, taste, smell, and colour were observed for samples treated with 1.0% acetic acid during 60 minutes of treatment. They develop a soft consistency, the meat becomes very soft, excessively tender and juicy and corresponds to a 5-point scale. Therefore, according to the results of organoleptic evaluation, acetic acid in all experimental samples has a positive effect on carp meat, and a more pronounced effect is manifested in the variant with a concentration of 0.7-1.0%. The characteristics of the change in USS in the muscle tissue of carp treated with acetic acid are shown in Figure 2.

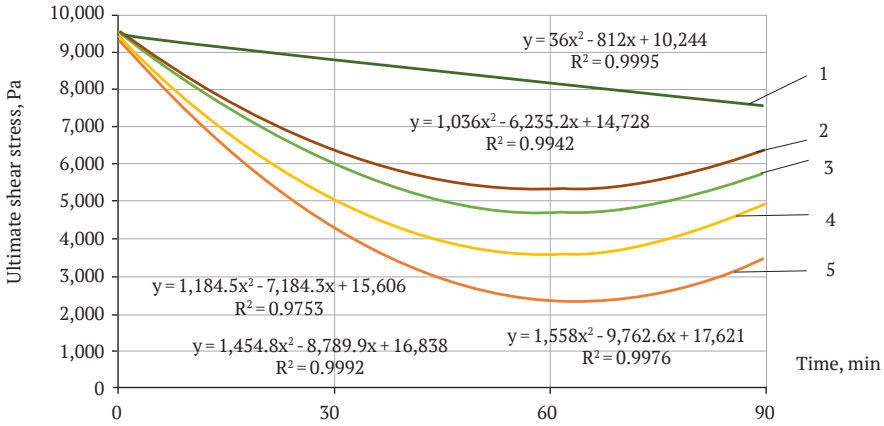

Figure 2. Dynamics of dependence of the USS of carp meat on the action of different concentrations of acetic acid for 90 minutes

Notes: (n=5, p⩽0.05): 1 – control; 2 – C=0.5%; 3 – C=0.7%; 4 – C=1.0%; 5 – C=1.5%

Source: developed by the authors

Analysing the data, it was concluded that the effect of all acetic acid concentrations is accompanied by a more significant decrease in the USS index compared to the control sample, which indicates the effect of acid on carp meat, resulting in its softening. Gradual decrease in this indicator is observed up to 60 minutes, after which it begins to grow again. It is proved that the consistency of fish meat under the action of acids has the ability to soften, which is confirmed by the activation of cathepsin enzymes and a decrease in pH to the acidic side. The results of this study are consistent with these theories. A linear dependence of changes in the pH of carp meat during exposure to different concentrations of acetic acid was observed, characterised by a decrease in the acidic side from 6.8 to 4.3 (Fig. 3).

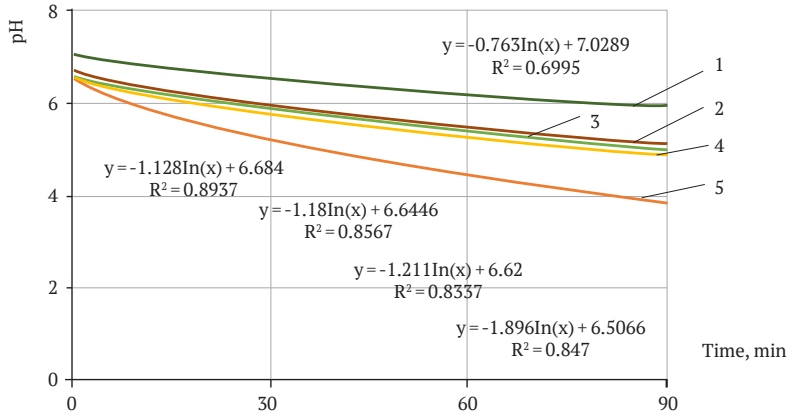

Figure 3. Dynamics of changes in the pH of carp meat under the influence of acetic acid of different concentrations depending on the processing time

Notes: (n=5, p⩽0.05): 1 – control; 2 – C=0.5%; 3 – C=0.7%; 4 – C=1.0%; 5 – C=1.5%

Source: developed by the authors

The indicator of changes in the consistency of meat – USS – indicates that after 60 minutes of acid treatment, the consistency is compacted against the background of a gradual change in pH to the acidic side and the organoleptic evaluation of softening of the consistency (Fig. 4).

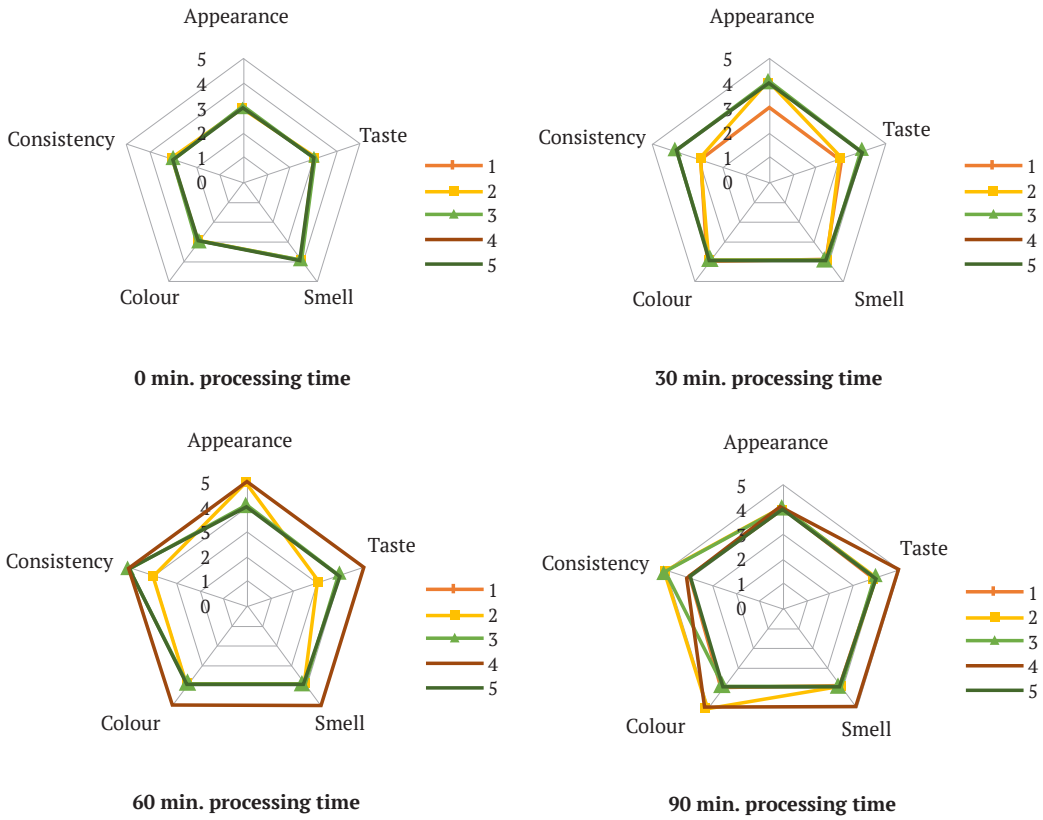

Figure 4. Changes in the organoleptic evaluation of carp meat under the influence of different concentrations of malic acid and dependence on the processing time

Notes: (n=5, p⩽0.05): 1 – control; 2 – C=0.5%; 3 – C=0.7%; 4 – C=1.0%; 5 – C=1.5%

Source: developed by the authors

These inconsistencies can be explained by the fact that when the period of processing carp meat with acids is extended, both protein hydrolysis and denaturation changes occur simultaneously. An organoleptic evaluation of carp meat treated with malic acid at different concentrations is shown in Fig. 4. After 30 minutes of treatment, the organoleptic characteristics of the experimental samples improved compared to the control, after 60 minutes of processing at a concentration of 0.5%, the appearance improved significantly; at 0.7; 1.5% – the consistency reached high quality, the meat became soft, extra tender and juicy; at 1.0% these samples correspond to a 5-point score. During 90 minutes of processing, the samples lose their quality in terms of such indicators as consistency, which becomes more rigid, and appearance of minor signs of white plaque on the surface. The results of the study of USS of carp meat treated with malic acid are shown in Fig. 5.

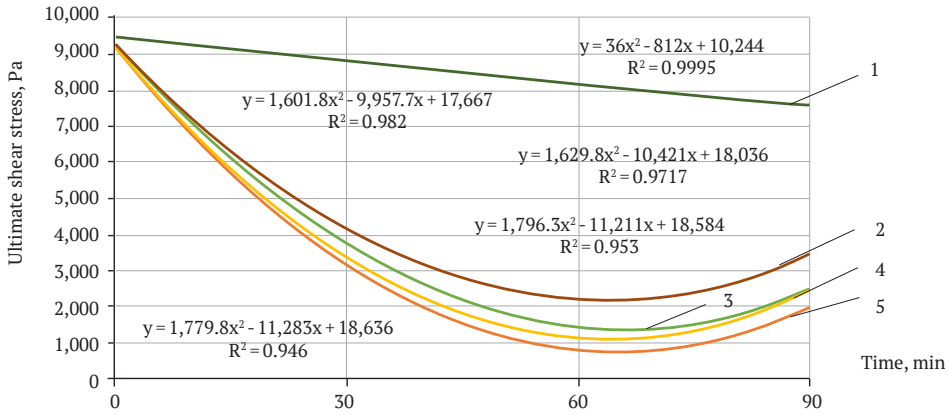

Figure 5. Dependence of the ultimate shear stress of carp meat on exposure to various concentrations of malic acid for 90 minutes

Notes: (n=5, p⩽0.05): 1 – control; 2 – C=0.5%; 3 – C=0.7%; 4 – C=1.0%; 5 – C=1.5%

Source: developed by the authors

The nature of exposure to different concentrations of malic acid is identical to that of acetic acid, but during its use, the greatest decrease in the USS index during the same time was noted. Changes in the pH value of carp meat are consistent with the results of a study of changes in this indicator after treatment with acetic acid, showing that in this case, cathepsin activation occurs after 45 minutes (Fig. 6) and is characterised by a gradual decrease from 6.8 to 4.2 in the acidic side. S. You *et al.* (2022) proved that a decrease in the value of active acidity negatively affects fish proteins due to the destruction of some essential amino acids, primarily lysine and sulphur-containing ones.

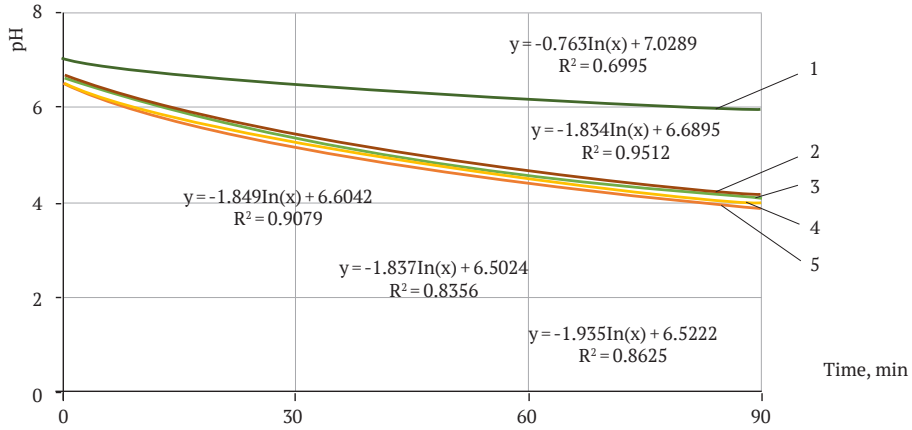

Figure 6. Dynamics of changes in the pH of carp meat under the influence of different concentrations of malic acid depending on the processing time

Notes: (n=5, p⩽0.05): 1 – control; 2 – C=0.5%; 3 – C=0.7%; 4 – C=1.0%; 5 – C=1.5%

Source: developed by the authors

M.M. Pobedash & O.V. Sydorenko (2014) investigated pH changes in fish preserves with the addition of sea buckthorn and cranberries, which were used as preservative components that are natural and with the assistance of which the pH stabilisation of the environment of preserves is achieved due to organic acids in their composition. In addition, their action affected the buffering indicators, it remained stable due to the action of plant components, also extended the shelf life and improved consumer properties. Researchers also explained the effect of organic acids (namely benzoic acid), natural antioxidants and pectin substances, which are found in plant additives, on the state of consistency of fish meat, which becomes softer and more tender. The organoleptic evaluation of carp meat treated with different concentrations of citric acid is not entirely consistent with the indicators of USS changes (Fig. 7).

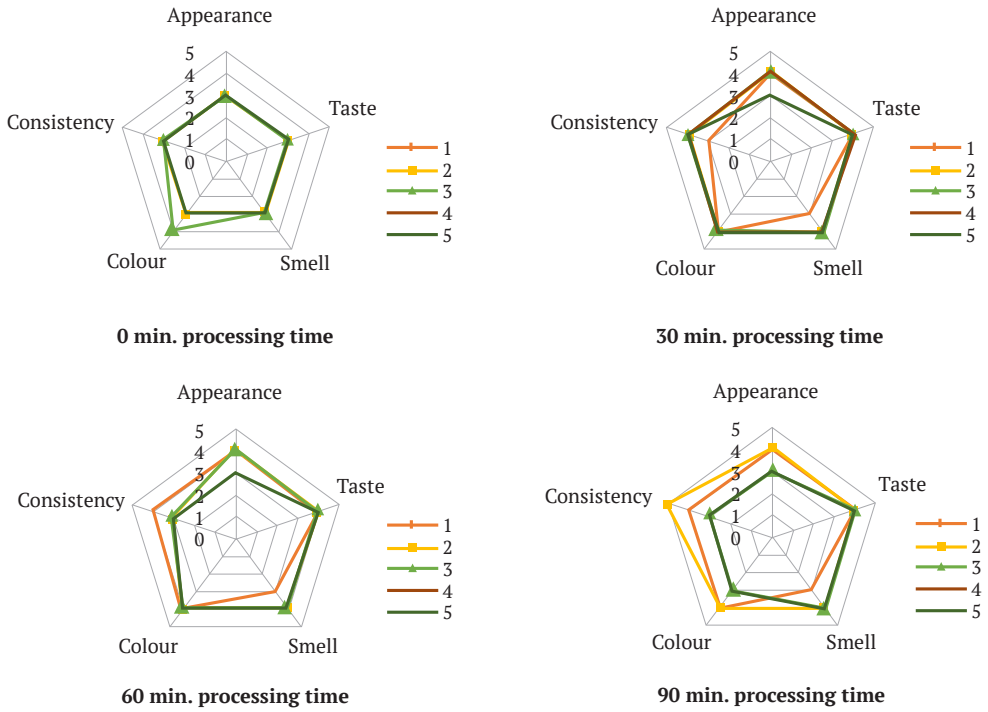

Figure 7. Changes in the indicators of organoleptic evaluation of carp meat under the influence of different effects of citric acid concentration

Notes: (n=5, p⩽0.05): 1 – control; 2 – C=0.5%; 3 – C=0.7%; 4 – C=1.0%; 5 – C=1.5%

Source: developed by the authors

These acid concentrations do not soften the consistency and according to organoleptic indicators, the higher the acidity, the larger the white layer and dense consistency. After 30-60 minutes of treatment, no significant organoleptic changes were observed in the control and experimental samples. In the study samples, organoleptic parameters improved compared to the control, and the consistency, on the contrary, was tougher than in the control with the first sign of white plaque. After 90 minutes, samples treated with citric acid at a concentration of 0.5% showed better organoleptic characteristics than other samples, and the consistency corresponded to 5 points.

The nature of exposure to different concentrations of citric acid is identical to that of acetic and malic acids, but the USS indicators decrease quite slightly (Fig. 8), which is not accompanied by softening of the consistency of carp meat. When measured after 45 minutes using all citric acid concentrations, the pH of carp meat changed to a degree indicating activation of its own cathepsin enzyme (Fig. 9) and is characterised by a gradual shift to the acidic side from 4.2-3.9.

The organoleptic evaluation of carp meat treated with tartaric acid at different concentrations is shown in Figure 10.

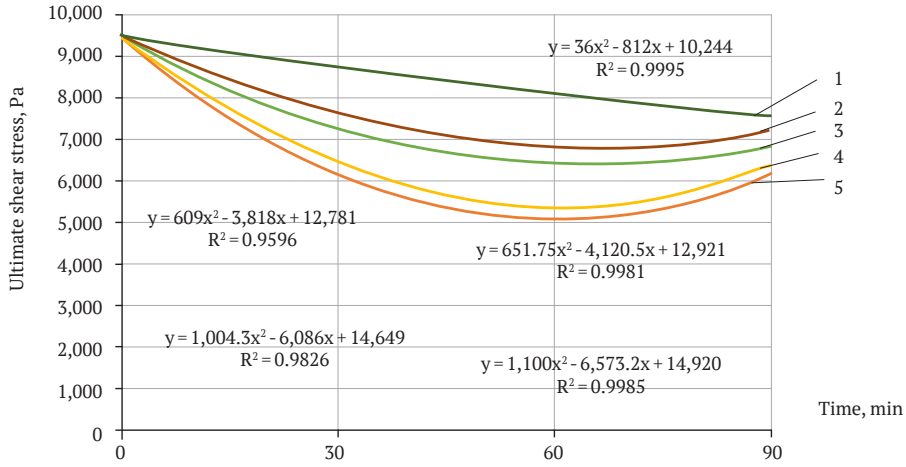

Figure 8. Dynamics of changes in the ultimate shear stress in carp meat under the influence of various concentrations of citric acid for 90 minutes

Notes: (n=5, p≤0.05): 1 – control; 2 – C=0.5%; 3 – C=0.7%; 4 – C=1.0%; 5 – C=1.5%

Source: developed by the authors

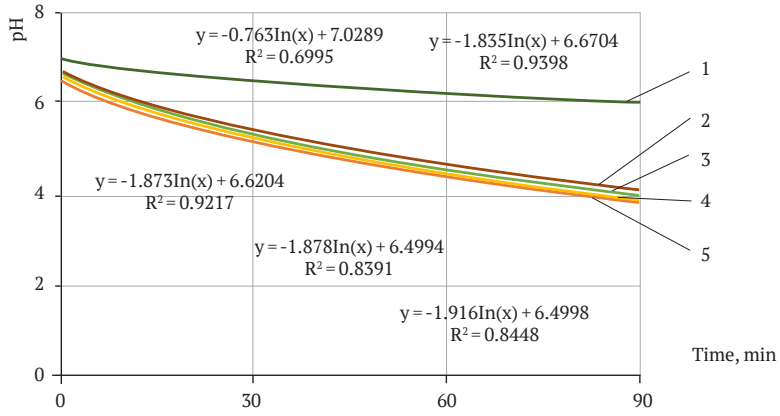

Figure 9. Dynamics of changes in the level of acidity in carp meat when interacting with different concentrations of citric acid, depending on the processing time

Notes: (n=5, p≤0.05): 1 – control; 2 – C=0.5%; 3 – C=0.7%; 4 – C=1.0%; 5 – C=1.5%

Source: developed by the authors

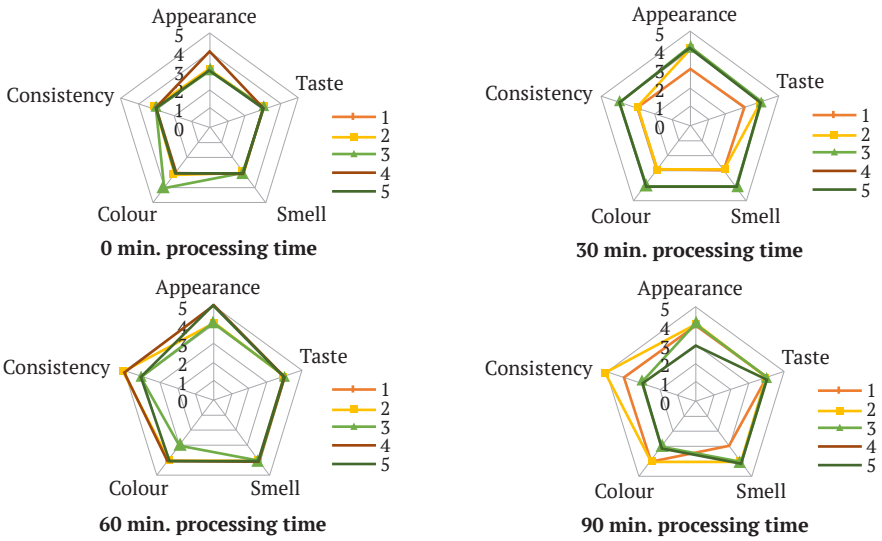

Figure 10. Dynamics of assessment of organoleptic characteristics of carp meat under the influence of different concentrations of tartaric acid during different processing periods

Notes: (n=5, p≤0.05): 1 – control; 2 – C=0.5%; 3 – C=0.7%; 4 – C=1.0%; 5 – C=1.5%

Source: developed by the authors

After 60-90 minutes of treatment, there were no significant sensory changes, the samples did not reach 5 points on the sensory score, the consistency was not too soft or juicy. After 90 minutes, samples treated with tartaric acid with a concentration of 0.5-1% showed better organoleptic characteristics than other samples, and the consistency was 5 points. The nature of the effect of different concentrations of tartaric acid on USS indicators differed from the effect of acetic, malic, and citric acids. On the control sample, with an increase in the processing time, this indicator gradually decreased and the changes had a linear relationship. Exposure to 0.5% tartaric acid was accompanied by a gradual increase in the USS index. Tartaric acid concentrations such as 0.7, 1.0, and 1.5% softened the consistency of carp meat and the best results were obtained when using 1.5% concentration for 60 minutes (Fig. 11).

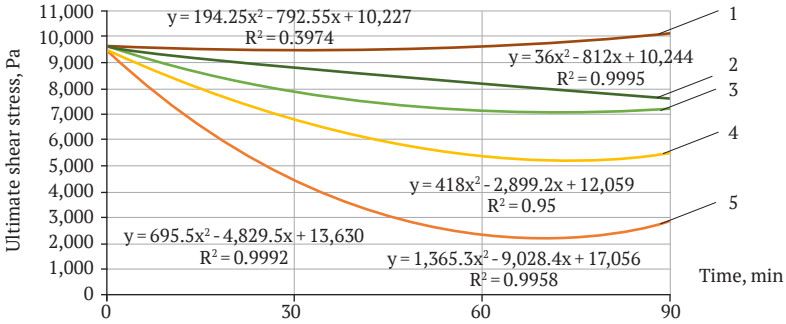

Figure 11. Relationship of changes in the acidity of carp meat under the influence of different concentrations of tartaric acid for 90 minutes

Notes: (n=5, p≤0.05): 1 – control; 2 – C=0.5%; 3 – C=0.7%; 4 – C=1.0%; 5 – C=1.5%

Source: developed by the authors

Under the influence of all tartaric acid concentrations, the dynamics of changes in the pH of carp meat had a linear relationship, which was characterised by a gradual shift from 6.8 to the acidic side to 5.1, which contributed to partial activation of cathepsins (Fig. 12).

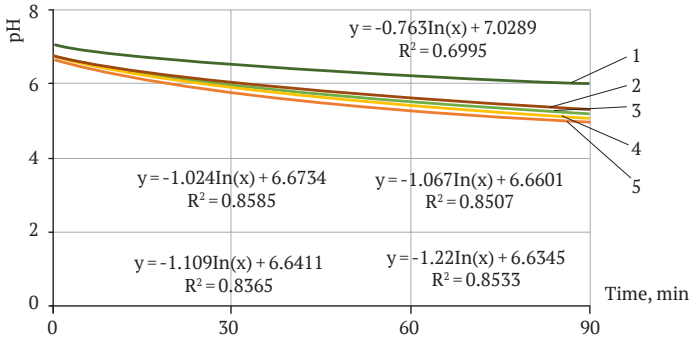

Figure 12. Changes in the level of acidity in carp meat depending on the processing time and different concentrations of tartaric acid

Notes: (n=5, p⩽0.05): 1 – control; 2 – C=0.5%; 3 – C=0.7%; 4 – C=1.0%; 5 – C=1.5%

Source: developed by the authors

The difference in the effect of organic acids on softening fish meat can be explained by the fact that acetic, malic, citric acids are carboxylic, which are widely used in the food industry as flavourings, acidity regulators, and preservatives. Acetic acid is monobasic, malic acid is dibasic, and citric acid is tribasic. In water, these acids easily dissociate and the degree of their dissociation due to the different number of bases is not the same, which probably determines such differences in the effect on the muscle tissue of fish. The oxygen-hydrogen bond of the carboxyl group (-COOH) in acetic acid is highly polar, so these compounds can easily dissociate and exhibit acidic properties, which can promote cathepsin activation and soften the consistency of muscle tissue. N.V. Novikova *et al*. (2023) also used acetic acid with a concentration of 9% in their research in the manufacture of preserves. A significant effect on the consistency of fish was influenced by the addition of elderberry and cranberry plant raw materials, due to which the fish became softened, and preserves were enriched with ascorbic acid, rutin and folacin, which took part in the metabolic processes, protecting the molecules from damage by reactive oxygen species. Organoleptic studies, USS, and pH confirmed the positive results of using organic acids of various concentrations in the treatment of freshwater fish both to ensure safety and to stimulate maturation.

Notably, organic acids such as lactic, malic, citric, etc. are known for their antibacterial, preservative, and antioxidant properties. Therefore, a number of studies are known that confirm this fact. The organoleptic properties of meat, such as colour, smell, taste, and texture, may be important for consumers. Research by X. Liu *et al*. (2019) confirm that adding organic acids to processed meat can improve its taste, reduce adverse odours, and preserve its natural colour, although the study does not specify the shelf life of such products, which is consistent with the results in the context of the current study. Structural and mechanical properties are also affected. Thus, the structural and mechanical properties of meat that were studied by M. Nikoo *et al.* (2021) indicate a possible positive effect of organic acids. For example, they can promote the breakdown of collagen.

O.A. Olopade *et al.* (2023) investigated the mechanisms of the effect of organic acids on

meat, in particular, their interaction with proteins. The researchers claim that different types of acids cause different processes of breakdown of meat proteins and affect the preservation of moisture in meat, which is confirmed by an objective analysis of the results, which include a wide range of factors that affect the properties of meat during interaction with organic acids. In addition, some researchers note that optimal conditions and concentrations of organic acids may vary depending on the type of hydrobionts and processed meat. The research presented by S. Rahayu *et al.* (2022) provides specific guidance on this hypothesis.

Prospects for the use of organic acids in industrial conditions to improve the quality of meat and its shelf life are described by K. Rahmanifarah *et al.* (2023). In industrial settings, the use of organic acids to optimise the processing and conservation processes of freshwater fish can be an important strategy for improving the quality and duration of product storage. An analysis of the study by the above-mentioned researchers may indicate possible advantages of implementing these approaches in industrial production. In the context of the current study, it is worth noting that organic acids can interact with fish meat, promoting protein breakdown and improving the texture and organoleptic characteristics of the product. Their antimicrobial activity can also provide an additional level of safety and shelf life, reducing the risk of micro-organisms and fish spoilage. However, the introduction of these technologies in industry requires additional research and process optimisation. It is important to consider different production conditions, types of freshwater fish, and compliance with regulatory standards for product safety and quality.

The study by N. Sadeghinejad *et al.* (2019) highlighted the safety issues and possible risks associated with the use of organic acids in food. The researchers provide a detailed comparative analysis of canned meat and fish, in particular, considering the influence of organic acids on their quality and safety. The study focuses on determining the interaction between organic acids and meat or fish during the conservation process. The possible effects of these acids on the organoleptic and chemical properties of products are analysed, and their impact on the safety of consumption is evaluated. One of the key aspects is to identify possible risks associated with the use of organic acids in food that may arise as a result of chemical interactions and potentially affect the quality and safety of products. This study opens up space for discussing the possible benefits and limitations of using organic acids in canned food, emphasising the importance of balancing improving the technical characteristics of products and ensuring their safety for the consumer. In particular, the results of the comparative analysis of meat and fish preserves carried out within the framework of the study can play an important role in shaping scientific approaches to the use of organic acids in the food industry and determining their optimal application in the context of ensuring food safety and quality. In general, these sources can serve as a basis for discussing the effect of organic acids on the meat of freshwater hydrobionts, and for determining the prospects for their use in the food industry.

N. Slobodyanyuk *et al.* (2018), O.B. Tkachenko *et al.* (2020) analysed scientific sources and studies on indicators of the biological value of freshwater fish in Ukrainian and foreign reservoirs. The prospects of using this raw material resource to expand the range of high-quality food products are indicated. The paper presents the results of the analysis of the chemical, fatty acid, amino acid, and mineral composition of carp, silver carp, and bream meat. A comparative analysis of the nutritional value of these fish species, which are promising for industrial processing, was carried out, revealing certain differences in the studied indicators. The presented results of the analysis of the total chemical composition show that fish from the

Ukrainian reservoir is characterised by a high protein content and moderate fat content, while protein contains all the necessary amino acids. The study of the mass fraction of toxic elements confirms that the level of heavy metals in fish is lower than acceptable standards, in particular in silver carp, carp, and bream.

Based on the results obtained by R.R. Veloso *et al.* (2019), H.P. Vieira *et al.* (2019), changes in rheological characteristics, microbiological and toxicological parameters were found during the storage of minced meat from freshwater hydrobionts with plant components, in particular ginkgo biloba powder in the amount of 1%, 3%, and 5%. During the experiment, the moisture retention capacity of the developed minced meat was investigated. Changes in the effective viscosity of minced meat from hydrobionts after freezing and during storage were also determined. The organoleptic properties of minced meat were analysed after freezing and during 6 months of storage. The values of fat constants, such as acid, peroxide, and iodine numbers, were obtained. The results show that minced meat from hydrobionts with plant components retains its high consumer properties for 6 months of storage.

After conducting a comparative analysis of the results obtained by the above-mentioned researchers, it is possible to predict possible areas and prospects for further research:

1. *Study of the effects of various organic acids:* investigation of various types of organic acids (for example, lactic, malic) and their concentrations to determine optimal conditions for improving the quality of hydrobiont meat.

2. *Investigation of organoleptic properties*: study of the effect of organic acids on the taste, aroma, colour, and texture of meat and the possibility of using organic acids to improve the organoleptic characteristics of finished products.

3. *Study* of *structural and mechanical properties*: effect of organic acids on meat structure and other mechanical properties, to determine optimal po concentrations for best results.

4. *Mechanisms of action of organic acids:* investigation of molecular and biochemical processes that occur when organic acids interact with meat components, which will help to better understand the mechanisms of improving meat quality.

5. *Study of long-term exposure:* investigation of the effect of organic acid use on the quality of freshwater hydrobiont meat, including storage and transportation of products.

6. *Innovative processing methods:* study of new methods for introducing organic acids into meat, such as marinating, impregnating, or using nanoparticles to improve the effectiveness of exposure.

7. *Safety and health aspects:* research on safety aspects and the impact of organic acid administration on consumer health.

Research in these areas can help identify the potential benefits of using organic acids to improve the quality of freshwater hydrobiont meat, which, in turn, can lead to the development of new production technologies and improve the competitiveness of products in the market.

Conclusions

The study was carried out to determine changes in a set of indicators of carp meat under the influence of acids of different concentrations during storage in a refrigerator at a temperature of 0 to 5°C for 90 minutes of processing. At the first stages of treatment, there were no noticeable organoleptic changes in the control and experimental samples. After 30 minutes of treatment, organoleptic parameters improved in the experimental samples compared to the control, namely at a concentration of 0.7-1.5%. Pieces of carp meat after treatment with different acid concentrations differed from the control sample in the best indicators of taste, smell, and colour.

Comparison of the results of studies on the dependence of organoleptic evaluation on USS revealed the following patterns: the positive effect of acetic acid on carp meat occurs at a concentration of 0.7-1.0% for 60 minutes providing

the appearance of a soft consistency; softening of the consistency of carp meat, which corresponds to high organoleptic indicators, occurs when treated with 1.0 and 1.5% malic acid for 60 minutes; for tartaric acid, the best result is determined by adding 0.5-1.0% acid for 60 minutes; analysis of the results of the study of the effect of citric acid on the consistency indicators of carp meat indicates that we have studied concentrations do not lead to softening of carp muscle tissue and show the inexpediency of its use. Thus, to soften carp meat, it is recommended to use 1.0 and 1.5% malic acid during 60 minutes of processing. Pretreatment of salty semi-finished products with 1.0-1.5% malic acid for 60 minutes to achieve a soft, tender, and juicy consistency is theoretically justified and experimentally confirmed.

Prospects for future research are to conduct comprehensive analyses of the effect of organic acids in the production of preserves with spicy-aromatic root vegetables and their impact on plant raw materials. This developed technology can be applied in the food industry in the production of fish preserves using freshwater fish, which will expand the range of fish products. However, to determine the regularity of the effect of different acid concentrations on both safety and maturation stimulation, it is necessary to conduct additional studies due to the specific features of the type of raw material. Therefore, this topic requires improvement and further investigation.

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Conflict of Interest

None.

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Вплив органічних кислот на органолептичні та структурно-механічні властивості м'яса прісноводних гідробіонтів

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Анотація. Дослідження використання органічних кислот у консервуванні прісноводної риби в аквакультурних господарствах набуває важливості через ростучий інтерес до якості та безпеки рибної продукції. Мета дослідження полягала у створенні та обґрунтуванні нової технології консервування прісноводної риби з додаванням пряних коренеплодів, а також у попередній обробці сировини органічними кислотами. Під час дослідження коропа обробляли за допомогою солі та різних концентрацій органічних кислот, зберігали при певних температурних умовах. Для оцінки результатів використовували сенсорну оцінку, pH і граничне напруження зсуву, а також проводили хімічний аналіз з використанням пенетрометра та потенціометричного методу. За результатами досліджень розроблено науково-обґрунтовану технологію консервування прісноводної риби з додаванням пряних коренеплодів з принципово новим способом обробки сировини органічними кислотами для розширення сфери застосування рибопродуктів, які матимуть більш високу біологічну цінність. Виявлено, що на початкових етапах обробки в досліджуваних зразках не спостерігалося відчутних органолептичних змін. Порівняльний аналіз результатів досліджень підтвердив позитивний вплив оцтової кислоти при концентрації 1,0 %, для винної кислоти найкращий ефект визначено при додаванні 0,5-1,0 %, а щодо лимонної кислоти дослідження

вказує на те, що її використання не призводить до розм'якшення м'язової тканини, свідчачи про доцільність її використання. Дослідженнями підтверджено використання попередньої обробки напівфабрикатів солоного коропа обробленого 1,0-1,5 % яблучною кислотою протягом 60 хвилин для отримання ніжної, соковитої та м'якої консистенції, що відповідає 5 балам за сенсорною оцінкою. За результатами проведених досліджень встановлено позитивний вплив застосування органічних кислот для розм'якшення м'яса коропа при виробництві пресервів з прісноводних риб. Практичне значення дослідження полягає в удосконаленні технологій обробки м'яса та виробництва продуктів харчування, а також визначити оптимальні умови для збереження смакових та текстурних якостей м'ясних продуктів

Ключові слова: короп; гранична напруга зсуву; гідробіонти; органолептичні показники; слабодозріваюча риба

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Efficiency of using different levels of mixed ligand complexes of Zinc, Manganese, and Cobalt in cow feeding

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Abstract. To increase productivity and reduce the negative impact on the environment, it is necessary to investigate the most optimal ratios of organic trace elements in the diets of cows involved in the production cycle, considering their physiological state and lactation stage. The purpose of the study was to determine the economic profitability of using mixed complexes of zinc, manganese, and cobalt in the diets of dairy cows. The experiment was carried out under production conditions on Holstein cows. 100 cows were divided into two groups. Cows of the control and experimental groups consumed feed mixtures made according to the same recipes, supplemented with mixed ligand complexes of zinc, manganese, and cobalt. It was found that with the optimal content of mixed ligand complexes of zinc, manganese, and cobalt in cows of the experimental group, body weight loss during the first hundred days of lactation was 16.4 kg or 2.88% less than in

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the control. Analysis of performance indicators of cows during the dry period and the first hundred days of lactation showed that in animals of the experimental group, whose diet contained mixed ligand complexes of trace elements at the rate of 54.7 mg of zinc and manganese and 0.7 mg of cobalt per 1 kg of dry matter, milk yields of natural fat content increased by 4.1 kg or 9.2%, the fat content in milk – by 0.02% compared to cows of the control group, whose diets provided the content of zinc and manganese at the level of 60.8 mg, cobalt – 0.78 mg/kg of dry matter due to mixed ligand complexes. There was also a decrease in the duration of the service period by 9 days and the insemination index by 0.17 semen doses. The cost of production remained unchanged, and the additional profit with a reduced concentration of these elements in the diet of cows of the experimental group amounted to EUR 27.24 or 9.7%. Thus, optimisation of mineral nutrition of cows by introducing mixed ligand complexes of zinc, manganese, and cobalt to achieve the concentration of trace elements of zinc and manganese – 54.7 mg each, and cobalt – 0.7 mg per 1 kg of dry matter of the feed mixture will improve milk production and reproductive capacity of cows and allow obtaining additional profit

Keywords: organic compounds of trace elements; dry cows; dairy cows; milk yield; milk fat content; economic efficiency

Introduction

According to researchers, in particular P.S. Erickson & K.F. Kalscheur (2020), in order to achieve full genetic potential, modern dairy cattle breeds need appropriate biologically complete feeding, which will ensure the restoration of the cow's body's production costs.

Zinc, iodine, cobalt, cuprum, manganese, and selenium play a significant role in increasing the biological usefulness of feeding cows. According to J. Batra *et al.* (2022) and R. Duffy *et al.* (2023) focus on the properties of zinc to enhance the activity of rumen microbial enzymes. I.M.T. Fadlalla *et al.* (2022) argue that a significant number of pathological changes that develop in the body of cattle are somehow associated with manganese deficiency. Attention is paid to the extremely low digestibility of this trace element from inorganic salts. The researchers note the exceptional role of cobalt in the synthesis of vitamin B_{12} by rumen microorganisms and also focus on the absorption of this trace element from organic and inorganic sources.

The important role of trace elements, and the importance of the source of their entry into the body of ruminants, was described by N.F. Suttle (2022), J. Arthington & J. Ranches. (2021) and R.A. Palomares (2022). They noted that the health and productivity of high-yielding cows depend on the availability of key micronutrients in their bodies. Animals can adapt to deficiencies in minerals, especially trace elements such as copper, cobalt, zinc, iodine, manganese, etc., but this can lead to abnormalities in protein metabolism and synthesis, reduced health, loss of reproductive ability, and reduced genetic productivity, which is determined by breed characteristics. P.K. Gupta (2021) emphasises that such changes develop both in the mother and in the offspring's body.

R.B. Corbett (2023) also notes that the absorption of trace elements in the gastrointestinal tract depends on their interaction with other nutrients and biologically active substances of feed and the synthesis of new forms of complex compounds in the body, which are significantly different from the forms of compounds in which they were contained in feed. The researcher also focuses on the physiological significance of the degree of stability and

solubility of the resulting compounds. The introduction of trace elements into the diets of cows in the form of inorganic salts due to their low digestibility in the body leads to environmental pollution with heavy metals, which is described by B. Mion *et al.* (2023). This group of researchers as well as J.W. Spears (2023) note that only 5-30% of trace elements obtained from salts in the form of sulphate or chloride compounds are absorbed by the animal body. However, the introduction of organic minerals in the diets of cows increases their absorption by the body up to 70-98%.

M.A. Abu El-Hamd *et al.* (2023) conducted a comparative assessment of the use of organic and inorganic sources of zinc, cuprum, and manganese and found a positive effect on the productivity, milk quality and reproduction qualities of cows of organic compounds of these trace elements. The optimal level of biotic trace elements contributes to the stable flow of metabolic processes in animals, supports their health, and ensures high productivity. Based on the findings of B. Mion *et al.* (2023), the best results are found when using complex metal compounds with amino acids, organic acids, etc., including metal compounds from amino acids, their derivatives, peptides, proteins, nucleic acids, nucleotides, carbohydrates, and carboxylic acids, which are complexons.

Metal ions show their activity when they enter into a complex with ligands, they are easily adsorbed into the bloodstream and penetrate through the cell membrane to the places of their localisation. Chelates move freely through the animal body, ensuring optimal metal transfer to the cell, and therefore, the need for ligands and carrier proteins disappears, which contributes to a more pronounced action of trace elements and determines their corresponding biochemical and physiological activity at significantly lower concentrations than when using trace elements in the ionic state.

Currently, it is recommended that 1 kg of dry matter in the diet of high-yielding cows should be: 60-80 mg of zinc; 60-80 mg of manganese; 10-12 mg of cuprum; 1-1.5 mg of cobalt; 0.8-1.4 mg of iodine; 0.3 mg of selenium; 0.5-1 mg (Suttle,2022). According to the recommendations of Ukrainian researchers, the concentration of zinc and manganese in 1 kg of dry matter in the diets of high-yielding cows with an average daily yield of 35 kg of milk or more should be 70 mg; cobalt – 0.9, cuprum – 11, iodine – 1, and selenium – 0.3 mg. However, the researchers found that the addition of mixed ligand complexes of zinc, manganese, and cobalt to the diets of dairy cows during the first 100 days of lactation to ensure the concentration of these trace elements in 1 kg of dry matter, respectively, 54.7 mg; 54.7, and 0.7 mg had a positive effect on their productivity per concentration (Bomko *et al.*, 2019).

The purpose of the study was to investigate the economic efficiency of using mixed ligand complexes of zinc, manganese, and cobalt in feeding dairy cows.

Materials and Methods

According to the experimental design, a production test was carried out at the "Terezyne" additional liability company in Kyiv oblast to determine the economic efficiency of using mixed ligand complexes of zinc, manganese, cobalt using 0.3 mg/kg organic selenium against the background of balancing rations for copper and iodine according to existing standards (12 mg and 1.1 mg/kg dry matter, respectively) at the expense of copper sulphate and potassium iodide. A production experiment in the feeding of German-bred Holstein cows using mixed ligand complexes of zinc, manganese, cobalt and Suplex Se was conducted during the dry period and the first 100 days of lactation. In total, the duration of the production test was 160 days.

Complete feed mixtures were used as cow feed, the nutritional value of which met the established requirements for the weight, physiological condition, and productivity of the animals. Cows of the control and experimental

groups consumed the same basic feed mixture, but the supply of zinc, manganese, and cobalt was different and corresponded to the scheme of the experimental design (Table 1).

Table 1. *Design of the production experiment*

Animal group	Number of animals	Feeding conditions
Control	50	Feed mixture + mixed ligand complexes of zinc, manganese, and cobalt. 1 kg of dry matter contains, mg: zinc – 60.8; manganese – 60.8; cobalt – 0.78.
Experimental	50	Feed mixture + mixed ligand complexes of zinc, manganese, and cobalt. 1 kg of dry matter contains, mg: zinc – 54.7; manganese – 54.7; cobalt – 0.7.

Source: developed by the authors

Cows were kept loose with free access to a complete feed mixture, which was distributed twice a day to the feed table. In addition to feed, the animals had free access to clean drinking water around the clock. The microclimate parameters met the established requirements. The animals were divided into sections: 1 group of 50 cows was kept in one section During the first 100 days of lactation, dairy cows were milked three times a day in the milking parlour. Cows of each group were milked separately, taking average milk samples to analyse the fat content of milk. Since the production test occurred during the insemination period, the cows were fitted with a sensor around their necks to determine their heat and monitor their health. This helped to determine the period of heat even in the absence of external signs, which is characteristic of animals of this breed. Insemination of cows was carried out no earlier than the 50th day (free waiting period) after calving. Stimulation or synchronisation of heat was not used.

During the production test, the live weight, productivity of cows, the duration of the service period, and the insemination index were recorded, and the economic efficiency of the proposed changes in animal feeding was calculated. For this purpose, the following methods were used:

- live weight – determined by weighing animals on a scale on the day following the start-up (beginning of the dry period), 6 weeks after start-up (end of the dry period), on the 5th day after calving (beginning of lactation), on the 100th day after calving (end of the first 100 days of lactation);
- cow productivity – determined by recording the daily milk yield from each cow separately, using software from the milking parlour;
- milk fat content – measured in an average milk sample from each group of cows using the Ecomilk device after each milking;
- milk yield of 4% fat content – calculated by multiplying the average daily milk yield of natural fat content by the fat content in milk and dividing by 4;
- milk yield of basic fat content – calculated by multiplying the average daily milk yield of natural fat content by the fat content in milk and dividing by 3.4;
- duration of the service period – measured by the time from calving to the first fertile insemination;
- insemination index – calculated the number of semen doses spent on one fertilisation. Studies on animals were conducted in compliance with the requirements of the "General ethical principles for conducting animal experiments", approved by the 1st National Congress on Bioethics (Law of Ukraine No. 3447-IV, 2006), and the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (1986).

Results and Discussion

The dry period, both early and late, is an important stage of the cow's production cycle, as it lays the foundations for future lactation. Any technological methods used during this period will have a certain impact on its success. However, it is difficult to control the course of this period – there are no criteria for daily assessment. But during the first 100 days of lactation, both the amount of milk received and its qualitative composition can be evaluated daily. This greatly facilitates the comparative assessment of the impact of the feed factor on the cow's productivity.

In the course of the production experiment, live weight was assessed, as this is one of the few criteria for assessing the dry period of dairy cows. During the dry period, cows of the control group increased their live weight by 49.6 kg or 8.2%, and in the experimental group – by 53.3 kg or 8.9% relative to the weight at the beginning of the experiment (Fig. 1).

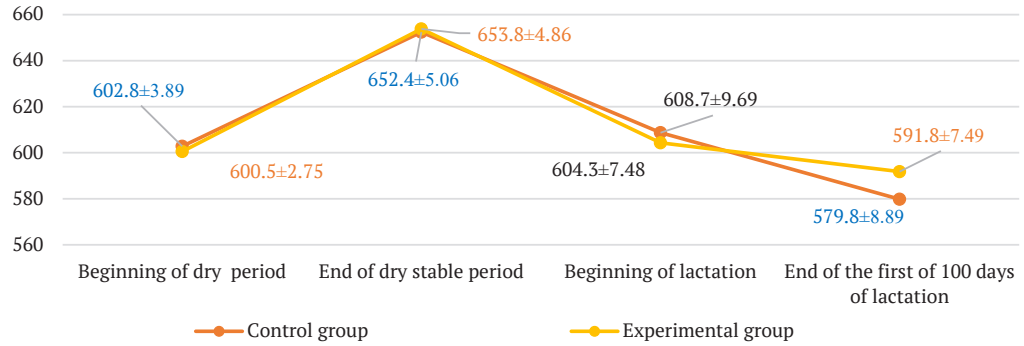

Figure 1. Dynamics of live weight, kg

Source: developed by the authors

After calving and before the start of lactation, cows of both groups lost live weight: control animals reduced their weight by 43.6 kg or 6.7%, and experimental animals – by 49.5 kg or 7.6%.

During the first 100 days of lactation, the animals of the control group lost another 28.9 kg or 4.7% of their weight, while the cows of the experimental group reduced their weight by only 12.5 kg or 2.1% (Fig. 2).

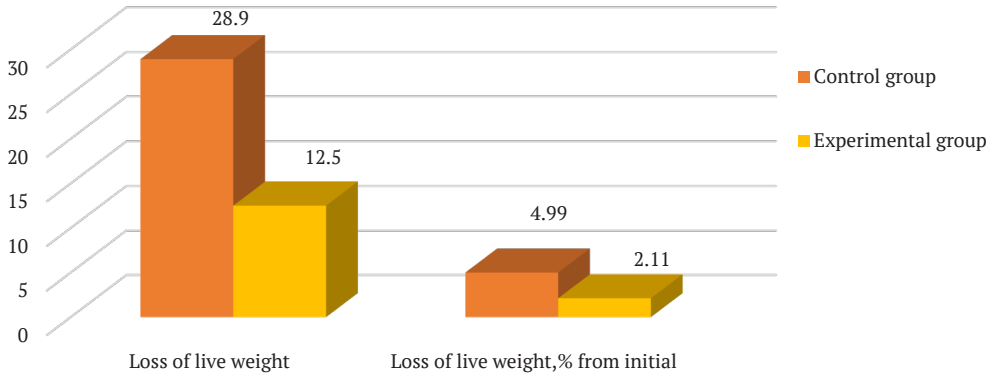

Figure 2. Dynamics of live weight loss, kg

Source: developed by the authors

Weight loss after calving is a natural process. It is typical for highly productive cows, since the animal is not able to consume enough feed during this period. In this case, the increase in productivity (divisions) is genetically determined. Thus, for a certain time, the cow gives up the reserves of its own body to ensure productivity. However, along with an increase in productivity, rumen development also occurs and animals recover quickly, including body weight.

The main production indicator in dairy cattle breeding is the average daily milk yield of natural fat content and directly the fat content of milk. From the data obtained as a result of the experiment, it can be seen that during the first 100 days of lactation, cows of the experimental group that consumed the feed mixture included mixed ligand complexes of zinc, manganese, and cobalt to ensure the content of these trace elements at the level of 54.7 mg, 54.7 mg, and 0.7 mg/kg of dry matter, on average synthesised 4.1 kg or 9.2% more natural fat milk per day compared to animals of the control group, the content of zinc, manganese, and cobalt in the dry matter of the diet which amounted to 60.8 mg, 60.8 mg, and 0.78 mg, respectively (Fig. 3).

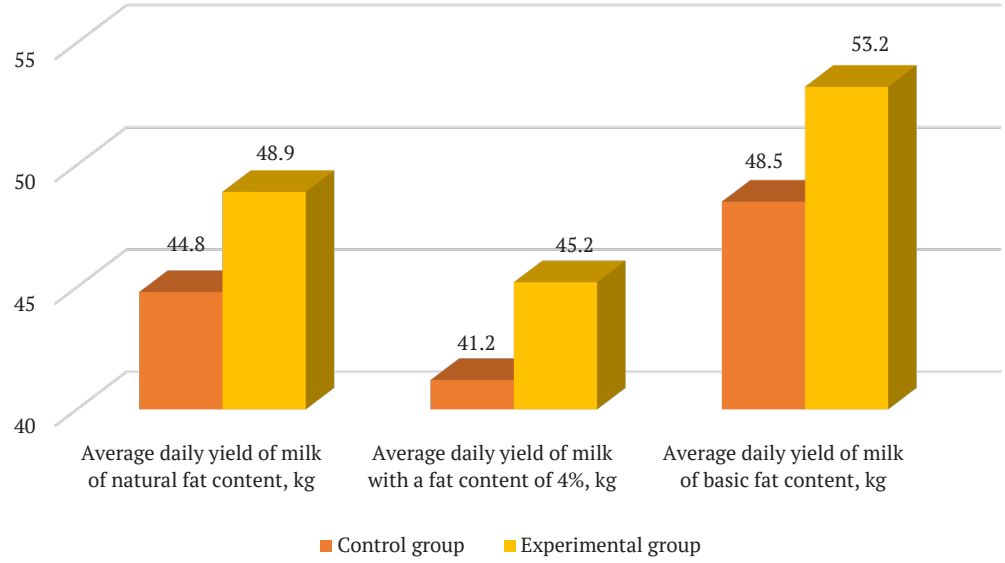

Figure 3. Milk productivity of cows

Source: developed by the authors

In addition to the increase in the average daily milk yield, an increase in the fat content in the milk of cows of the experimental group by 0.02% compared to control animals was recorded (Fig. 4). It can be assumed that the reason for this was the positive effect of the optimal content of these trace elements on the cellulolytic bacteria of the rumen. Normalisation of their vital activity directly affected the fibre digestibility, and accordingly, the fat content in milk. Since the difference in fat content in milk between the groups is quite large, converting the productivity of animals to fat content of 4% noted an increase in milk productivity of this fat content in cows of the experimental group by 4.0 kg or 9.7% relative to the control. A similar difference between the groups, namely 4.7 kg or 9.7%, occurred in terms of milk yield of basic fat content, which is a technological indicator. When selling milk, such recalculation is carried out by the milk processing enterprise and directly affects the profit.

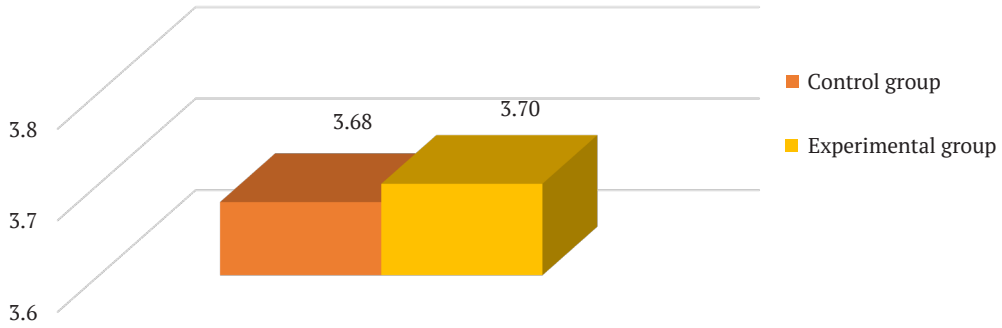

Figure 4. Fat content of cow milk, %

Source: developed by the authors

The first 100 days of lactation are the period of heat and insemination in cows. In highly productive cows, the manifestation of heat can be visually invisible, so special sensors placed on the animal's neck were used to record this physiological phenomenon. Insemination was carried out no earlier than 50 days after calving.

Studying the reproductive performance of cows of both groups, it was noted that with a lower concentration of zinc, manganese, and cobalt, the service period was reduced by 9 days, and the insemination index decreased by 0.17 (Fig. 5). The duration of the service period is a very important indicator, since the duration of lactation depends on it. The longer the service period lasts, the longer the lactation decline phase will be, which will lead to an increase in the cost of milk production. Admittedly, this indicator depends on the insemination effectiveness, since infertile insemination increases the duration of the service period, and accordingly lactation of the cow, by an average of 19-22 days.

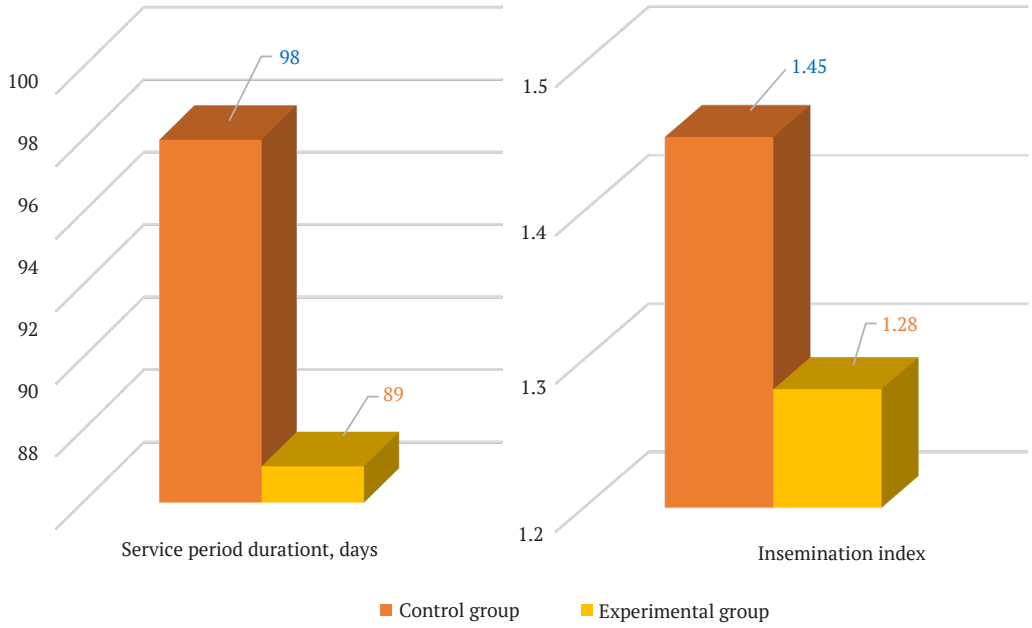

Figure 5. Reproductive indicators of cows

Source: developed by the authors

During the first 100 days of lactation, cows of the experimental group who consumed a mixture with mixed ligand complexes of zinc, manganese, and cobalt, and the content of these elements in 1 kg of dry matter was 54.7 mg, 54.7 mg, and 0.7 mg, provided 9.7% more milk of basic fat content, compared to control animals, in the mixture of which there were also mixed ligand complexes, but they supplied zinc at the level of 60.8 mg; manganese – 60.8; cobalt – 0.78 mg/kg of dry matter. At the same selling price, revenue from milk sales in the experimental group was also 9.7% higher, but production costs also exceeded the control indicator by 9.7%. Thus, the cost of production was the same in both groups. However, due to the increase in cow productivity, the additional profit per animal was EUR 31.04, which is 9.7% more than in the control (Table 2).

Table 2. *Economic efficiency*

Indicator	Group	
	control	experimental
Gross milk yield of basic fat content, hwt	2,425.0	2,660.0
Sales price of 1 metric centner of basic fat milk, EUR	23.7	23.7
Cost of gross output, thousand EUR	57.52	63.10
Total production costs, thousand EUR	41.5	45.5
including: salary	8.92	9.79
feed	25.40	27.86
other direct costs	4.63	5.05
overhead costs	2.57	2.82
Cost of 1 metric centner of basic fat milk, EUR	17.116	17.116
Profit, thousand EUR	16.02	17.57
Overall economic effect, EUR	-	1,552.09
Economic effect per 1 animal, EUR	-	31.04

Source: compiled by the authors

Thus, the results of the production test showed that the amount of pure trace elements per 1 kg of the feed mixture is important when using chelated compounds of zinc, manganese, and cobalt. With a reduced amount of them, namely 54.7 mg/kg of dry matter of zinc and manganese mixed with 0.7 mg of cobalt compared to the currently recommended dry matter concentration of 60.8 mg/kg of zinc and manganese and 0.78 mg/kg of cobalt, milk productivity, quality composition of milk, reproduction indicators, and economic efficiency in Holstein cows were the best.

The positive impact on the health and productivity of cows when feeding mixed ligand complexes of trace elements is described in numerous studies. Thus, G.M. El Ashry *et al.* (2016) noted that the replacement of inorganic salts of zinc, manganese, and copper with their methionates at the same concentration of these trace elements in the diets of dry cows had a positive effect on their milk productivity and fat content in milk during subsequent lactation. During the experiment on dairy cows, comparing the action of chelated compounds of trace elements (Co, Cr, Cu, Fe, Mn, Se, Zn) and amino acid complexes, M.D. Banadaky *et al.* (2021) found that the introduction of a combination of these trace elements produced using chelated compound technology to the diet of dairy cows helps to increase milk productivity and fat content.

According to J. Illek *et al.* (2023), feeding cows chelated complexes of trace elements

during the late dry period and the first 98 days of lactation resulted in a significant increase in milk productivity, fat and protein content in milk, and a decrease in the number of somatic cells in it. In this case, the effect of additives on udder health can be confirmed. The positive effect on the productivity of beef cows using organic sources of trace elements in diets was described in the monograph by R.A. Hill (2012). However, R. Marques *et al.* (2016) also noted an increased body weight of calves during weaning after feeding cows with diets containing chelated compounds of trace elements. A meta-analysis of 20 research papers on the amount of milk yield, composition and yield of milk components, and the reproductive function of cows with the consumption of organic sources of trace elements, conducted by A.R. Rabiee *et al.* (2010), concluded that organic micromineral supplements can improve the productivity and reproduction of dairy cows during lactation.

Ukrainian researchers, namely M. Dolgaya *et al.* (2016), according to the results of experimental studies, noted a positive effect of a 50% reduced concentration of zinc, cobalt, and manganese introduced into the diet in the form of chelated compounds in the dry matter of a complete cow feed mixture. During the experiment, an increase in milk yield during the first 100 days of lactation and an increase in the proportion of fat in it with a reduced concentration of zinc, manganese, and cobalt were recorded. A similar trend was observed in the course of this study. It is obvious that the norms of trace elements recommended for feeding animals, in particular cows, are based on the use of inorganic compounds of trace elements. Automatic recalculation of the required amount of chelated compound by the number of pure trace elements is erroneous, since with better digestibility of trace elements from organic sources, the animal needs a lower concentration of them. Therefore, when using a lower concentration of zinc, manganese, and cobalt in their chelated compounds in feed, productivity results are improved. Under such conditions, another side effect is a reduction in ecosystem pollution.

S. Kulibaba *et al.* (2017), according to the balance of cuprum, zinc, and manganese in the body of dairy cows, proposed to use microelement chelates in concentrations below the norm of their introduction in the form of sulphuric acid salts by 25% for Cu, 50% for Zn, and 65% for Mn. Thus, it can be assumed that improving the functioning of the scar microflora due to the optimal content of certain trace elements (cuprum, zinc, manganese, cobalt) contributes to positive changes in the digestion of nutrients, as mentioned by Y. Kropyvka *et al.* (2022) and milk synthesis, as discussed above.

In the course of the current experiment, the dynamics of changes in live weight of cows from start-up to the end of the first 100 days of lactation was investigated. During this period, cows lost and gained body weight, which is physiologically natural. However, in cows whose diet contained reduced amounts of zinc, manganese, and cobalt, body weight loss during lactation was significantly less than in control animals. The effect on the meat qualities of livestock was also reported by R. Raths *et al.* (2023). As for dairy cows, reducing body weight loss during the period of active increase in milk secretion in their body (energy pit) is very relevant. This will affect the further productivity of the animal, its achievement of the peak of lactation, timely heat, and successful insemination.

The researchers found a significant decrease in the duration of the service period in cows (by 9 days) and an improvement in the insemination index (by 0.17). Similar results were obtained in a study by a group of researchers led by K. Uchida *et al.* (2001), when feeding cows organic compounds of trace elements. They noted a reduction in the service period and an increase in the insemination rate of cows, but there were no changes in productivity and fat and protein content in milk. It can be assumed that improving reproductive functions in cows

is the body's response to normalising the dose of zinc, since it directly affects the reproductive function of animals – oocyte maturation and fertilisation (Spears, 2023). Thus, an increase in the fertilisation rate of cows of the experimental group leads to a decrease in the number of semen doses for successful insemination and the duration of the service period and lactation in general. This can be considered a significant production achievement as it helps to reduce the duration of the least productive phase of lactation of the cow.

The goal of most changes in animal feeding is to make a profit. The calculation of economic efficiency showed that with a reduced dose of zinc, manganese, and cobalt, total production costs increased, but productivity also increased. When these factors were combined, it was found that the cost of milk production was the same in both groups. With increased productivity, additional profit was also recorded, which is an obvious fact. For each cow that consumed a diet containing 54.7 mg of zinc and manganese, and 0.7 mg of cobalt/kg of dry matter, an additional profit of EUR 31.04 was obtained. However, it is worth noting that these calculations apply only to the first 100 days of lactation. Reducing the duration of the service period by 9 days will automatically reduce the duration of the lactation decline stage, which occurs after 200 days of lactation and is the least productive period in the cow's production cycle. This will reduce the cost of feeding an animal of relatively low productivity and provide additional profit, but the calculation of this amount was not part of the design of this study.

Thus, it was established that under the conditions of the same sales prices for products, the use of mixed feeds with zinc and manganese levels of 54.7 mg and cobalt of 0.7 mg/kg of dry matter in feeding German-bred high-yielding Holstein cows contributes to an increase in the level of profitability of milk production compared to the option with the levels of zinc, manganese 60.8 mg and cobalt 0.78 mg/kg of dry matter due to their mixed ligand complexes. Such data are consistent with the results obtained by numerous Ukrainian and foreign researchers.

Conclusions

A number of studies on the use of various types of chelated complexes of trace elements in animal feeding, in particular zinc, manganese, and cobalt, have shown that their optimal doses are slightly lower than recommended. Obviously, this is due to better absorption of trace elements from organic sources. Similar results were obtained in the course of this research. Analysing changes in the productivity of cows, the composition and quality of their milk, the preservation of reproductive ability with the introduction of mixed ligand complexes of zinc, manganese, and cobalt, it can be argued that their optimal content in feeding high-yielding Holstein cows of German selection is slightly lower than currently recommended. It was found that feeding cows during the dry period and during the first 100 days of lactation of complete feed mixtures containing 54.7 mg of zinc and manganese and 0.7 mg of cobalt per 1 kg of dry matter compared to the recommended level (zinc – 60.8 mg, manganese – 60.8, cobalt – 0.78 mg/kg of dry matter) contributes to an increase in the daily yield of natural milk by 9.2%, and its fat content by 0.02%. When calculating the average daily milk yield with a fat content of 4%, the advantage of animals of the experimental group over the control group by 4.0 kg or 9.7% was established.

Analysis of body weight dynamics showed that when 1 kg of dry matter in the diet contains 54.7 mg of zinc and manganese and 0.7 mg of cobalt instead of 60.8 mg and 0.78 mg/kg of dry matter, respectively, the weight loss after calving was 12.5 kg or 2.1%. A decrease in the concentration of zinc, manganese, and cobalt in chelated compounds also contributed to a reduction in the service period by 9 days, and a decrease in the insemination index by 0.17. This

result helps to reduce the least productive phase of lactation, which affects the cost of milk production. The economic effect of using this level of feeding was EUR 31.04 per animal during the experiment period. The results obtained complement the previously suggested assumptions that the use of organic compounds of trace elements in animal feed requires a number of studies to determine their optimal level. Therefore, it is important to continue experiments on animals of other species and groups, especially those that will participate in herd reproduction.

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None.

Conflict of Interest

None.

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Ефективність використання різних рівнів змішанолігандних комплексів Цинку, Мангану та Кобальту у годівлі корів

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Анотація. Для підвищення продуктивності та зменшення негативного впливу на навколишнє середовище необхідно науково досліджувати найбільш оптимальні співвідношення органічних мікроелементів у раціонах корів, що беруть участь у виробничому циклі, враховуючи їх фізіологічний стан та стадію лактації. Мета дослідження полягала у визначенні економічної вигідності використання змішаних комплексів цинку, марганцю та кобальту у раціонах дійних корів. Дослід проводили у виробничих умовах на коровах Голштинської породи. 100 корів поділили на дві групи. Корови контрольної та дослідної груп споживали кормосуміші, виготовлені за однаковими рецептами, у яких були додані змішанолігандні комплекси Цинку, Мангану, Кобальту. Встановлено, що за оптимального вмісту змішанолігандних комплексів цинку, мангану та кобальту у корів дослідної групи втрата маси тіла за перші сто діб лактації була на 16,4 кг або 2,88 % меншою, ніж у контролі. Аналіз показників продуктивності корів упродовж сухостійного періоду та перших сто днів лактації засвідчив, що у тварин дослідної групи, раціон яких містив змішанолігандні комплекси мікроелементів з розрахунку по 54,7 мг Цинку і Мангану та 0,7 мг Кобальту на 1 кг сухої речовини надій молока натуральної жирності збільшився на 4,1 кг або 9,2 %, вміст жиру в молоці – на 0,02 % порівняно з коровами контрольної групи, в раціонах яких за рахунок змішанолігандних комплексів забезпечували вміст Цинку і Мангану на рівні 60,8 мг, Кобальту – 0,78 мг/кг сухої речовини. Також було зафіксовано зменшення тривалості сервіс-періоду на 9 діб та індексу осіменіння – на 0,17 спермодози. При цьому собівартість виробленої продукції залишалася без змін, а додатковий прибуток за зниженої концентрації вказаних елементів у раціоні корів дослідної групи становив €27,24 або 9,7 %. Таким чином, оптимізація мінерального живлення корів шляхом уведення змішанолігандних комплексів цинку, мангану та кобальту для досягнення концентрації мікроелементів у 1 кг сухої речовини кормосуміші Цинку та Мангану – по 54,7 мг, Кобальту – 0,7 мг дасть можливість покращити показники молочної продуктивності та відтворної здатності корів, а також отримати додатковий прибуток

Ключові слова: органічні сполуки мікроелементів; сухостійні корови; дійні корови; надій молока; жирність молока; економічна ефективність

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Relationship between the chemical composition, physical, technological, and sensory properties of beef and the colour of muscle tissue

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Abstract. In Japan, the United States, Korea, and Australia, the colour of muscle tissue is used to assess the quality of beef carcasses, while in the EU and Ukraine, this feature is not considered. The relevance of the study was to substantiate the need to consider the colour of muscle tissue to assess the consumer properties of beef. The purpose of the study was to evaluate the chemical composition, sensory and physical, and technological properties of beef in accordance with the colour of muscle tissue. The study used carcasses of 21-month-old bulls (n=13) of Ukrainian black-and-white dairy cattle slaughtered after a 24-hour fast with free access to water in a slaughterhouse (Kalynivka village) in Brovary district of the Kyiv oblast. The colour of muscle tissue in the carcass was evaluated according to the method of the Japan Meat Grading Association. The total fat content, moisture content, and protein content of minced *m. longissimus dorsi* were analysed. Beef acidity (pH), penetration, and bound moisture content were also investigated. Consumer properties of beef were determined by tasting boiled meat (by aroma, juiciness, tenderness, ease of chewing) and broth from it (by colour, taste, thickness). It was found that the darker colour of muscle tissue had a likely (p>0.99) inverse correlation (r=-0.737) with beef boiling down. It showed a tendency for direct correlation with the cross-sectional area of back muscle (r=0.341), water binding capacity (r=0.326), penetration (r=0.295), adipose tissue colour (r=0.267), marbling (r=0.258), pH (r=0.231),

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and inverse correlation with mineral content (r=-0.309) and overall tasting score of cooked meat (r=-0.487). There was no correlation between muscle staining and the content of moisture, dry matter, protein, and fat (r= from -0.005 to 0.094). The practical significance of the study lies in the possibility of using the acquired knowledge to sort beef carcasses according to their purpose, and consumers will be provided with additional information for objective meat selection, considering the relationship between muscle colour and sensory and physical and technological properties of beef

Keywords: meat productivity; marbling of muscles; physical; technological; and sensory properties of beef; chemical composition of meat; bovine cattle

Introduction

There are certain differences between the requirements for evaluating beef carcasses in countries with developed cattle breeding in the European Union and the world and in Ukraine. According to DSTU 4673:2006 (2011), beef is to be evaluated by live weight and carcass weight and fatness. In the European Union, cattle carcasses are evaluated according to the EUROP classification system (Commission Regulation (EC). No 1249/2008, 2008), which describes their conformation (E, U, R, O, P), external fat coverage (from 1 to 5), and its thickness. These regulations focus only on describing the yield of meat products, which weakly correlate with the nutritional value of muscle tissue at the consumer level and do not reflect the qualitative composition of carcasses and consumer satisfaction. The study by O. Kruk *et al.* (2023) proved that with a better assessment of the development of adipose tissue on carcasses according to the EUROP system, there was a tendency to reduce beef boiling and deterioration in the overall assessment of the sensory properties of cooked meat and its broth. According to the beef classification systems of the Ministry for Food, Agriculture, Forestry and Fisheries in Korea (MFAFF, 2007), Japan Meat Grading Association (JMGA, 2000), Beef Grading System in the United States (United States standards for Grades of Feeder Cattle, 2000) and Meat Standards Australia (Meat Standards Australia, 2015) there is a parallel assessment of the quality of carcasses by the colour and marbling of muscle tissue and its intended sensory and physical and technological characteristics.

For the consumer to make a decision on the purchase of meat from cattle, the most important factor (36%) is the amount of visible fat, the second place (25%) is the price, and the third (19%) is the colour (Ramanathan *et al.,* 2023). M. Hanson (2023) claims that consumers prefer beef, which has a bright "cherry-red" appearance, which they associate with the good quality of the product and consider this colour to be a criterion for freshness. Appearance defects associated with poor quality of meat and deviation from bright red towards discolouration (Pale Soft Exudativ – PSE) or darkening (Dark Firm Dry – DFD). PSE beef, which has excessive losses in water, observed in carcasses for a rapid drop in pH, and rapid metabolism in the muscles. Beef at a high (>6.09) final pH has a dark colour. Dark firm dry meat is one of the main defects in its quality associated with colour, tenderness, juiciness, taste, and shelf life. It is caused by the depletion of glycogen in the muscles and limits the synthesis of lactic acid. As a result, the pH of the muscles does not decrease enough. Meat with signs of DFD and PSE is unacceptable for consumers and leads to a decrease in the price of it or even to recycling.

According to R. Ramanathan *et al.* (2023), in the life cycle of beef production in the United States, due to colour changes, the industry annually disposes of 194.7 million kg of meat, and at the same time loses natural resources

totalling USD 3.9 billion. Referring to statistics (Maia Research Analysis), the researchers state that due to the discolouration of beef in the world in 2015-2020, losses averaged USD 14.2 billion per year. Despite the significant economic costs that are incurred for the meat industry, the decisions made by consumers regarding the purchase of beef by colour do not guarantee its nutritional quality. Colour had no significant effect on the chemical composition of bovine muscle tissue (Moloney *et al.,* 2022). To date, the academic literature in Ukraine has not discussed the relationship of colour with other beef quality indicators. Therefore, an urgent problem in the context of implementing and following European standards in the field of cattle breeding is the substantiation of the need to include the colour of muscle tissue and its other qualitative features in the regulatory documents on the classification of cattle carcasses, in order to increase the sustainability of beef production and limit the negative impact on its quality and prevent consumer diseases and reduce the loss of natural resources and energy consumed.

The purpose of the research at this stage was to evaluate the ability to predict the quality of carcasses in accordance with the colour of muscle tissue, signs of beef quality by chemical composition, sensory and physical and technological properties in bulls of Ukrainian black-and-white dairy cattle.

Materials and Methods

The study was conducted at the "Zhuravushka" farm in Brovary district of Kyiv region. From birth to the age of 4 months, the animals were kept in groups of 25 units. During the pre-weaning period, they were fed 547.2 kg of whole milk and 182.4 kg of skimmed milk. Cattle had free access to hay. It was also provided with concentrated feed at an early age. Rearing and fattening of bulls was carried out on fattening grounds with free access to feeders and water. In the farm, the animals' need for feed was provided at the expense of their own feed base. On the sites, animals were placed considering the age and live weight in the amount of up to 50 animals. Bulls had free access to coarse, juicy, green, concentrated feed and mineral supplements, which were fed from self-feeders in accordance with the diets developed on the farm. During the period from birth to reaching the age of 21 months, bulls consumed feed with a nutritional value of 34,232 MJ (Table 1).

Table 1. *Feed consumption by bulls, MJ*

Consumed	Feed								
	concentrated	fresh, total	silage	haylage	rough, total	hay	straw	green	during the growing period
MJ	6,344.0	4,894.5	3,290.7	1,603.8	4,226.6	2,951.2	1,275.4	9,645.8	34,232
%	18.5	14.3	9.6	4.7	12.3	8.6	3.7	28.3	100

Source: compiled by the authors

The pre-slaughter live weight of animals was determined by weighing them before and after 24-hour fasting with free access to water. The slaughter of 13 animals was carried out in the slaughterhouse of the "Zhuravushka" farm in the Kalynivka village. Age difference between bulls in the group was up to 5%. During the experiment, the animals were kept in accordance with the Order of the Ministry of Agrarian Policy and Food of Ukraine No. 652 (2012) and guidelines for the care and use of laboratory animals (National Research Council of the National

Academies, 2011) and acted in accordance with ARRIVE (2010) guidelines for reporting experiments on the use of live animals.

After slaughtering and skinning, the colour of muscle tissue in the carcass was visually classified according to the Japan Meat Grading Association (2000) method using a colour scale from 1 to 7 (Fig. 1), adhering to the existing regulatory document in Ukraine – "Rules of pre-slaughter veterinary inspection of animals and veterinary sanitary examination of meat and meat products" (2002).

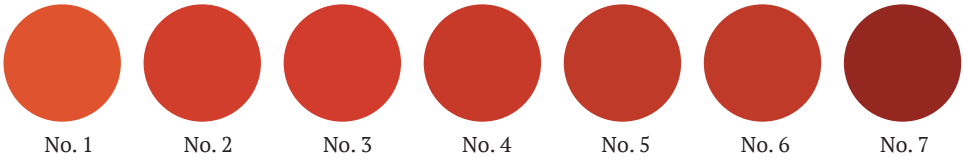

Figure 1. Muscle tissue chroma scale

Source: JMGA (2000)

After slaughtering, a piece of meat (300 g) was selected from *m. longissimus dorsi* for mincing and chemical analysis. Assessment of the total fat content in it was carried out in accordance with DSTU ISO 1443:2005 (2007), total ash content – DSTU ISO 936:2008 (2008), moisture content – DSTU ISO 1442:2005 (2008), protein (Shkurin *et al.,* 2002). The following physical and technological features of beef were also studied: acidity (pH) according to DSTU ISO 2917:2001 (2003) 48 hours after slaughter of bulls and penetration (Guts & Koval, 2007). The sensory characteristics of boiled beef (by smell, juiciness, tenderness, ease of chewing) and broth from it (by colour, taste, thickness) were evaluated by the tasting commission consisting of 8 people, and the content of bound moisture – by the "press method", in accordance with the recommendations (Antoniuk, 2020). The data obtained were processed statistically using Microsoft Excel 2016 in combination with XLSTAT. The studied indicators were evaluated by correlation coefficients calculated using appropriate methods (Osadcha, 2021).

Results and Discussion

Most carcasses are classified as grades 5 and 6 in terms of muscle colour. The chemical composition of beef was determined to assess its nutritional value. According to the study data, shown in Table 2, there was no significant correlation between muscle colour and total fat, protein, dry matter, and moisture content in *m. longissimus dorsi*. Its values (r) ranged from -0.005 to 0.094. These results are consistent with data from studies A. Diler *et al.* (2022). The researchers also found that the shade of muscle tissue in cattle is not a source of variability in their chemical composition.

Table 2. *Correlation between muscle tissue colour and chemical composition of beef*

Chemical composition	r
Moisture	-0.005
Dry matter (DM)	0.005
Protein	0.054
Total fat content	0.094
Total ash weight	-0.309
Acidity (pH)	0.231
Water binding capacity	0.326

Source: compiled by the authors

The relationship between the colour of muscle tissue and its total fat content – a key component of beef quality for consumers – was absent. Fat in the middle of the muscles gives the meat a special taste and lubricates its fibres. This improves the consumer's perception of the tenderness and juiciness of meat and gives it taste and aroma. There is a tendency for a weak positive correlation between the water content in the muscles and their colour. Water is important in the development of muscle structure and affects the quality of meat associated with taste, tenderness, and juiciness. There was a tendency for a weak direct correlation between muscle tissue colour and protein content in *m. longissimus dorsi*. According to B. Santiago *et al.* (2023), protein in muscle plays a key role in adenosine triphosphate (ATP) production, energy metabolism, oxidative stress, and redox processes in cells.

Lack of correlation between the colour of muscle tissue and the total content of fat, protein, dry matter, and moisture in *m. longissimus dorsi* indicates that it is not possible to accurately predict these elements of the chemical composition of beef by the appearance of muscles in carcasses. They depend on the prominence of meat forms in cattle (Ugnivenko *et al.,* 2023), the slaughter age of animals, the type of muscle and adipose tissue, their placement in the carcass (Diler *et al.,* 2022). When increasing the assessment of the colour of muscle tissue, there was a tendency to reverse weak correlation (r=-0.309) with the total weight of ash, whose macro- and microelements, including iron, zinc, selenium, play a crucial role in the evolution and nutrition in human health.

An increase in the colour rating of muscle tissue weakly (r=0.231) correlated with its acidity and indicated a darkening of beef with an increase in its pH. Deterioration of muscle colour due to an increase in their acidity (pH) was found by B. Santiago *et al.* (2023) also in crossbreed (½Aberdeen-Angus x ½ Charolais) animals. An increase in the colour rating of beef positively and weakly correlates with its acidity due to the fact that an increase in the pH of muscle tissue affects its physical and biochemical properties, which lead to a decrease in oxygen saturation and darkening of meat. Muscle colour is correlated with the chemical form and concentration of myoglobin contained in them (Raza *et al.*, 2019). After slaughtering animals and exsanguinating carcasses, myoglobin in muscle cells acts as a short-term oxygen reserve. The colour of meat depends not only on the content of myoglobin in the muscles, but also on the state of its oxidation. Fresh meat contains deoxymyoglobin, oximyoglobin, and metmyoglobin. Red pigmentation of beef is given by deoxymyoglobin. In the presence of oxygen, it is oxidised to oxymyoglobin and shows a bright pink-red colour. Thus, the higher the proportion of "red" muscle fibres that are rich in myoglobin due to high respiratory activity, and the fewer "white" muscle fibres, the redder the meat looks. When deoxymyoglobin and oximyoglobin are oxidised to metmyoglobin, muscle tissue becomes reddish-brown, which causes rejection in the consumer.

According to the authors of the current study, water binding capacity had a weak direct relationship (r=0.326) with an increase in muscle colour score, which indicates a slightly better ability of beef to retain moisture in this case. The water retaining ability of fresh beef determines its visual perception by the consumer, which affects their willingness to purchase the product. It also determines possible water losses during future transportation, storage, processing, and cooking of meat, and also characterises the juiciness, tenderness, and other technological properties of meat products. In raw muscle, and in unprocessed and cooked meat, the main factors determining water retention in the meat structure are the swelling that occurs in the myofibrillary structure and the forces that promote water intake or retention, and the deployment and aggregation of proteins that determine the formation of gels or

separated insoluble proteins. M. Ijaz *et al.* (2020) explained possible mechanisms of association between beef colour and other features of its quality, including water retention capacity. According to their data, typical dark firm dry beef (DFD) with a pH of more than 6.09 showed the lowest values of oximyoglobin content, which accompanied the highest values of water retention capacity and deoxymioglobin. The fact that the colour change of beef is affected by its ability to retain water is also proven by D. King *et al.* (2023). Meat becomes darker with increased water retention capacity because DFD absorbs light rather than reflects it off the surface. In order not to lead to exhaustion in the muscles of inadequate acidification and an increase in pH, which leads to darkening of beef and a decrease in its tenderness, juiciness and taste, it is necessary to carefully handle the animals during transportation to the slaughterhouse, and with raw materials after slaughter, in order to reduce the loss of water from the meat, to ensure a moderate drop in pH in the muscles of the carcass.

The physical characteristics of beef, such as fat colour, subcutaneous fat development, fat thickness, and marbling, were determined by their appearance. Manifested (Table 3) in the tendency to a direct weak correlation between an increase in the assessment class of muscle tissue colour and such physical quantities of *m. longissimus dorsi* as the cross-sectional area, penetration, marbling, and development of subcutaneous fat. Due to the increased assessment of muscle colour as a result, beef became tougher, which indicated an improbable weak direct correlation (r=0.295) between its hue and penetration – the ease with which the needle penetrated the meat.

Table 3. *Correlation between the colour of muscle tissue and the physical properties of beef*

Indicator	r
Penetration	0.295
Marbling	0.258
Subcutaneous fat thickness	-0.230
Cross-sectional area	0.341
Subcutaneous fat development	0.245
Colour of adipose tissue	0.267

Source: compiled by the authors

The background stiffness associated with connective tissue mainly depends on meat tenderness (Liu *et al.,* 2022). The stiffness of meat is increased by proteins that are present in the muscles. They protect myofibrillary proteins from destruction by proteolytic enzymes. M. Ijaz *et al.* (2020) proved that atypical DFD beef had a higher value of shear strength indicators, which were positively associated with the level of metmyoglobin, and concluded that in addition to the main acidity factor (pH), meat colour is affected by other characteristics of meat quality, including the ability to retain water, and shear strength, with a greater contribution of relative myoglobin content and glycolytic potential.

There was a tendency for a weak direct correlation (r=0.256) between the shade of muscle tissue and marbling of *m. longissimus dorsi*, which indicates that the colour of beef cannot accurately describe its good sensory properties. Marbling of muscles is the main factor determining the quality of meat (Sakowski *et al.,* 2022). Due to the strong association between marbling assessment and beef taste, the quantity and distribution in m. longissimus dorsi is a critical criterion for evaluating carcasses in the USDA and JMGA classification systems. This can also serve

as a theoretical basis for introducing the marbling indicator into the classification system of cattle carcasses in Ukraine.

There was a tendency to weak correlation (r=-0.230) between the colour of muscle tissue and the thickness of subcutaneous fat, which led to faster cooling of carcasses and caused the loss of beef tenderness. Subcutaneous fat protects the carcass from weathering and loss of moisture, which ensures juiciness of the finished meat product. With the worst thickness of fat cover, bulls increased catabolic processes (glycolysis), oxidative stress, muscle structure and contraction, which affected the colour of meat. A tendency for a weak direct correlation (r=0.341) between the shade of muscle tissue and the cross-sectional area of *m. longissimus dorsi* indicates that the colour of beef is not possible to accurately judge the content of valuable cuts in carcasses. With an improved assessment of beef colour, there is a tendency for a slightly better correlation (r=0.245) with the development of subcutaneous fat. A.P. Moloney *et al.* (2022) found that there is no or inverse relationship between the colour of muscle and adipose tissue. According to the study, there was a tendency to a weak positive relationship (r=0.267) between these traits due to a small proportion of concentrates in the diet and significant green, juicy, and coarse feeds, the feeding of which leads to darkening of muscle and yellowing of adipose tissue.

The technological properties of beef related to storage and processing (yield during cooking) of beef products were investigated. According to the data (Table 4), the colour of muscle tissue can be used as an indicator of its degraded technological properties, since a probable (p>0.99) strong inverse correlation (r=-0.737) with its boiling is established.

Table 4. *Correlation between the colour of muscle tissue and the technological and sensory properties of beef*

Indicator	r
Boiling down of beef	-0.737**
Broth tasting	-0.047
Boiled beef tasting	-0.487

Note: **P>0.99
Source: compiled by the authors

Cooking beef greatly affects the sensory quality during consumption. This is considered only in the beef classification system (Meat Standards Australia, 2015). The consequence of the deterioration in the boiling down rate of meat was a lower yield during cooking and often to "dry (without juiciness)" beef. This led to high losses of cooked meat and meat products during water runoff, which also affected the yield and quality of processed meat. The loss of fluid from cooked meat is important for the meat industry because of its impact on the economy. As water is lost from the muscles during cooking, the proteins become less flexible and more rigid. With such a loss of water from beef during heat treatment, the product from it is coarser (Rahman *et al.,* 2020). Although with longer heating, the sarcoplasmic protein collagen becomes gelatinised and is able to retain water. The deterioration of the technological properties of muscle tissue with an improvement in the assessment of its colour can also be explained by the fact that collagen fibrils contract during heating. This leads to a loss of fluid in the meat, and to a decrease in its tenderness. Water is lost during the preparation of meat products due to denaturation of muscle proteins at different temperatures, which causes transverse and longitudinal shrinkage (Warner, 2023). The loss of moisture in meat

during its boiling also increased with an increase in its acidity (pH). The sensory quality of beef is becoming increasingly important to consumers.

To obtain data on the description of the nutritional quality of meat, it is determined by sensations during beef consumption and preferences for individual sensory responses during its consumption for taste, tenderness, juiciness, aroma, freshness, and leanness. The results of the correlations between the assessment of muscle tissue colour and the average values of sensory properties (aroma, juiciness, tenderness, chewiness) of cooked beef and beef broth (colour, taste, thickness). It is indicated that with an increase in the colour class of muscles, there was a tendency to worsen the values of tasting meat and broth, since the correlation coefficients between them were weak and inverse, respectively (r=-0.487 and -0.047). The main reason for the feedback between better beef colour development and its sensory properties is the lack of correlation (r=0.094) between the appearance of muscle tissue and its fat content. Beef tasting scores are not affected by its colour, also due to the fact that juiciness, which affects the quality of consumption and the amount of juice during chewing, also closely correlates with the fat content of beef (Listrat *et al.,* 2020), the amount of which, according to the researchers of the current study, does not increase due to colour changes. Beef, which is characterised by a low muscle fat content, is darker in colour, firmer, and drier.

Thus, the qualitative characteristics of beef are key. Studies by A. Hosseini (2022) have shown that the main problems with the quality of meat from cattle are associated with the non-compliance of consumer preferences with their consumer characteristics. The quality of beef is evaluated by the degree of marbling, colour of meat and fat, sensory characteristics – tenderness, aroma, juiciness, taste, smell, and appearance (Sakowski *et al.,* 2022). The main factor determining the quality of beef is a fat deposit in the middle of muscles or marbling. In the future, it is necessary to investigate the effect of intramuscular fat (marbling) on the quality characteristics of beef.

The variability of beef quality is very significant between animals, so according to A. Clinquart *et al.* (2023), it is not easy for consumers to evaluate it, except visually, only by the colour of muscle tissue and its fat content. Conventional beef quality assessment, which involves chemical and biological experiments, is tedious, time-consuming, destructive, and sometimes environmentally harmful. The consumer pays special attention to the sensory properties of beef, which are almost difficult to assess during the purchase. Predicting the sensory characteristics of meat was the main task. This was achieved based on the collected data after slaughter of animals and pre-determined relationships between them. In this paper, the approach to beef quality is applied in terms of providing an answer to the question of what its quality means for consumers, how they form expectations from it during purchase, and how it correlates with the quality that they perceive during consumption. Based on this, the relationship between the colour of meat and its physical (colour of meat and fat, marbling), sensory (sensations during consumption and preferences for individual reactions – taste, tenderness), technological (related to storage and processing, output during cooking), chemical (pH, water retention capacity, moisture and dry matter content, protein, total fat content, and ash weight) characteristics was studied.

According to the data obtained, the authors came to the conclusion that the colour of beef does not characterise its good sensory and culinary properties. With an increase in the colour class of muscles in the carcass, there is a deterioration in the boiling of meat, there is a tendency to reduce the fat content in the muscles, and some increase in the marbling of beef, and the deterioration of its tasting properties (taste, aroma, tenderness) and broth from it. In the

market, the cost of beef depends on the colour of muscle tissue, which the consumer uses to assess the freshness and healthiness (acceptability) of meat, since its colour affects the preferences and probability of its purchase. Colour measurement is more important for the visual perception of beef than the actual quality parameters. Therefore, it is vital to maximise the benefits of beef production by understanding the factors that affect the colour of meat and its deviation from bright red colour. Life-long factors that affect muscle colour include (Sakowski *et al.,* 2022) sex and age of animals, time spent on fattening them, seasonality, stress before slaughter, nutritional status, slaughter process, and meat maturation time.

In turn, consumers are interested in the nutritional value and sensory characteristics of beef and the prevention of their diseases (Randhawa *et al.,* 2021) from eating meat, which has become a pressing issue in recent years. For people's perception of beef quality information, about its sensory assessment used to describe the nutritional quality of meat becomes more complex as consumers' purchasing decisions become more sensitive to internal and external challenges, including food quality and safety, and environmental sustainability (Liu *et al.,* 2022). There are many problems regarding the assessment of Ukrainian black-and-white dairy cattle by the colour of muscle tissue, including the deterioration of many qualitative indicators of beef. Despite recent advances in the world regarding the possible regulation of the development of muscle tissue colour in cattle to improve the quality of beef, this problem remains insufficiently solved in Ukraine and deserves further investigation. In this regard, Ukraine raises the question of solving the problem of producing beef that is attractive to buyers, which would combine the quality of meat with the prevention of their diseases.

In the future, it is necessary to conduct research on more animals of different breeds regarding the relationship between the colour of muscle tissue and the qualitative characteristics of beef, including meat, the colour of which, according to M. Hanson (2023), is most consistent with consumer preferences.

Conclusions

The EUROP classification system of beef carcasses in Europe by conformation, external fat content and its thickness has little to do with food quality for the consumer, does not reflect the composition of the carcass and the level of marbling (main quality characteristics).

Although the colour of muscle tissue does not characterise the good sensory and culinary properties of bulls of the Ukrainian black-and-white dairy breed, but it should be included in the quality characteristics of carcasses, since it is the first to indicate its freshness and affects the preferences of consumers and the probability of its purchase. The colour of muscle tissue inversely (r=-0.737) significantly (p>0.99) correlated with its boiling, which indicated a deterioration in its technological properties, showed a tendency to increase the water binding capacity (r=0.326), penetration (r=0.295), marbling (r=0.258), acidity (pH) (r=0.231), the cross-sectional area (r=0.341), the highest class of carcasses in the colour of muscle tissue it tended to have a relatively positive association with the overall tasting score of cooked meat (r=-0.487) and broth from it (r=-0.047). There was no correlation between the colour of muscle tissue and its chemical composition (moisture, dry matter, protein, and fat content) (r=-0.005 to 0.094). The tendency to correlation (r=-0.230) between the colour of muscle tissue and the subcutaneous fat thickness led to faster cooling of carcasses and caused an increase in beef stiffness, drying out, and a darker colour of muscle tissue

In the future, it is advisable to conduct similar studies on other breeds of cattle common in Ukraine and develop technologies for the production of beef that is attractive to buyers in colour, which would combine its qualitative

sensory characteristics. In the future, it is necessary to investigate the effect of intramuscular fat (marbling) on the quality characteristics of beef. Special attention should be paid to establishing the optimal thickness of subcutaneous adipose tissue, which ensures an adequate meat yield with a significant protein content and low fat concentration and a high amount of edible meat for consumers. Future research should focus on reducing water loss during beef storage, as this will reduce the amount of waste and improve its visual and sensory quality. Such data will provide consumers with additional information that will help them make an objective, useful choice when purchasing beef. This will become a theoretical basis for introducing indicators of the colour of muscle tissue, marbling of meat, the development of subcutaneous fat and its thickness into the carcass classification system in Ukraine.

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Conflict of Interest

None.

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Взаємозв'язок між хімічним складом, фізико-технологічними та сенсорними властивостями яловичини і кольором м'язової тканини

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Анотація. У Японії, США, Кореї, Австралії колір м'язової тканини застосовують під час оцінювання якості туш яловичини, в той час як в країнах ЄС та Україні цю ознаку не враховують. Актуальність роботи полягала в обґрунтуванні необхідності врахування кольору м'язової тканини для оцінки споживчих властивостей яловичини. Метою дослідження було оцінити хімічний склад, сенсорні і фізико-технологічні властивості яловичини відповідно до забарвлення м'язової тканини. У дослідженні використовували туші 21-місячних бугайців (n=13) української чорно-рябої молочної худоби, забій яких провели після 24-годинного голодування за вільного доступу до води, в забійному цеху (с. Калинівка) Броварського району Київської області. Колір м'язової тканини у туші оцінили згідно з методикою японської асоціації класифікації яловичини. У фарші із *m. longissimus dorsi* дослідили загальний вміст жиру, вміст вологи та протеїну. Вивчили також кислотність (pH) яловичини, пенетрацію та вміст зв'язаної вологи. Споживчі властивості яловичини визначали шляхом проведення дегустації вареного м'яса (за ароматом, соковитістю, ніжністю, легкістю жування) і бульйону із нього (за кольором, смаком, міцністю). Було встановлено, що більш темний колір м'язової тканини мав вірогідну (P>0,99) зворотню кореляцію (r=-0,737) із уварюванням яловичини. Він проявляв тенденцію щодо прямої кореляції з площею «м'язового вічка» (r=0,341), водозв'язуючою здатністю (r=0,326), пенетрацією (r=0,295), кольором жирової тканини (r=0,267), мармуровістю (r=0,258), pH (r=0,231) і зворотньої – з вмістом мінеральних речовин (r=-0,309) і загальною оцінкою дегустації вареного м'яса (r=-0,487). Між забарвленням м'язів і вмістом у них вологи, сухої речовини, білку та жиру кореляція була відсутньою (r=від – 0,005 до 0,094). Практичне значення роботи полягає в можливості використання отриманих знань для сортування яловичих туш відповідно до призначення, а споживачам нададуть додаткову інформацію для об'єктивного вибору м'яса за урахування зв'язку між забарвленням м'язів та сенсорними і фізико-технологічними властивостями яловичини

Ключові слова: м'ясна продуктивність; мармуровість м'язів; фізико-технологічні і сенсорні властивості яловичини; хімічний склад м'яса; велика рогата худоба

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Use of unmalted cereals with enzyme preparations in brewing

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Abstract. The use of unmalted grain is one of the methods for improving quality, reducing production costs, and creating new types of beer. However, the enzymatic activity of such raw materials is zero, so the use of enzyme preparations is relevant. The purpose of the study was to compare the quality indicators of 100% malt beer and beer with the replacement of malt with unmalted barley using enzymes. Methods of technical and chemical analysis were used to determine the organoleptic and physico-chemical parameters of beer. Recommendations have been developed for intensifying the preparation of wort using various types of grain raw materials – unmalted barley and rice grits with the addition of enzyme preparations from Novozymes (Denmark) at different stages of brewing were developed. When testing new enzyme preparations (β-glucanase complex, xylanase), the filtration time, wort viscosity, and the content of β-glucans and arabinoxylans decreased by about 20% compared to the control samples. The use of a new enzyme preparation resulted in better breakdown of substances, which affected the quality and duration of filtration. This leads to an improvement of wort quality, reducing the duration of the process and reducing the cost of raw materials. To ensure the required ratio of substances in the wort, it is necessary to introduce complex enzyme preparations – peptidhydrolase, α-amylase, endo-β-glucanase (Ceremis Plus). Preparations containing endo-β-glucanase (Ultraflo Max) should be used at the beginning of mashing to improve the rheological properties of the wort and reduce its viscosity, which improves the filtration process. To obtain beer with the required degree of fermentation during mashing, it is better to use new enzyme preparations (glucoamylase). The practical significance of the study lies in the use of large amounts of unmalted grain together with exoenzymes such as Ultraflo Max and Ceremis Plus, which will allow brewers to produce wort and beer of the same composition and quality as premium wort and beer, using only malt

Keywords: barley; beer; wort; malt; quality

Introduction

Barley is a traditional raw material for beer production. To turn barley grains into fermented wort, enzymes are needed to break down cell walls, release amino acids, and break down starch into fermented sugar. For brewing with a very small amount of malt, barley is a very good choice to replace some of the malt, as it has some special features. Firstly, barley contains β-amylase and peptidase. Secondly, it can be added to the mash, since barley starch has a low gelatinisation temperature. Thirdly, it has a husk that ensures good filtration in the lauter tank when properly crushed (Zamai *et al.*, 2022). The researchers used the replacement of 30% corn and 70% malt with 30% corn grits, 35% barley and 35% malt, and proved that it is possible to produce high-quality beer. However, there is a need to use exoenzymes (Park *et al.*, 2023).

According to G. Birta *et al.* (2021) found that the use of corn grits in brewing allowed to increase the quality indicators and dietary value of beer, and the optimal value of corn grits content ranges from 15 to 20%. A. Fujita *et al.* (2020) proved that single-grain, emmer or spelt grains can be used to make malt of acceptable quality and produce craft beer with a specific taste and aroma. Researchers have investigated the brewing potential of 100% unmalted barley, wheat, oats, and rye compared to 100% malt and proved that in this way, along with the use of enzyme preparations, it is possible to reduce the level of colour, the content of higher alcohols and esters in the finished beer. Results of the study by A. Dabija *et al.* (2021) showed that buckwheat can be used as a raw material for the production of gluten-free beer.

M.E. Ciocan *et al.* (2023) suggested using malted and unmalted sorghum instead of traditional barley malt, which included 60% sorghum malt and 40% unmalted sorghum. N. Cela *et al.* (2022) investigated the combined effect of the composition of sorghum:quinoa and at a ratio of 6 and 34% unmalted sorghum and quinoa, respectively, it was found that the use of unmalted sorghum and quinoa in an optimised combination in brewing allows the development of beer brewed from unconventional grains, without negative impact on consumer perception.

Ukrainian researchers also used unmalted materials to replace some of the malt during brewing. Thus, the analysis of the results obtained by S.P. Orel (2021) indicates the possibility of replacing malt with 20% spring and winter barley, while wort quality indicators decrease, but remain within the normal range. When replacing malt in the amount of 20% spring barley, such qualitative indicators of wort as extractivity, amine nitrogen, reducing substances are slightly higher than when replacing 20% with winter barley. When replacing malt with 25% barley, when using Termamil enzyme preparation in the amount of 0.6 kg/t of barley, a significant increase in the quality indicators of wort was observed. The researcher established that the optimal dose of malt replacement with rye (without the use of enzyme preparations) is 10%. S. Loiko & Z. Romanova (2018) in their research replaced 25% of malt with unmalted barley using the Tetramil enzyme in the amount of 0.15 kg/t of barley and proved by technological indicators the possibility of obtaining high-quality beer using external enzymes and unmalted barley.

According to L.M. Puzik (2019), the main quality indicators of spring barley grain for use in brewing in the first months of its storage under an unregulated temperature regime are intensively improved compared to its storage under a regulated temperature regime. The researcher claims that it is advisable to store spring barley grain with humidity within critical and with good initial quality Indicators in storage facilities for no more than 9 months, with a longer shelf life, such grain should be stored under a controlled temperature regime. Post-harvest grain ripening of brewing spring barley varieties is more intensive under unregulated conditions. Nevertheless, the best preservation of the technological qualities of grain of brewing varieties of spring barley is ensured by its storage in a controlled temperature regime.

The exergy performance of beer produced using the traditional malt and brewing process is compared with that of beer produced using the fermentation process. Therefore, the purpose of the study was to compare the quality indicators of 100% malt beer and beer with the replacement of malt with unmalted barley and rice grits with the additional use of Ondea Pro enzyme preparation from the Danish company Novozymes.

Literature Review

While beer was produced using various types of unmalted grain as excipients along with malt, brewing from 100% unmalted grain combined with the addition of mashing enzymes remains largely unknown. Barley, as one of the oldest crops, has great potential to regain its role as a food grain, mainly due to its high nutritional value. W. Kunze (2004) proved that barley's food enzymes, in particular lipase, amylase, and protease, stimulate the breakdown of proteins, fats, and carbohydrates.

As noted by J. Mallett (2014) in the textbook on malt theory, barley is the most suitable cereal for malting in terms of the composition of extractives before fermentation. The main varieties are those that contain a lot of starch and a small amount of protein, which leads to a better yield of extractives. According to F. Guzmán-Ortiz (2019), malt production has provided suitable conditions for the production of various brewing varieties of malt from Doña Josefa barley. Malt had total and reduced sugar values that correspond to brewing. The

researcher found that temperature and soaking time are determining factors for the time of enzyme activation. At higher temperatures, enzymatic activation is usually faster, but there are some exceptions. The use of germination can be a promising resource for modifying grain properties and increasing enzymatic activity.

H. Gupta *et al.* (2020) noted that the presence of non-starchy polysaccharides in barley in the form of mixed bonds (1-3), (1-4)-β-d-glucans and arabinoxylans together with enzymes is responsible for its modification. Malt production is a rather complex process that involves important enzymes such as α-amylase, β-amylase, α-glucosidase, and limit-dextrinase. During the malting and brewing process, the by-products left over from wort separation are rich in protein, fibre, arabinoxylanes, and β-glucans. Therefore, the researchers point out the feasibility of using by-products and summarise the properties of barley in relation to the nutritional, functional, and compositional changes that occur during malting and brewing.

F. Nocente *et al.* (2023) showed that fermented beverages made from single-grain, tritordeum, edible sorghum, and teff may be of interest for light and gluten-free beer markets. L. Van Donkelaar *et al.* (2019) found beer produced with added enzymes reduced the use of water, raw materials, and natural gas by 7, 14, and 78%, respectively. In this case, the loss of exergy in the enzyme production process is compensated by preventing the loss of exergy in the overall brewing process. M. Salailenbi *et al*. (2019) used a mixed dough fermentation culture from Khamei dough to produce beer from black rice, which increased the shelf life of the beer and lowered its alcohol content.

The use of enzyme preparations can be carried out with any amount of barley to produce high-quality beer. Therefore, the desired taste is obtained according to the developed brewing recipe or by blending beer, which is confirmed by the results of H. Kábelová-Ficová *et al.* (2018). It was these authors who proved that the highest level of fermentation, which corresponds to 61.7%, is inherent in beer made from spring barley. To confirm the quality of beer, an organoleptic assessment was carried out, which corresponded to the originality of the beer sample.

The use of enzyme preparations can increase the stability of beer and improve its consumer characteristics along with improving the filtration process. Zh. Zamai *et al.* (2022) experimentally investigated and substantiated the possibility of using enzyme preparations available on the market to optimise the filtration process of craft beer. The use of enzyme preparations helps to speed up the technological cycle of production and to obtain finished products with high indicators of colloidal resistance. The effect of the enzyme preparations Laminex® MAX Flow 4G, Amylex 5T, Beerzym® AMYL HT, Beerzym® BG, EnerZyme® P7 on the wort filtration process was studied and evaluated by determining the content of extractives in beer pellets at the premises of the "Bierwelle" brewery.

According to sources (Sustainable brewing starts..., n.d.), beer is produced with standard brewing equipment using a standard mashing profile and time. Ondea Pro enzyme preparation is also particularly suitable for the production of flavoured beer or any type of soft grain beverages, and for the production of extracts from unmalted grains. Standard technological instructions for beer production provide for the use of established modes of brewing beer wort. However, the modern operating conditions of breweries of any capacity require intensification of production processes, which is impossible using old technological methods. One of these techniques, as noted by M. Zheplinska *et al.* (2022a), may be the use of enzyme preparations. There are currently no universal recommendations for the effective operation of the brewery shop. This indicates that there is a need to develop an intensive technology of high-quality beer wort, adapted to the processing of various raw materials using exogenous

enzymes, which can replace a certain amount of malt with unmalted material in the form of rice or barley and obtain the wort and finished product – high-quality beer using the method of optimising beer fermentation. This was the issue addressed by A. Rodman & D.I. Gerogiorgis (2021), who found that to accelerate the fermentation process and reduce its duration, it is necessary to optimise it through dynamic modelling.

Materials and Methods

Barley malt, unmalted barley and rice grits were used for the study. Danish enzymes Novozymes Ondea Pro were used to replace some of the barley malt with unmalted barley or rice grits in order to provide effective viscosity, turbidity reduction, and protein degradation. The enzymes work in synergy with endogenous barley enzymes to produce fermented wort. In combination with endogenous barley proteases and β-amylases, Ondea Pro enzymes create a synergistic effect to achieve the best brewing results. The study used enzyme preparations containing the same enzymes as brewing barley malt. The research was conducted in the laboratories of the National University of Food Technologies and the National University of Life and Environmental Sciences of Ukraine.

The physico-chemical parameters of wort and finished beer were determined using a PBA-B generation M beer analyser (Anton Paar, Austria). Physical and chemical parameters (moisture, fat, protein, colour, acidity, amine nitrogen, coagulation nitrogen, pH values, polyphenols and reducing sugars) were determined by standard methods in accordance with DSTU 7103:2009 (2016); DSTU 3888:2015 (2017). Dynamic viscosity was determined using the Hepler device according to the method provided by D. Kumar *et al.* (2017). Determination of organoleptic parameters was carried out in accordance with DSTU 7103:2020 (2023). Barley and rice were crushed on a hammer mill (with adjusted power consumption), which allowed for perfect filtration of the mash. For better roll grinding, barley of uniform size with a particle diameter of more than 2.2 mm was used.

The mashing mode corresponded to a temperature of 53°C for a duration of 15-30 minutes to prevent grain deposition and ensure good enzyme efficiency (Zheplinska *et al.,* 2022b). The saccharification time did not exceed 64°C to maintain the productivity of heat-sensitive barley β-amylase. The pH value of barley grits did not decrease below 5.6 units. The wort was acidified to a pH of 5.2 during boiling to maintain a drop in the pH value during fermentation to the beer pH value of 4.5. The quality of wort and beer obtained from 100% malt with the replacement of 11% malt with rice and 14% malt with barley was investigated. Pre-filling processes were carried out at 50-52°C and at 37-39°C. The main mash was kept at 52 and 63°C for 15 and 40 minutes, respectively, and the total mash was kept for 15 minutes at 72°C until it was completely saccharified, heated to 78°C, and filtered. Pre-mashing processes were carried out at temperatures of 50-52°C, which corresponds to the modes (1.1, 2.1, 3.1) and at 37-39°C (modes 2.2, 2.3, 3.2, 3.3, which are given in the Section "Results and Discussion"). The main mash was kept at 52 and 63°C for 15 and 40 minutes, respectively, and the total mash was kept for 15 minutes at 72°C until completely cooled. The mash was then heated to 78°C and filtered.

Application rates of exogenous enzyme preparations: α-amylase – ≈100 g/t; α-glucanase+xylanase complex – ≈100 g/t. Ultraflo Max was used to accelerate the mash filtration, having previously determined its rational dosage. 100% malt mash was prepared according to mode 1.1 with standard pauses (mash 50, pauses 52, 63, and 72°C). During wort filtration for 10 brews, the filtration time was recorded and the wort viscosity, β-glucan and arabinoxylane content were determined with and without using the Ultraflo Max enzyme according to the classical brewing scheme. Statistical analysis data were obtained using Microsoft Excel and Statistica 15 software suites. All experiments

were conducted in 5 replicates, and the results are averages of these replicates with standard deviations. The Student's t-test was used for statistical processing of the results obtained. The data are presented as the mean ± standard deviation from the mean (SDM). The smallest acceptable difference for probes from a single sample is indicated at 5%. The confidence value was chosen as $p<0.05$.

Results and Discussion

A study was conducted on the production of beer with the replacement of part of the barley malt with unmalted barley and rice grits using Novozymes preparations. The research data covers the entire process from raw material processing to the production of finished beer and the obtaining of physicochemical and organoleptic parameters that comply with the DSTU for beer.

Table 1 shows the indicators of grain raw materials, namely, barley malt, barley and rice grits, which were used in the manufacture of beer. Indicators of barley and rice grits as solid raw materials are represented by the mass fraction of moisture, extract, fat, and protein.

Table 1. *Indicators of grain raw materials*

Values	Raw material		
	Barley malt	Barley grits	Rice grits
Number of grains (%): flouriness	85.0±0.5 3.0±0.1	- -	- -
Mass fraction of moisture (%)	4.5±0.05	14.5±0.05	13.2±0.05
Mass fraction of the extract in the dry matter of finely ground malt (%)	77.8±0.07	63.6±0.06	82.2±0.06
Mass fraction of fat (%)	1.8± 0.01	2.0± 0.02	1.1± 0.01
Difference in the mass fractions of extracts in the dry matter of fine and coarse malt (%)	1.4±0.01	-	-
Mass fraction of protein substances (%)	10.1±0.02	10.9±0.02	7.6±0.03
Duration of saccharification (min.)	15	-	-
Colour (chromaticity units)	0.18±0.005	-	-
Acidity (units)	0.9±0.02	-	-
Amine nitrogen (mg/dm^3)	4.6±0.1	-	-
Viscosity (mm^2/cwt)	1.58±0.02	-	-

Source: compiled by the authors

As can be seen from Table 1, the moisture content of barley and rice grits differs by only 1.3%, but a significant difference in these two solids is observed in the mass fraction of the extract in the dry matter. Thus, for rice grits, this value was 18.6% higher than for barley grits, but barley grits have a higher amount of fat and protein. Therefore, the results obtained for the above indicators allow conducting experiments with such types of unmalted grain, replacing them with barley malt, for which the results obtained for determining the number of grains, flouriness, saccharification time, colour, acidity, amine nitrogen content, and viscosity correspond to DSTU 4282:2018 (2019). Table 1 indicators show a well-ground dispersion of raw materials, which predicts enzymatic hydrolysis to values acceptable for obtaining an optimal extract. The mass fraction of protein substances does not exceed 11%, which predicts the production of wort with an optimal nitrogen composition. Research conducted by Z.M. Romanova *et al*. (2021) confirmed the optimal nitrogen composition by automatic

control of the brewing process, which considers the features of the chemical composition of raw materials, the structure and quality of auxiliary materials. The researchers proved that the development and implementation of modern software with its practical use in the preparation of existing and new types of beer will contribute to further intensification of brewing, which is of great importance in the context of compliance with high quality standards for hop beverages.

The optimal effect of drugs and the influence of various factors on their stability have been studied and confirmed, because in general, the quality of beer depends on many factors, such as the chemical composition, physico-chemical characteristics of the wort, the type of beer, the degree of fermentation, etc. The results of the study of the optimal action and activity of enzyme preparations are presented in Table 2.

Table 2. *Characteristics of enzyme preparations*

Enzyme preparation	Manufacturer	Active substance	Optimal conditions actions	
			temperature (°C)	pH value
Ultraflo Max	Humicola insolens	cellulase	70.0±5	5.0±0.5
Ceremix Plus	Bacillus amuliquefacien	α-amylase Endo-®-glucanase endoproteases	70.0±2 50.0±2 45.0±2	5.5±0.1 7.0±0.1 6.0±0.1
Neutraz 0.5 L	Bacillum amynoliquefaciens	neutral protease	40.0±2	6.0±0.1
Attenuzim Pro	Asperglus niger	glucoamylase	60.0±2	4.3±0.1

Source: compiled by the authors

Based on the results obtained in Table 2, it can be seen that the first two enzyme preparations Ultraflo Max and Ceremix Plus are best suited for use in brewing, with the optimum effect on unmalted raw materials at 70°C. In addition, these enzyme preparations are active in an environment with a pH value from 5.0 to 5.6 units, which is exactly what is necessary for the mashing process. I. Zeida (2023) investigated and substantiated the possibility of using enzyme preparations available on the market to optimise the beer preparation process. The use of enzyme preparations helps to speed up the technological cycle of production, to obtain finished products with high indicators of biological and colloidal resistance. The effect of Laminex® MAX Flow 4G, Amylex 5T, Beerzym® AMYL HT, Beerzym® BG, EnerZyme® P7 enzyme preparations on the brewing of beer wort and finished beer was investigated and evaluated.

In the course of the study, it was found out that the use of Hytemphase EP in the mashing process can increase the content of extractives and maltose in the wort, reduce its viscosity, and therefore, improve the quality of beer, reduce the duration of filtration, speed up the fermentation of beer wort, and control its composition. Due to its high thermal stability, the enzyme preparation under study allows carrying out the mashing process at high temperatures, while malt's own enzymes can already be inactivated.

Proteolytic components work in synergy with endogenous barley enzymes. The generation free amino nitrogen compounds (FAN) is directly related to the protein content of the grain. According to an online source (Features of growing..., n.d.), the use of high protein barley can have some negative effects, as barley is naturally high in protein, and high protein levels can affect the enzymatic processes during brewing. This can lead to less efficient breakdown of starch and other components of barley, which affects the quality of beer. A high

protein content can also lead to the formation of bilge substances during brewing. This leads to a darkening of the beer and an unpleasant taste. Protein can increase the viscosity of the wort, which can complicate the filtration and separation of wort from the waste. A high protein content can lead to more waste during filtration, which can be inefficient from a production standpoint. In general, brewers choose barley with the appropriate protein content to ensure beer quality and optimal conditions for enzymatic processes. High protein content may be undesirable if its effects on beer production are not controlled.

Figure 1 shows the dependence of FAN content on protein content in barley grain. Using barley with a high protein content can be an advantage when brewing using Ondea Pro enzymes. The amine-nitrogen compounds in barley wort are more easily absorbed by yeast, so barley wort requires only 9-12 mg FAN/L/°P to ensure good fermentation.

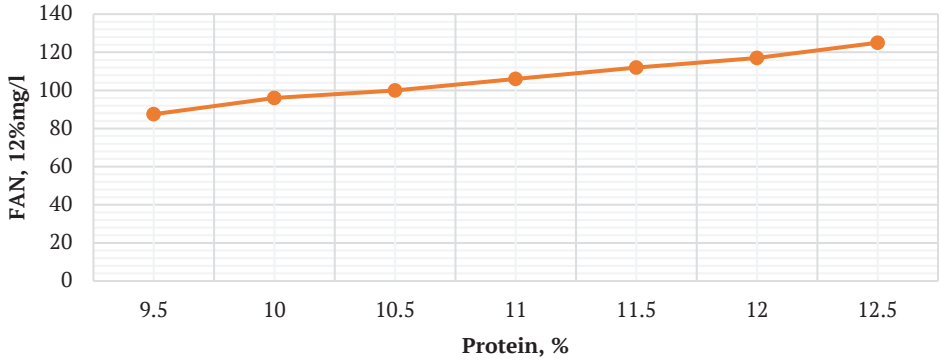

Figure 1. Dependence of FAN on grain protein content

Source: compiled by the authors

T. Pryimachuk *et al.* (2018) note that the quality of beer is largely determined by the characteristics of wort, which depend on the indicators of raw materials, which is why the beer and hop markets are interconnected and respond to economic (prices, purchasing power) and technological (change of equipment, overproduction) indicators. I. Mascia *et al.* (2019) point out the need to use appropriate mashing modes and the ratio of the main components to ensure good physico-chemical and organoleptic characteristics of the finished beer. It is impossible to brew high-quality beer without the appropriate equipment for grinding grain products, mashing them, and filtering them. According to (The brewing equipment..., 2022), due to well-chosen equipment, it is possible to control the degree of grinding, which is used to grind the grains for brewing different types of beer. Therefore, it is important to investigate the effective mashing mode, which was carried out based on composition of raw materials, their ratios and parameters given in Table 3.

Table 3. *Mash composition and ratio*

No. of mode	Dry matter content of initial wort (%)	Ratio			Raw material (%)		
		Decoction	Main part	Total part	Light malt	Barley	Rice grits
1	11.0±0.01	1:4.8	1:3.3	1:3.6	100.0	-	-
2	11.0±0.01	1:5.3	1:3.7	1:4.1	83.0	-	12.0
3	11.0±0.02	1:4.2	1:4.0	1:4.1	81.0	14.0	-

Table 3. Continued

No. of mode	Dry matter content of initial wort (%)	Ratio			Raw material (%)		
		Decoction	Main part	Total part	Light malt	Barley	Rice grits
4	11.6±0.02	1:4.5	1:3.1	1:3.6	83.0	-	-

Source: compiled by the authors

The quality of wort and beer obtained from 100% barley malt (mashing mode 1.1, control) was studied, replacing 12% malt with rice malt (mashing modes 2.1, 2.2, 2.3), 14% malt with barley malt (mode 3.1, 3.2, 3.3). Ceremix Plus enzyme preparation was added to the wort at the rate of 100 g per tonne. This particular drug was chosen because, as can be seen from Table 2, the activity of enzymes has broad actions in the temperature range. Physical and chemical parameters of the obtained wort, which is brewed according to the classical scheme and using unmalted raw materials, are given in Table 4.

Table 4. *Physical and chemical parameters of wort*

No. of mode	Viscosity (mm^2/cwt)	Content in wort				
		Amine nitrogen (mg/dm^3)	Coagulating nitrogen ($mg/100\ cm^3$)	Polyphenols (mg/dm^3)	Reducing sugar (g/dm^3)	Ratio of polyphenols:amine nitrogen
1.1 Control	1.33±0.015	150.0±2.3	1.55±0.05	202.1±2.5	58.5±0.11	1.35
2.1 Rice	1.50±0.020	147.7±1.9	1.58±0.04	190.3±3.1	62.2±0.15	1.29
2.2 Rice	1.46±0.015	137.2±1.5	1.43±0.04	204.4±3.2	63.1±0.10	1.49
2.3 Rice	1.42±0.015	145.2±1.4	1.58±0.04	204.8±1.8	62.9±0.13	1.41
3.1 Barley	1.54±0.02	145.9±2.0	1.54±0.05	213.6± 3.7	59.4±0.10	1.46
3.2 Barley	1.49±0.015	140.7±1.3	1.57±0.05	223.2±3.4	61.6±0.15	1.59
3.3 Barley	1.47±0.015	143.7±1.6	1.45±0.04	221.3±3.3	69.6±0.12	1.54

Source: compiled by the authors
Note: figures are given for 10% wort

Table 4 shows that partial replacement of malt with rice and unmalted barley gives good results regarding the final content of amine nitrogen and coagulating nitrogen, increases the amount of polyphenols and reducing sugars by 9.4 and 18.97%, respectively, especially in samples 3.2 and 3.3. This will contribute to the development of protein-polyphenolic complexes during boiling of the wort, high-quality clarification of the hopped wort, and the richness of taste of the finished beer. Filtration of wort using "classic" technology is the longest technological stage of brewing wort. In modern conditions, it is possible to reduce its duration due to the use of modern equipment and the use of enzyme preparations, the action of which is aimed at hydrolysis of biopolymers, mainly β-glucan. The use of the Ultraflo Max enzyme preparation helped to reduce the viscosity and content of β-glucans in the wort at a dosage of 0.01%, which corresponds to 100 g/t.

V.M. Gulyaev *et al.* (2020) found that the addition of Jerusalem artichoke wort to malt wort causes, as in our case, an increase in the degree of fermentation and the mass fraction of alcohol in young beer. The researchers also observed an increase in the colour of young beer compared to the control sample. But replacing

10% barley malt with Jerusalem artichoke can only be recommended for making dark beers, as opposed to light beers obtained in this study. In brewing technology, classic beer malt wort can be diluted with Jerusalem artichoke wort. The yeast works equally well in the wort specified by the composition. The addition of Jerusalem artichoke wort improves the fermentation process and promotes the accumulation of yeast biomass. The researchers noted that partial replacement of malt wort with Jerusalem artichoke tuber wort will be economically feasible, since it will lead to a reduction in the cost of beer and brewer's yeast production. Jerusalem artichoke raw materials are affordable and cheaper than malt. The product obtained as a result of fermentation – dark beer has satisfactory organoleptic properties, sweet taste, and a light sunflower aftertaste.

Figures 2-4 present the results obtained in determining the viscosity of wort and the content of β-glucan and arabinoxylan, from which the following conclusions can be drawn. The total filtration time using enzyme preparations is reduced due to the lower viscosity of the wort (Fig. 2).

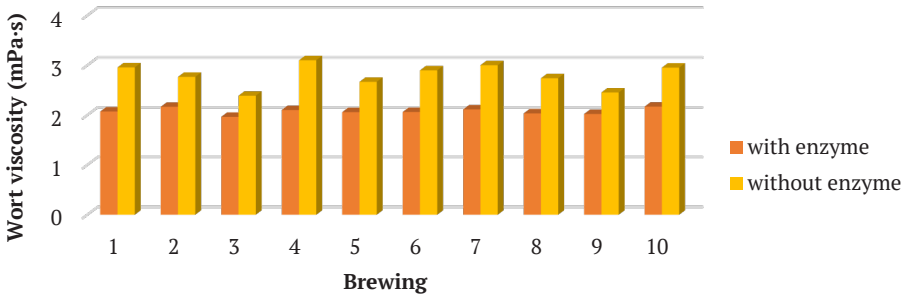

Figure 2. Change in wort viscosity depending on the number of brews

Source: compiled by the authors

As can be seen from Fig. 2, the use of the Ultraflo Max enzyme preparation resulted in a decrease in the wort viscosity by an average of 0.8 mPa·s, which reduces the filtration process by up to 20%. The viscosity of the wort depends on the presence of enzymes, so the amount of β-glucan residues (Fig. 2) and arabinoxylan (Fig. 3) for the variant using an enzyme preparation, it is significantly lower than in the case of traditional wort brewing, in which only barley malt was used without adding an enzyme.

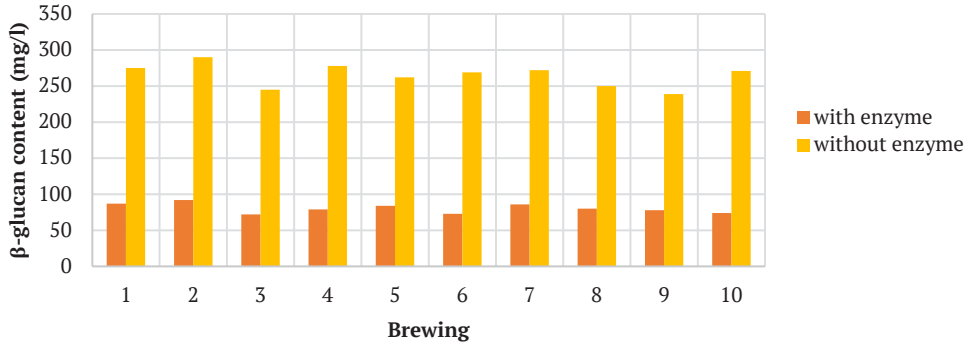

Figure 3. Change in β-glucan depending on the number of brews

Source: compiled by the authors

Arabinoxylane is one of the most important components of the yeast cell walls used for beer production, so its amount should be large. T.V. Bytka *et al.* (2019) found that arabinoxylan itself as a biologically active substance is a promising component for the creation of food and dietary supplements. Therefore, its increased amount in beer will contribute to positive physiological effects for humans. However, as can be seen from Figure 4, the arabinoxylan content in the wort is very low. Accordingly, the effect of enzyme complexes will stimulate its growth for yeast cells during fermentation.

In the brewed beer, physico-chemical parameters were determined, which are shown in Table 5.

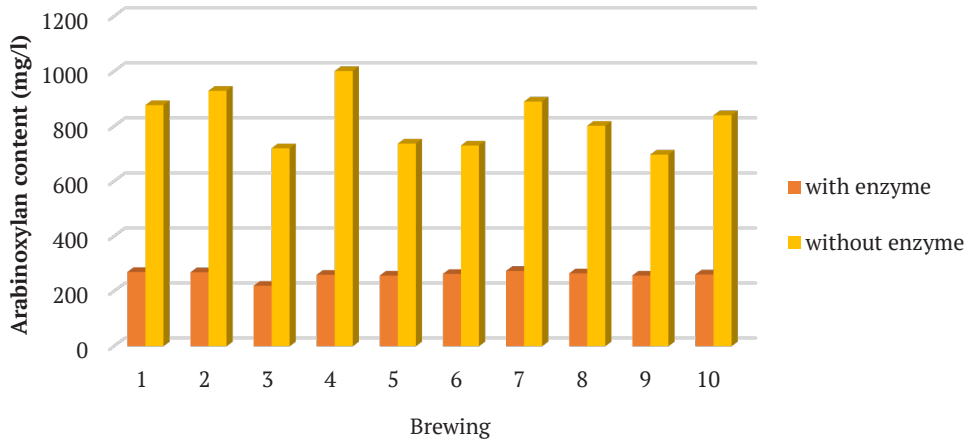

Figure 4. Change in arabinoxylan content depending on the number of brews

Source: compiled by the authors

Table 5. *Physical and chemical parameters of beer obtained according to the classical scheme and using barley and rice grits*

Mashing mode	Final degree of fermentation (%)	Actual degree of fermentation (%)	Dry wort substance (%)	Alcohol content (vol.%)	Total extract (%)
1.1 Control	87.0±0.1	67.5±0.1	11.09±0.02	5.13±0.02	3.61±0.02
2.1 Rice	85.1±0.1	66.2±0.1	11.02±0.015	4.95±0.01	3.72±0.03
2.2 Rice	85.1±0.1	67.6±0.1	11.07±0.01	5.10±0.02	3.60±0.01
2.3 Rice	89.6±0.1	68.0±0.1	11.08±0.02	5.15±0.02	3.53±0.02
3.1 Barley	84.6±0.1	66.8±0.1	11.07±0.01	4.94±0.01	3.79±0.03
3.2 Barley	86.4± 0.1	67.5± 0.1	11.10± 0.01	5.10± 0.01	3.51± 0.01
3.3 Barley	87.6±0.015	66.9±0.01	10.95± 0.01	5.00±0.01	3.63±0.015

Source: compiled by the authors

As can be seen from Table 5, there is a higher final fermentation rate in beer compared to the control sample 1.1 and almost the same result in terms of alcohol content in beer. A higher final fermentation rate in beer can be a consequence of a higher fermentation temperature, which can lead to an increase in the content of esters and higher alcohols in beer. For mode 2.3, the alcohol content is even more important. This follows from Table 4 for this mode, since it has a high content of amine nitrogen. This indicates the presence of protein

compounds, which are important components of beer. Amine nitrogen is one of the key indicators of beer quality, as it affects its taste, aroma, and foam stability. Consequently, the complex of enzyme preparations hydrolyses the nutrients of raw materials to values that, in the process of fermentation of the finished wort, give indicators at the level or higher than the indicators of the control sample (Table 5).

K. Habschied *et al*. (2022) evaluated the possibility of using Lutra® Kveik yeast for the production of pseudo-lager. The beer produced showed good physical and chemical quality indicators, comparable to ordinary light beer made using unmalted grain and enzyme preparations. Faster fermentation and no diacetyl residue, no maturation, and no settling time – all in favour of this strong yeast. In terms of sensory characteristics, this yeast can offer a good, balanced, and refreshing light beer, similar to a regular lager. Beer brewed with Ondea Pro enzyme preparations will be successfully produced with 100% barley inclusion on an industrial scale. In the finished product, physico-chemical parameters were determined, which are represented by density, alcohol content, colour, pH value, carbon dioxide content, foam stability, and the actual degree of fermentation in Table 6. The typical composition of Pilsner beer was achieved in terms of its basic physical and chemical parameters.

Table 6. *Physical and chemical parameters of Pilsner beer using Ondea Pro enzyme preparations*

Density (g/cm^3)	Alcohol content (vol.%)	Colour, units	pH value	Content CO_2 (%)	Foam stability	RDF
10.47±0.03	4.9±0.02	5.9±0.05	4.48±0.15	0.5±0.01	258±5	69.8±2.2

Source: compiled by the authors
Note: RDF – real degree of fermentation

When using enzyme preparations, the physical and chemical parameters of Pilsner beer are important for ensuring the quality of the drink. As can be seen in the Table 6 indicators correspond to light beer of Pilsner type: the content of ethyl alcohol in beer usually ranges from 3.4 to 4.5%. In Table 6, the value corresponds to 4.9 vol.%, which is obtained through the use of enzymes (the dissolution processes took place deeper, a larger extract was obtained, more nitrogenous components for yeast nutrition). The beer density is 10.47±0.03 g/cm^3, which corresponds to a light beer such as Pilsner. Mass fraction of CO_2 according to DSTU for beer should be at least 0.3%. According to the data obtained, this value is within 0.5±0.01%. Beer contains dextrins (75-85% of the total amount of carbohydrates), simple sugars (glucose, fructose, sucrose) and complex sugars (polysaccharides, etc.). The actual degree of fermentation for light beer is about 68-75%, that is, it is included in the limits for beer, as well as the values of colour, pH value, and foam stability. The values of the physical and chemical parameters of beer, which are shown in Table 6, is within the normal range.

Thus, Table 6 shows that the physical and chemical parameters of beer obtained using unmalted barley and enzyme preparations correspond to the indicators of the typical composition of Pilsner beer. The conducted organoleptic assessment of beer indicates its excellent quality, which can be seen from Tables 7-8. The developed beer was a transparent straw-yellow drink based on such organoleptic parameters as aroma, taste, smell, and bitterness. The taste of the beer was pronounced, characteristic of raw materials, with a slight aftertaste that did not change the overall flavour. A light original smell was also noted. Despite the added same amount of hops, the bitterness for beer using 100% barley and enzyme preparations was at the highest level, which affected the total score.

Table 7. *Organoleptic parameters according to GAS*

Values	100% barley		100% malt	
	fresh	used	fresh	used
Flavour	4.3	3.8	4.2	3.6
Taste	3.9	3.7	4.0	3.8
Smell	4.4	4.3	4.3	4.3
Bitterness	4.6	4.0	4.0	3.9
GAS score	4.3	4.0	4.1	3.9

Note: GAS – German Agricultural Society. Taste score: 5 – very good, 1 – unacceptable
Source: compiled by the authors

As noted by R. Yerolla *et al.* (2022), the temperature of the wort during fermentation affects the content of acids, higher alcohols, and esters of beer. The researchers developed a taste model based on the available literature and built a growth model in MATLAB and compared it with industry data to establish important kinetics of taste development. The beer taste model used by US confirms the obtained data on organoleptic parameters of the developed beer using unmalted raw materials and enzyme preparations according to the methodology of the German Agricultural Society. As can be seen from Table 7 according to such indicators as aroma, smell, and bitterness, beer produced from unmalted barley using enzyme preparations has higher indicators than beer produced using classical technology using only barley malt.

Table 8. *Organoleptic parameters with an emphasis on ageing*

Values	100% barley		100% malt	
	fresh	used	fresh	used
Flavour	1.0	1.4	1.0	1.8
Taste	1.0	1.6	1.0	1.8
Bitterness	1.0	1.6	1.0	1.7
Score	1.0	1.5	1.0	1.7
Acceptance (%)	**100**	**77**	**100**	**70**

Note: Ageing conditions: 24 hours of shaking (100 g) at 20°C and 4 days of storage at 40°C: 1 – fresh, 4 – very aged
Source: compiled by the authors

The obtained data on organoleptic parameters of aged beer brewed in two ways also confirmed the high quality of beer in all indicators of aroma, taste, and bitterness, which can be seen in Table 8. Laboratory wort and subsequently prepared beer were examined in terms of transparency and colour (5.13-0.17 units), acidity (0.93-1.0 units), the final degree of fermentation (79.2-80.3%), viscosity (1.32-1.40 mPa·s), and the content of β-glucans (12-140 mg/l) correspond to the indicators of Pilsner beer. The results obtained confirm compliance in all experiments with the current requirements of the standard.

Brewing with Ondea Pro enzyme preparation requires specific ideas and know-how from the brewery to achieve the desired taste of a particular type of beer or brand, similar to making malt-based beer. Therefore, it is recommended to dose Ondea Pro at the beginning of mashing (when approximately 30% of the grain is crushed) at a dose of 1.5-2.5 kg per tonne of raw barley, depending on the mashing mode and recipe. Beer, as a liquid with a dynamic viscosity coefficient of 1.2 mPa·s, is ideal for automatic dosing equipment. Optimisation of saccharification time and dosage is possible to achieve the desired wort profile.

Therefore, the conducted comparative studies of the indicators of barley malt and unmalted grain on the example of barley and rice grits confirmed the possibility of replacing malt with unmalted raw materials. Well-chosen dispersion of unmalted raw materials, which allows obtaining wort with optimal nitrogen content. The optimal effect of enzyme preparations of the Danish company Novozymes was investigated and confirmed. It was found that the use of Ultraflo Max enzyme preparation reduces the duration of the wort filtration process by 20% due to its lower viscosity. The use of enzyme preparations can significantly increase the content of β-glucan and arabinoxylan, which will lead to the growth of yeast cells during fermentation. Studies have shown that the replacement of malt with unmalted grain and the use of enzyme preparations has a higher final fermentation rate and a similar result in relation to the alcohol content in beer. In terms of physico-chemical and organoleptic parameters, the resulting beer corresponds to the indicators of the typical composition of Pilsner beer.

Conclusions

It was found that the selected barley and rice grits as unmalted raw materials for brewing do not exceed 11% in terms of protein content, which allows obtaining wort with an optimal nitrogen composition. The optimal effect of enzyme preparations and the effect of temperature and pH on their stability were investigated and confirmed. Partial replacement of malt with rice and unmalted barley gives good results in terms of the final content of amine nitrogen and coagulating nitrogen, increases the amount of polyphenols and reducing sugars by 9.4% and 18.97%, respectively. The use of the Ultraflo Max enzyme preparation helped to reduce the viscosity by an average of 0.8 mPa·s and the content of β-glucans in the wort at a dosage of 0.01%, which corresponds to 100 g/t. It is recommended to introduce complex enzyme preparations at the beginning of mashing – Peptidhydrolase, α-amylase, endo-β-glucanase (Ceremis Plus) to ensure the necessary ratio of substances in the wort. It was found that to improve the rheological properties of the mash and reduce the viscosity of the wort, which intensifies the filtration process, it is necessary to use preparations containing endo-β-glucanase (Ultraflo Max).

In experimental samples of wort after the introduction of enzyme preparations, the following results were obtained: a higher final degree of fermentation in beer compared to the control by 0.69 and 2.99% for rice and barley grits, respectively, which may be a consequence of a higher fermentation temperature and lead to an increase in the content of esters and higher alcohols in beer. Using large amounts of unmalted grain together with external enzymes such as Ultraflo Max and Ceremis Plus allows brewers to produce wort and beer of the same composition and quality as high-quality wort and beer using only malt. Studies have established that replacing part of barley malt with such unmalted materials as barley and rice grits using exoenzyme preparations Ultraflo Max and Ceremis Plus leads to high-quality beer, which is confirmed by organoleptic indicators of the aroma, taste, and bitterness of beer.

Promising areas for further research are the use of other unmalted materials, such as buckwheat and oats. Therefore, this work will be continued with studies replacing barley malt with buckwheat and oat malt with the addition of enzyme preparations and comparing the results.

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None.

Conflict of Interest

None.

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Використання несоложеної сировини з ферментними препаратами в пивоварінні

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Анотація. Застосування несоложеної сировини є одним із методів для підвищення якості, зниження собівартості і створення нових сортів пива. Але при цьому ферментативна активність такої сировини дорівнює нулю, тому актуальним є використання ферментних препаратів. Метою дослідження було порівняння показників якості 100 % солодового пива та пива з заміною солоду несоложеним ячменем із застосуванням ферментів. В роботі використовувалися методи техніко-хімічного аналізу для визначення органолептичних та фізико-хімічних показників пива. Розроблено рекомендації щодо інтенсифікації приготування сусла з використанням різних видів зернової сировини – несоложеної ячмінної та рисової крупки з додаванням на різних етапах пивоваріння ферментних препаратів фірми Novozymes (Данія). При випробуванні нових ферментних препаратів (бета-глюканазний комплекс, ксиланаза) після контрольних зразків зменшилася тривалість фільтрації приблизно на 20 %, в'язкість сусла, вміст бета-глюканів, арабіноксиланів. Завдяки застосуванню нового ферментного препарату відбулося краще розщеплення речовин, що вплинуло на якість і тривалість фільтрації. Це призводить до поліпшення якості сусла, скорочення тривалості процесу і зменшення у витратах сировини. Для забезпечення необхідного співвідношення речовин у суслі на початку затирання необхідно вводити ферментні препарати комплексної

дії – пептидгідролазу, α-амілазу, ендо-бета-глюканазу (Ceremis Plus). Препарати, що містять ендо-бета-глюканазу (Ultraflo Max), необхідно використовувати на початку затирання для поліпшення реологічних властивостей сусла і зниження в›язкості сусла, що покращує здійснення процесу фільтрації. Для отримання пива з необхідним ступенем бродіння при затиранні краще застосовувати нові ферментні препарати (глюкоамілазу). Практична цінність роботи полягає у використанні великої кількості несоложеного зерна разом із зовнішніми ферментами, такими як Ultraflo Max і Ceremis Plus, що дозволить пивоварам виробляти сусло та пиво такого ж складу та якості, що й сусло та пиво преміум-класу, використовуючи лише солод

Ключові слова: ячмінь; пиво; сусло; солод; якість

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Quality characteristics of fish sausages made from African catfish (*Clarias gariepinus*)

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Abstract. In the context of the problem of insufficient consumption of fish products, the possibility of creating high-quality sausage products based on new types of raw materials is considered. The development of high-quality fish sausages from new raw materials will expand the range of sausage products and provide the population with seafood products. The purpose of the study is to establish the feasibility of using African sharptooth catfish in the technology of fish sausage products. The study used a complex of generally accepted organoleptic, chemical, physico-chemical methods of research: moisture content – by drying the sample to a constant weight; fat content – by the Soxhlet method; protein content – by the Kjeldahl method; ash – by burning the sample. The penetration stress was determined using a penetrometer; water activity index – using a hygrometer; the hydrogen index – using a pH meter. As a result of research, the feasibility of using the African sharptooth catfish as a raw material for making fish sausages is theoretically substantiated and experimentally confirmed (*Clarias gariepinus Burchell, 1822*). The effectiveness of combining catfish meat with other hydrobionts and animal raw materials is shown. New recipes for raw smoked sausages based on African catfish with the addition of mackerel, scallop, and lard have been developed. Organoleptic and physico-chemical indicators of quality and nutritional value of finished products were studied. The results of organoleptic evaluation showed the similarity of catfish sausages to conventional meat sausages. Fish flavour and aroma were poorly expressed in sample 2, which included only African catfish meat from fish raw materials, which determined its consumer preferences. Sample 3 obtained by combining African catfish meat with mackerel and scallop received high organoleptic scores. The results of a study of the chemical composition show a high nutritional value of sausage products based on the improved recipe. The moisture content in all samples did not exceed 50%, which is the standard for raw smoked sausages. The highest protein content (26.19%) was observed in sample 2, the amount of protein in other sausage samples exceeded the minimum recommended value of 19%. The fat content in all samples was in the range of 18.93-21.41%, which does not exceed the recommended standard of 25%. Based on the results of physical and chemical studies, the indicators were found to be in line with the established standards. Studies of changes in quality and safety indicators during storage determined the permissible shelf life of raw smoked fish sausages at temperatures from 0 to +5°C for no more than 15 days

Keywords: fish; sausage products; recipe; technology; organoleptic indicators; nutritional value

Introduction

Modern principles of creating high-quality sausage products are based on the choice of raw materials, substantiation of the ratio of ingredients to ensure the appropriate quality of products, high organoleptic parameters and certain technological food characteristics (Rahayu *et al*., 2022). A valuable raw material for food production is fish, due to the high nutritional benefits associated with chemical composition. Fish meat is at the heart of current dietary trends and is recommended for consumption, as it is a source of easily digestible protein, polyunsaturated fatty acids, vitamins and minerals. Fish processing enterprises in Ukraine face a limited range of products that do not meet the growth of demand and high requirements of consumers. However, with the help of classification of sausage products and the possibility of combining different components and additional raw materials, fish processing enterprises have a chance to expand their range of goods (Vieira *et al*., 2019). The advantage of sausage products is that they are made based on minced fish, which solves the

issue of rational use of raw materials, creating products accessible to all segments of the population, with specified taste properties, biological and structural characteristics. Due to the use of fish meat in the technology of sausage products as a structural component of the minced meat system, it is possible to increase its water binding capacity and juiciness of finished products.

Technologies for sausage products made from aquatic organisms are being successfully developed and implemented in many countries around the world. Despite the world experience, the production of fish sausages in Ukraine is limited. Therefore, the necessary developments are aimed at using new types of raw materials, improving technology for further introduction into production at Ukrainian enterprises.

N. Bozhko *et al*. (2021) investigated the use of freshwater fish in sausage technology. Researchers have proven the positive effect of combining duck meat (*Anas platyrhynchos*) and freshwater fish (*Hypophthalmichthys molitrix*) in semi-smoked meat sausages. An increase in protein content by 17.90-21.34% was observed in semi-smoked sausages with a different ratio of duck and fish meat, compared to the analogue. A sample containing 50% duck and 30% fish showed an ideal protein-fat ratio. It is proved that the addition of silver carp improves the elasticity of minced meat and increases the stability of the emulsion. The expediency of combining meat and fish raw materials is proved by the high organoleptic characteristics of sausage products.

The above-mentioned researchers, together with other scientists, also developed meat-containing boiled sausage with duck meat and freshwater silver carp (*Carassius gibelio*) and meat-containing semi-smoked sausages with musk duck and grass carp meat (*Hypophthalmichthys molitrix*). It is established that the use of freshwater fish with duck meat increases organoleptic, functional, and technological properties, does not have a negative impact on the physical and chemical parameters of finished sausage products, and allows obtaining products that meet the requirements of the standard. The results obtained indicate an increase in the consumer value of sausages and confirm the effectiveness of using freshwater fish raw materials with waterfowl meat in the technology of sausage products (Bozhko *et al*., 2018). Meat from various types of fish can be used to make fish sausages, but it is not recommended to use dark meat from mackerel and tuna species, as blackish-red spots appear after heat treatment. Known technologies of fish sausages made from cod, pollock, horse mackerel, rainbow trout, carp, silver carp, sea catfish (Coban *et al*., 2018). Sausages made from washed minced fish surimi are also popular (Liu *et al*., 2019).

A.M.T. Lago *et al.* (2019) developed and investigated the quality of fish sausages made from filleting waste of Nile tilapia (*Oreochromis niloticus*), which were stored frozen at a temperature of -10°C. It was found that the food and microbiological quality of tilapia sausage was maintained within the recommended standards during the established shelf life. A comparative analysis of the quality of sausage from Nile tilapia (*Oreochromis niloticus*) is covered by P.C. Alda *et al.* (2019). Researchers have established the advantages of sausage made from this type of raw fish compared to chicken sausage and pork sausage. Fish sausage had higher levels of moisture, ash, calcium, and collagen compared to other species. Tilapia sausage had a higher pH value and lower water activity. The texture of the fish sausage was better in terms of hardness, stickiness, and chewiness.

A decrease in the volume of fish and seafood production, changes in the species composition of raw materials, make it necessary to use new aquaculture facilities. Recently, African catfish (*Clarias gariepinus*) has been receiving a lot of attention as a promising object of cultivation and processing. It is characterised by high growth rates, the use of inexpensive feed, and is undemanding to keeping conditions (Olopade *et al*., 2023). African catfish

has high-quality meat rich in essential amino acids, biologically effective lipids, vitamins and minerals (Adebisi & Oshibanjo, 2019), therefore, the issue of developing sausage products from African catfish using an improved recipe and technology to expand the range and provide the population with fish products is becoming particularly relevant.

The purpose of the study was to determine the quality indicators of sausage products made from African catfish and establish the feasibility of using this type of raw material in the technology of raw smoked fish sausages.

To achieve this goal, the following tasks were defined:

- study organoleptic parameters;
- investigate chemical composition and energy value;
- determine physico-chemical parameters and establish their compliance with the standards;
- investigate changes in organoleptic and physico-chemical parameters during storage.

Literature Review

Fish sausages are products that are increasingly common on the global market. These products have high potential due to their convenience (ready to eat), lack of bones, good sensory properties, and they are often used as a model for testing new ingredients and/or additives (Rahmanifarah *et al.,* 2023). Therefore, the research by many foreign researchers is devoted to the development and improvement of the technology of sausage products made from hydrobionts, including African catfish.

To increase organoleptic parameters and nutritional value, the recipe for fish sausages includes pork fat, seafood, seaweed, vegetable components, and spices. The technology of fish sausages made from sea catfish with the addition of 30% smoked pork fat was developed, which improved the sensory properties of smell, taste, and general perception, increased fat content, ash content, energy value, and reduced moisture content and water activity (Vieira *et al*., 2019). Fresh sea catfish sausages stored at low temperatures were manufactured and evaluated. Sea catfish sausages had good nutritional quality, sensory properties, and a shelf life of 21 to 25 days when stored in the refrigerator and four months when frozen (Veloso *et al.,* 2019*)*. Smoked catfish sausages are made both by traditional smoking and using liquid smoking. The results show that both methods provide products with good physico-chemical, microbial, and biochemical parameters during storage (Filho *et al*., 2021). It is important to ensure the appropriate texture and consistency of the finished product (Chattopadhyay *et al*., 2019). For this purpose, various types of raw materials and variations of components are used, protein-fat emulsions and hydrocolloids are introduced.

In modern technologies of sausage production, including those made from hydrobionts, considerable attention is paid to the quality and safety of finished products. Researchers (Estevez *et al*., 2021) proposed a partial replacement in the NaCl formulation with KCl, which are suitable solutions for reducing Na levels while maintaining the quality of sausage products and allowing for a K-rich product, which can help protect the cardiovascular system. In order to extend the shelf life of smoked carp sausages (*Cyprinus carpio* it is suggested to use propolis extract (Coban *et al*., 2018). According to the results of studies, treatment with 1% propolis increased the shelf life of sausage by 3 weeks compared to control samples. Researchers have also proven the antioxidant and bactericidal effect of using goji berries in smoked carp sausage recipe (Fadiloglu & Coban, 2019).

Due to the high content of polyunsaturated fatty acids, fish sausages are a healthier alternative to conventional meat sausages. However, hydrobiont sausages can quickly become rancid due to oxidation, which leads to a loss of quality. To reduce the oxidative effects and preserve the quality of the lipid component, researchers suggest using natural antioxidants such as

polyphenols from spices, herbs, green tea, as well as tocopherol nanoemulsion. The existing range and innovations in the technology of fish sausage products determine the feasibility and prospects of producing high-quality products based on global trends (Nikoo *et al*., 2021).

Materials and Methods

Materials of the study included raw smoked sausages based on African catfish meat. In the production of sausages, the following raw materials were used: live African catfish (*Clarias gariepinus*), autumn catch, grown on a farm in the Hrebinki village, Kiev oblast, according to DSTU 2284:2010 (2010); frozen Alaska pollock (*Theragra chalcogramma*) and Atlantic mackerel (*Scomber scombrus*) according to DSTU 4868:2007 (2007); frozen scallop (*Mizuhopecten yessoensis*) according to DSTU GOST 30314:2009 (2009); chilled pork fat, unsalted according to DSTU 4590:2006 (2006). Table salt and spices were used as additional raw materials. The recipe composition of sausages is shown in Table 1.

Table 1. *Recipe composition of fish sausages*

Raw material name	Content of ingredients required for sausage production, %			
	Control	Experimental samples		
		No. 1	No. 2	No. 3
Alaska pollock (pieces)	30	-	-	-
Alaska pollock (minced meat)	30	-	-	-
African catfish (pieces)		30	40	30
African catfish (minced meat)		45	50	30
Atlantic mackerel (minced)	10	15	-	10
Scallop muscle (pieces)	20	-	-	20
Unsalted pork fat	10	10	10	10
Auxiliary raw materials, g/100 g of raw materials				
Boiled table salt “Extra” grade	2.0	2.0	2.0	2.0
Ground black pepper	0.1	0.1	0.1	0.1
Whole coriander	0.1	0.1	0.1	0.05
Ground nutmeg	0.1	0.1	0.1	0.05
Dried ground red pepper	0.05	0.05	-	0.05
Dried ground garlic	-	0.1	-	0.1
Ground paprika	-	-	0.1	0.1
Cumin seeds	-	0.05	0.05	0.05
Dried ground ginger	-	0.1	-	0.1

Source: developed by the authors

The sausage without African catfish meat was used as a control, the recipe of which is given in Table 1. The technology of fish sausages included preliminary preparation of raw materials (defrosting, washing, cutting into fillets), dry salting to a salt content of 3%. Part of the fish raw material was crushed in a grinder, and the other part was added to the minced meat in the form of pieces of 1.0-2.0×10-2 mm in size to obtain a fibrous structure of the finished sausage products. Unsalted pork fat was cut into 5×5 mm pieces. Food and flavouring additives were added to the minced meat mixture. Prepared natural shells (salted pork stomachs)

were filled with ready-made minced meat and loaves were formed. The resulting loaves were kept in the maturation chamber at a temperature of 0°C to 10°C and a relative humidity of not more than 75% for 12-36 hours to an average buffering value of at least 70°. Smoking was carried out by convective method at an air temperature of 45-60°C until a homogeneous state and an average humidity of no more than 55% was reached. After smoking, the resulting sausage products were dried convectively at an air temperature of 19°C-25°C and a relative humidity of 75-85% to an average moisture content of no more than 50%.

Determination of organoleptic parameters was carried out by the profile method using a 5-point scale (Tkachenko *et al.*, 2020). The chemical composition of the sausages was studied using the following methods: mass fraction of moisture – by drying the product sample to a constant weight in a SNOL drying oven (Labimpeks LTD, Ukraine) at a temperature of 100-105°C, according to DSTU 8029:2015 (2015); mass fraction of ash – by weighting, after mineralisation of the product sample in a SNOL muffle furnace (Labimpeks LTD, Ukraine) at a temperature of 500-600°C, according to DSTU 8718:2017 (2017); mass fraction of lipids – by the Soxhlet extraction-weight method, according to DSTU 8717:2017 (2017) on the SOX 406 Fat Analyser (Hanon Instruments, China); mass fraction of protein – by the determination of total nitrogen by the Kjeldahl method, which is based on the ability of the organic matter of the product sample to be oxidised by concentrated sulphuric acid in the presence of a catalyst, according to DSTU 8030:2015 (2015), the ashing of the samples was carried out on a DK6 digester (Velp Scientifica, Italy) with a JP vacuum pump, distillation was carried out on a UDK 129 steam distillation apparatus (Velp Scientifica, Italy). The penetration stress was determined using an Ulab 3-31 M penetrometer, using a needle indenter at a sample temperature of (20±0.5)°C, converting the indenter penetration value to stress, in Pa. The active acidity (pH) was measured using a portable pH meter. The water activity index was determined using a highly sensitive Hygro Palm HP23-AW device (Great Britain). The acid value of lipids was determined by titration (neutralisation) of free fatty acids with alkali in the presence of an indicator, and the peroxide value of lipids was determined by titration with a solution of sodium thiosulphate. This process was based on the interaction of fat oxidation products with potassium iodide in a solution of acetic acid and chloroform, followed by the determination of the amount of iodine. The mass fraction of volatile basic nitrogen (VBN) was studied by the titrometric method, according to which free and bound volatile bases were distilled with steam, the resulting ammonia interacted with sulphuric acid, and the excess sulphuric acid was titrated with alkali (Slobodyanyuk *et al.*, 2018).

Results and Discussion

Organoleptic characteristics of food are an important criterion for assessing consumer perception of finished products. They depend on the type of raw material and manufacturing technology. As a result of organoleptic evaluation, it was found that the addition of minced meat of fatty varieties, namely mackerel, lard pieces, and scallop, to minced meat from low-fat fish increases the taste properties and appearance, and creates an elastic consistency and integral structure of the sausage. Taste and aroma indicators are improved by spices and seasonings, the addition of cumin, paprika, ginger, and garlic the sausages a spicy and original taste. The colour of the experimental samples had a pinkish tint caused by the natural colour of the catfish, which was their advantage over the control. Due to the natural colour of African catfish, there is no need to use natural or artificial colourings in sausages, which makes the product healthier. A special feature of the prototypes was the weakly expressed taste and aroma of fish, which is due

to the practical absence of taste and aroma characteristics in African catfish. For the purpose of better visual perception of the results of organoleptic evaluation of samples, in comparison with the control, a quality polygon was constructed (Fig. 1).

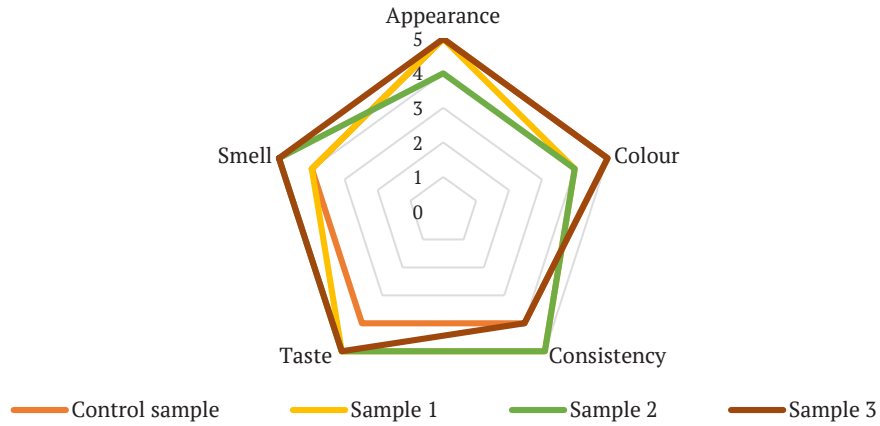

Figure 1. Organoleptic evaluation of fish sausage samples

Source: developed by the authors

According to the results of the organoleptic evaluation, sample 1 received maximum points for the appearance and taste of the product. Fish taste and aroma were poorly expressed in sample 2, which included only African catfish meat from fish raw materials, which determined its consumer preferences. The maximum score was also estimated for the consistency of sample 2, which is due to the high functional and technological properties of African catfish meat. Sample 3, made by combining African catfish meat with mackerel and scallop, received high organoleptic scores for taste and aroma characteristics, appearance, and colour. O.F. Adebisi & O.D. Oshibanjo (2019) also proved that smoked African catfish sausages are characterised by high sensory characteristics. R.R. Veloso *et al*. (2019) showed the colour preferences of African catfish sausages compared to small carp sausages and minced meat sausages from Nile tilapia fillet waste.

Nutritional value is one of the main indicators of food quality, which is determined by their chemical composition. The chemical composition and energy value of fish sausages are shown in Table 2.

Table 2. *Chemical composition of fish sausages*

Sample name	Content, %				Energy value, kcal/100 g
	moisture	fat	protein	ash	
Control sample	49.92±0.7	21.41±0.3	23.72±0.2	4.95±0.1	287.57
Sample 1	49.83±0.6	21.33±0.2	24.11±0.2	4.73±0.1	288.41
Sample 2	49.95±0.4	18.93±0.5	26.19±0.1	4.93±0.2	275.13
Sample 3	49.84±0.5	20.04±0.2	25.24±0.1	4.88±0.1	281.32

Source: developed by the authors

Analysis of the results shows that the chemical composition of the experimental formulations does not significantly differ from the control sample. The moisture content in the experimental samples and control did not exceed 50%. In terms of protein content, all study samples exceeded the control. The highest protein content (26.19%) was observed in sample 2. Sufficient protein intake is crucial for health and development. Due to its amino acid composition and good digestibility, hydrobiont proteins are valuable and necessary for the human body. The developed sausage samples will complement the human diet with high-quality easily digestible protein. The fat content in all samples was in the range of 18.93-21.41%, which does not exceed the recommended norm. High protein and lipid content was found due to the inverse relationship between humidity and lipid content, as well as moisture and protein. As a result of the smoking process, the moisture content of sausages decreased, thereby increasing the fat and protein content. The addition of seasonings, mainly salt, resulted in the corresponding ash content in sausages from 4.73% to 4.95%.

The obtained chemical composition values meet the requirements for commercial raw smoked sausages and are consistent with the results of previous studies by other researchers. According to global recommendations, the minimum protein content in smoked fish sausages is 19%, the recommended fat rate is no more than 25%. The amount of ash is not regulated by Ukrainian and international standards for fish sausages, so it is not possible to compare its content with any established regulations. However, ash is made up of minerals such as iron, zinc, and phosphorus, which are important for achieving a balanced diet (Veloso *et al.*, 2019; Ernawati *et al.*, 2021).

E. Ernawati *et al.* (2021) highlighted the chemical composition of smoked African catfish sausages made using smoking liquid. It was found that depending on the concentration of liquid smoke and the duration of immersion, the protein content ranged from 42.96% to 43.88%, the fat content from 11.34% to 12.89%, and the average moisture content was 20%. It is confirmed that the chemical composition of smoked sausages does not depend on the concentration of liquid smoke and the duration of immersion, and the protein and fat content changes due to heat treatment and dehydration. Energy value reflects the amount of energy provided to the body by the proteins and fats found in the product. Sample 2 had the lowest energy value due to its lower lipid content. This is due to the recipe composition, namely, the absence of fatty mackerel fish in sample 2. The physical and chemical characteristics of fish sausages, which are shown in Table 3, indicate that the products meet the standards and are stable during storage.

Table 3. *Physical and chemical parameters of fish sausages*

Indicators	Control	Sample 1	Sample 2	Sample 3
pH	6.68±0.09	6.40±0.10	6.37±0.09	6.50±0.08
Water activity indicator	0.88±0.01	0.87±0.01	0.85±0.01	0.83±0.01
Penetration stress, Pa	1.910±0.19	1.670±0.16	1.860±0.17	2.020±0.19

Source: developed by the authors

The results of the pH test are in the range of 6.37-6.68, which is below the established value of 6.8 and indicates that the products are of good quality. The obtained pH values will be consistent with the results obtained in the study of fish sausage products from other raw materials highlighted in the paper by R.R. Veloso *et al.* (2019). Thus, the pH of

chilled catfish sausages during storage for four days was 6.40. In Japanese bream sausages, the initial pH value was 6.75.

Water activity (a_w) indicates the microbiological stability and shelf life of food products. This indicator interacts well with the rate of various destructive processes and can be used to assess the quality of water in food and its suitability for chemical and biological changes. The values of the water activity index for fish sausages range from 0.83 to 0.88, which is less than the optimal level of water activity for most microorganisms (from 0.90 to 0.98). This indicates that sausages are resistant to the development of many types of bacteria, but can promote the growth of mould and yeast.

The obtained values are consistent with the water activity indicators of African catfish fish sausages obtained using industrial liquid smoke and improved technology by treating with a liquid concentration of 20% for 30 minutes (Ergawati *et al*., 2021). Other researchers (Fasuan *et al*., 2022) also noted the influence of the water activity index on the microbiological stability of smoked products. Thus, in the samples of smoked catfish obtained in the conventional and improved way contained traces of *Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus and Penicillium*. This is due to the water activity values of these samples of 0.85 and 0.81, which is higher than the Codex standard for smoked fish, which is 0.75.

The values of the stress penetration index indicate the texture density of sausage products due to dehydration of the semi-finished product during smoking. Sample 3 and control have higher penetration stress values, which is explained by the presence of scallop muscle pieces in their composition, which have a dense consistency even after heat treatment. The lower indenter penetration limit of sample 2 compared to sample 1 indicates a denser consistency of African catfish sausage without the addition of mackerel meat. The results of rheological studies confirm the organoleptic evaluation of consistency. Preservation of quality and safety of sausage products during storage is determined by the chemical composition of raw materials, quantitative and qualitative composition of microflora, pH level, humidity and surface condition of the product, type of packaging, presence of bactericidal agents, and environmental conditions. In the technology of raw smoked fish sausages, the preservative effect is due to salting, dehydration, and exposure to smoke compounds that exhibit bactericidal properties (Filho *et al*., 2021). Dynamics of changes in organoleptic parameters of manufactured sausage samples during storage at a temperature of 5-6°C for 15 days is shown in Figure 2.

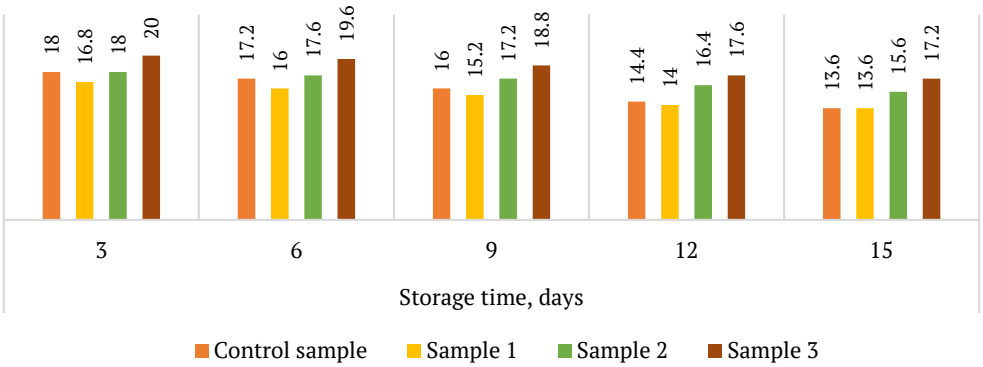

Figure 2. Organoleptic evaluation of fish sausages during storage

Source: developed by the authors

During storage, the highest scores of organoleptic parameters were observed in experimental samples that were stored for 3-6 days. The overall rating of sausage products after the 6th day decreased with an increase in the shelf life. The decrease in sensory parameters during storage is conditioned by the processes of proteolysis and denaturation changes in proteins, hydrolysis and oxidation of lipids, and the development of microorganisms. The discolouration of fish sausages during storage may be due to lipid oxidation and changes in blood pigments and heme and sarcoplasmic proteins (Sadeghinejad *et al.*, 2019).

The developed samples of sausage products contain an increased content of fish and animal fats. Therefore, an important indicator is the evaluation of the quality of the lipid component of products during storage. The course of hydrolysis and oxidation of fats in fish sausages was evaluated by acid and peroxide values. The acid value of fat characterises the presence of primary products of fat hydrolysis. The dynamics of free fatty acid accumulation is shown in Figure 3.

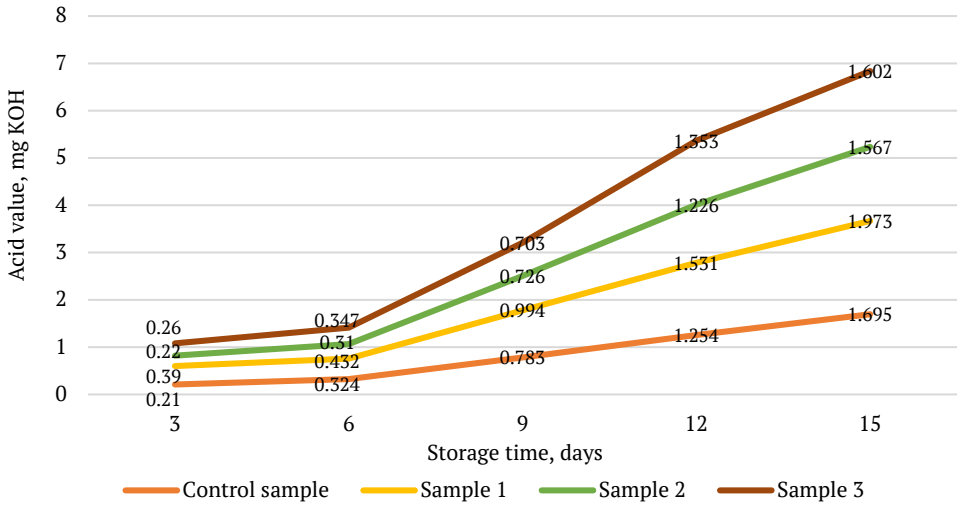

Figure 3. Changes in the acid value of fat during storage of fish sausages

Source: developed by the authors

During the storage of fish sausages, there is a linear trend of increasing the acid value of fat, which is due to the processes of hydrolysis of triglycerides. It was noted that from day 6, the breakdown of complex lipid compounds to simple ones occurred more intensively. The accumulation of free fatty acids during the storage of fish products is associated with enzymatic activity. During the entire shelf life of fish sausages, the acidic number of lipids in all samples did not exceed the established values for fish fats for food production – 4 mg KOH/1 g of fat.

K. Rahmanifarah *et al.* (2023) noted a similar trend towards an increase in the acid value levels during the storage of sausages from washed minced silver carp in a frozen state. Along with the lipidhydrolysis, oxidation processes also occur. Lipid oxidation negatively affects not only the sensory, but also the functional characteristics of products. Various primary and secondary byproducts are formed during the process, depending on the types of fatty acids, the presence of oxygen, and the presence of pro- and antioxidants. Some of the lipid oxidation products only affect product quality, while others affect human health. Therefore, the study of lipid oxidation processes in fish sausages is important for assessing

their quality, as well as for human health. As a result of fat oxidation, peroxide compounds, aldehydes, and ketones are formed. The presence of lipid oxidation processes in the initial stages characterises the peroxide value. This constant indicates the content of peroxide compounds in fat, and allows detecting the presence of spoilage products much earlier than it can be determined organoleptically. According to R.R. Veloso *et al.* (2019), lipid oxidation in fish sausages may be related to the amount of lipids, the type of fatty acids, the degree of fillet grinding, and the presence of oxygen. Thus, the inclusion of minced mackerel, lard, and shredded catfish fillets may have contributed to the lipid oxidation of sausages in this study. The dynamics of accumulation of peroxides and hydroperoxides during storage of fish sausages is shown in Figure 4.

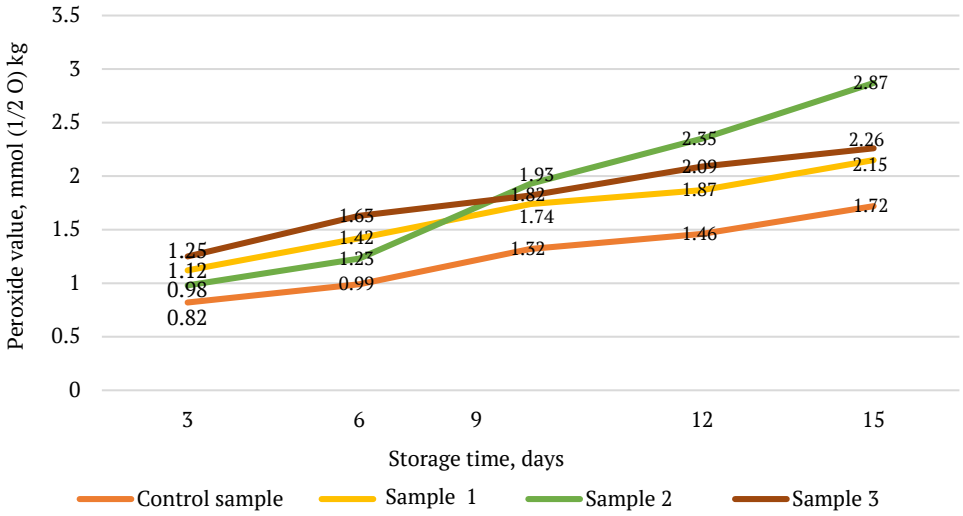

Figure 4. Changes in the peroxide value of fat during storage of fish sausages

Source: developed by the authors

Lipid oxidation processes were more intense in sample 1, which included the maximum amount of mackerel meat, compared to other fish sausage samples. This is due to the high lipid lability of aquatic organisms. The obtained peroxide values of fat in sausages on the 15th day of storage are significantly lower than the established norm – 10 mmol (1/2 O)/kg of fat and below the threshold value for detecting rancid taste, which indicates the good quality of the lipid component. Fish oil, especially marine and oceanic fish, is a source of omega-3 fatty acids, especially eicosapentaenoic and docosahexaenoic, which are polyunsaturated and very susceptible to oxidative changes (Nikoo *et al.*, 2021). A significant amount of saturated fatty acids present in the fat added to all fish sausage samples contributed to the inhibition of lipid oxidation processes, which is consistent with the conclusions of S. You *et al.* (2022). The low content of fat oxidation products in smoked sausages is also explained by the presence of antioxidants in the smoke, in particular phenols, which can inhibit the oxidation process (Abeyrathne *et al.*, 2021; Ernawati *et al.*, 2021)

The degree of proteolysis and microbiological processes was studied by changes in the volatile basic nitrogen content (VBN). The nitrogen content of volatile bases increases during storage under the influence of enzymatic processes and the vital activity of microorganisms

and is accompanied by the breakdown of amino acids to form ammonia, mono-, di-, and trimethylamines. The dynamics of VBN content during storage is shown in Figure 5.

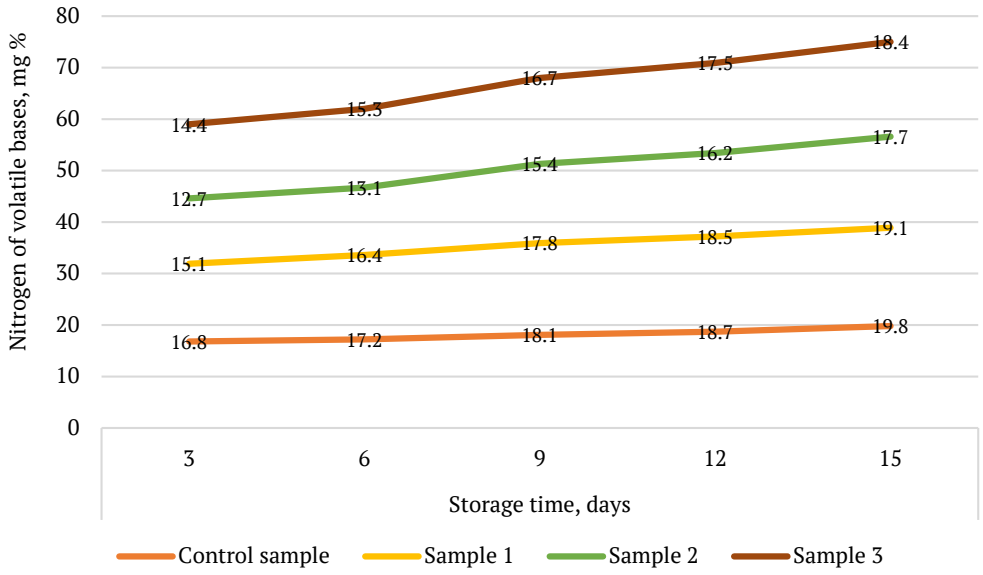

Figure 5. Changes in volatile basic nitrogen during storage of fish sausages

Source: developed by the authors

The study results show that fresh fish sausages had the lowest VBN values, which increased during storage. At the maximum storage period of the product, after 15 days, the content of volatile basic nitrogen in the control sample is 19.8 mg%, in sample 1 – 19.1 mg%, in sample 2 – 17.7 mg%, in sample 3 – 18.4 mg%, with an acceptable level of 30 mg%. The highest content of volatile basic nitrogen in the control is explained by the presence of trimethylamine, which is formed as a result of the breakdown of trimethylamine oxide, which is found mainly in marine aquatic organisms.

An increase in VBN values in fish products, according to S. You *et al*. (2022), is associated with bacterial metabolism and oxidation of protein and non-protein components. In other studies of fish sausages, an increase in VBN values was also observed during storage. In particular, in chilled catfish sausages, the VBN index increased from 14.9 mg% after four days of storage to 43.1 mg% at the end of 32 days of storage. There was also a significant increase in VBN values from 12.2 mg% to 16.5 mg% during the four months of frozen sausage storage (Veloso *et al.*, 2018). Based on organoleptic evaluation and physico-chemical indicators, the permissible shelf life for raw smoked fish sausages at temperatures from 0 to +5°C was set for no more than 15 days.

Conclusions

Based on the analysis of experimental studies, it was found that fish sausages based on African catfish are characterised by high sensory characteristics, nutritional value, and meet the requirements for raw smoked sausages. Organoleptic parameters indicate the taste and aroma advantages of African catfish sausages in comparison with sausages made from other fish raw materials. This is confirmed by the maximum scores for the taste, aroma, and

consistency characteristics of sample 2, which included only African catfish meat from raw fish. Sample 3, made by combining African catfish meat with mackerel and scallop, received high organoleptic scores for taste and aroma characteristics, appearance and colour.

The results of chemical composition studies indicate a high nutritional and energy value of sausages from African catfish. The moisture, protein and fat content in all samples comply with international regulations for raw smoked sausages. The moisture content in the experimental samples and control did not exceed 50%. In terms of protein content, all the test samples exceeded the control, so the developed sausages can be considered a source of high-quality, easily digestible protein. The fat content in all samples was in the range of 18.93-21.41%. Physical and chemical indicators are within the established standards and indicate the good quality of the finished product. According to the obtained values of the water activity index (0.83-0.88), raw smoked fish sausages are resistant to the development of most bacteria, but mould and yeast can develop in the products. The results of the study of penetration stress indicate the texture density of sausage products due to dehydration of the semi-finished product during smoking.

During the storage of fish sausages for 15 days, a gradual deterioration of sensory characteristics and quality indicators of protein and lipid components was observed. The highest scores of organoleptic parameters were observed in experimental samples that were stored for 3-6 days. During the entire shelf life of raw smoked sausages, the acid and peroxide lipid numbers of the prototypes did not exceed the established values for fish fats for food production, which confirms the quality of the lipid component. The quality of proteins in African catfish sausages was proved by the obtained values of the nitrogen content of volatile bases, which did not exceed the permissible level during the established shelf life. Further studies will focus on the development of regulatory documents and the implementation of the technology at fish processing plants.

Acknowledgements

None.

Conflict of Interest

None.

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Якісні характеристики рибних ковбас з африканського сома (*Clarias gariepinus*)

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Анотація. У контексті проблеми недостатнього споживання рибних продуктів розглянуто можливість створення високоякісних ковбасних виробів на основі нових видів сировини. Розроблення високоякісних рибних ковбас з нових видів сировини дозволить розширити асортимент ковбасних виробів та забезпечить населення рибними продуктами. Мета роботи полягає у встановленні доцільності використання африканського кларієвого сома у технології рибних ковбасних виробів. В роботі використовували комплекс загальноприйнятих органолептичних, хімічних, фізико-хімічних методів досліджень: вміст вологи – методом висушування зразка до постійної маси; вміст жиру – методом Сокслета; вміст білка – методом К'єльдаля; золи – спалюванням наважки. Пенетраційну напругу визначали за допомогою пенетрометра; показник активності води вимірювали за допомогою гігрометру; водневий показник – pH-метром. В результаті досліджень теоретично обґрунтовано й експериментально підтверджено доцільність використання африканського кларієвого сома (*Clarias gariepinus Burchell, 1822*) в якості сировини для виготовлення рибних ковбас. Показано ефективність поєднання м'яса сома з іншими гідробіонтами та тваринною сировиною. Розроблено нові рецепти сирокопчених ковбас на основі африканського сома з додаванням скумбрії, морського гребінця та шпику. Досліджено органолептичні,

фізико-хімічні показники якості та харчової цінності готової продукції. Результати органолептичної оцінки показали подібність ковбас із сома до традиційних м'ясних ковбасних виробів. Рибний смак і аромат був слабко виражений у зразку 2, який включав з рибної сировини лише м›ясо африканського сома, що зумовило його споживчі переваги. Високі бали органолептичної оцінки одержав зразок 3, виготовлений шляхом комбінування м'яса африканського сома зі скумбрією та морським гребінцем. Результати дослідження хімічного складу показують високу харчову цінність ковбасних виробів за удосконаленою рецептурою. Вміст вологи в усіх зразках не перевищував 50 %, що відповідає нормі для сирокопчених ковбас. Найвищий вміст білку (26,19 %) відмічено у зразку 2, кількість білка в інших зразках ковбас перевищувала мінімальне рекомендоване значення 19 %. Вміст жиру в усіх зразках перебуває в діапазоні 18,93-21,41 %, що не перевищує рекомендовану норму 25 %. За результатами фізико-хімічних досліджень встановлено відповідність показників встановленим нормам. Дослідження змін показників якості та безпечності в процесі зберігання визначили допустимий термін зберігання рибних сирокопчених ковбас за температури від 0 до +5 °С не більше 15 діб

Ключові слова: риба; ковбасні вироби; рецептура; технологія; органолептичні показники; харчова цінність

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Impact of biotechnological transgenesis procedures on duck productivity

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Abstract. The use of poultry as a unique model of biological research was characterised by a high level of efficiency, however, methods for creating transgenic ducks, complicated by the structure of waterfowl eggshells, are of low efficiency. The purpose of the study was to determine the influence of various biotechnological procedures for creating transgenic ducks on their productive qualities and reproductive ability to identify the optimal method for creating transgenic poultry for further

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use in scientific, research, or economic purposes. Weighting, morphometric and statistical analysis of productive traits were used during the study. 40 ducks (4 experimental groups of animals and about 3,000 of their eggs) were studied. The lowest value of the egg productivity index was obtained in the group created by busulfan injection (79.5±11.8%), the highest – in the group created by sperm-mediated gene transfer (91.8±2.3%), the group of direct injection of transgenic construct – 89.0±2.0%, which indicates that this biotechnological method of introducing transgenic construct did not have a clear effect on this indicator. The weight of ducks in different experimental groups ranged from 1,323.50±65.36 g (using the sperm-mediated gene transfer) to 1,608.08±94.76 g (in the group created using busulfan). Ducks that received direct injections had an average weight of 1,480.42±35.01 g. In the control group, the average weight at sexual maturity was 139.5±9.67 g, in the busulfan group – 148.2±13.13 g, in the direct injection group – 143.16±7.25 g, and in the sperm-mediated gene transfer group – 140.67±13.13 g. It was found that the method of injection into the embryo of a recipient sterilised with busulfan and the introduction of donor blastodermal cells negatively affect the reproductive qualities of ducks. The practical significance of the study lies in the fact that as a result of the analysis of the productivity of ducks obtained by various methods of transgenesis, it was determined that the most effective of the evaluated methods is the transfection of DNA of the transgenic construct with sperm (Sperm-mediated gene transfer, SMGT)

Keywords: egg size; egg weight; egg productivity; injections; blastodermal chimera; busulfan

Introduction

Due to its compact size, simple rearing conditions, zootechnical manipulations (artificial insemination, automated feeding, egg collection) that do not require highly qualified personnel, reproductive potential, generation interval, and embryo development without the mother's body, the bird can be used in a wide range of applied and fundamental research (Kim *et al.,* 2023). A significant advantage of a bird that serves as an object of transgenesis is the possibility of using its short generation interval, reproduction rate, and high feed conversion rate, which significantly exceeds the capabilities of other species (Ziaei & Amini, 2020). As noted by J.S. Park *et al.* (2020), transgenesis and cloning methods have become convenient tools for creating highly productive animals, animal development models, diseases, animal bioreactors, and producers of valuable biologically active drugs. As of 2023, transgenesis has become a routine procedure that allows creating an animal producer of unique high-molecular compounds in the pharmaceutical and pharmacological industries, an animal model of evolution (and hereditary diseases (Hamernik*,* 2019*;* Kim *et al*., 2020; Dehdilani *et al*., 2022). J. Lee *et al.* (2020) suggest that the first and most effective method of obtaining transgenic animals was to make changes to the zygote genome, which is successfully used on mammals, but is not effective in similar manipulations with the bird genome, which is due to the differences in the reproductive systems of mammals and birds, the uniqueness of the development of the embryo in the egg.

The creation of transgenic poultry is carried out using three main methods: 1) sperm-mediated gene transfer (SMGT) 2) injection of a DNA vector under the embryo cavity of a newly laid egg; 3) introduction of transfected blastomeres into the embryo (Kwon *et al.,* 2018*;* Konoval *et al*., 2019; 2021). The creation of transgenic poultry by injection is complicated by the lack of transparency of its egg, the presence of a large yolk, and the uniqueness of the reproductive system of the Avis class (Ibrahim &

Stadnicka, 2023). The wide pores of the shell of waterfowl cause infection of embryos in eggs opened for manipulation and prevent the success of the formation of the duck germ chimera, reducing the effectiveness of intervention procedures in embryonic development. This, according to T.L. Saunders (2023), significantly complicates the creation of appropriate conditions for carrying out the necessary biological procedures and has an impact on the efficiency of various methods of introducing transgenic constructs. SMGT is one of the effective methods for obtaining transgenic animals from the period when it was established that sperm can be used as a vector for delivering a gene to an egg. The advantages of SMGT are related to its simplicity, efficiency, speed, and cheapness.

An urgent problem in the study of biotechnological transgenesis procedures is their impact on the productive qualities of created ducks. The consequences of chimerisation and transgenesis, and their possible impact on the productivity of chimera offspring, are still poorly understood. The purpose of the study is to determine the effect of biotechnological transgenesis procedures (busulfan injection, direct injection of a transgenic construct into the subgerminal cavity of embryos at stage X, sperm transfection) on the productive qualities and reproductive ability of ducks.

Literature Review

In order to create a transgenic animal, when all the cells of its body carry foreign genetic information, the transfer of genetic constructs is carried out in the earliest periods of embryogenesis, starting with the zygote, so injecting genetic constructs into the nucleus of an egg is the most common method for creating transgenic animals. Successful transgenesis can be considered if the introduced genetic construct is present in every cell of the created organism, or the ability of this organism to produce gametes with the target genetic construct, which will allow obtaining offspring with the desired genotype. However, the reproductive system of poultry complicates the process of transgenesis compared to mammals. The duck oocyte has a one-day maturation period. The large size of poultry oocytes makes them vulnerable to damage when used in biotechnological manipulations. Normal embryonic development involves the presence of three membranes – protein, subcutaneous, and shell. The fertilised egg begins to break up in the oviduct (protein section), which leads to the presence of 40-60 thousand blastomeres in the newly laid egg, worsening the level of effectiveness of transgenesis procedures (Kim *et al.*, 2023).

Among other methodological approaches to transgenesis that are more effective for poultry is the creation of transgenic chimeras by transplanting early embryonic cells of donors after delivery of the target gene construct to their nuclei. Delivery of plasmid DNA to embryonic cells is one of the most difficult problems of non-viral transfer of exogenous genes. Synthetic peptides or non-cationic lipids (liposomes) are used to increase the efficiency of plasmid DNA transfer, which reduces cytotoxicity and increases resistance to inactivation by intracellular mechanisms, since minimal loss of pluripotent cells can negatively affect the normal development of the body (Longmuir *et al.*, 2001). Various strategies for creating a genetically modified transgenic poultry described above are currently being proposed, including the use of viral vectors in stage X embryos, and transfer of exogenous genes by injection into zygotes or embryonic stem cells, however, these methods have been little used in poultry farming, as the transmission of the transgene to subsequent generations using these methods is not stable. Another strategy for transgenesis in poultry is the cultivation of primordial germ cells in vitro (Han & Park, 2018).

The experience of creating blastodermal chimeras is described by J.N. Petitte *et al.* (1990). In the study, blastoderm cells were isolated from chicken embryos of a line with black

feathers corresponding to the recessive allele at locus 1 and transferred to chicken embryos with white feather pigment. As a result, 6 embryos out of 53 had black feathers. That is, 11.3% of the embryos were phenotypic chimeras by feather colour. One embryo with black feathers survived to hatch and was crossed to analyse the offspring, resulting in two chicks out of 719 with black plumage. This proves the possibility of using blastodermal cells to create a germ line and transmit it in generations.

The reproductive potential of poultry can be influenced by factors such as the breed of ducks, the ratio of males and females in the group, and housing conditions (for example, temperature). Reproductive capacity and fertilisation also depend on the ability of females to ovulate, the absence of pathological conditions of the reproductive system, and the provision of an appropriate environment for egg formation and development (Yakubu, 2013). The quality and quantity of sperm deposited by males is also an important factor for obtaining a high fertility rate. In addition, a significant factor of influence is the age of ducks; it affects the reproductive characteristics of both males and females (Morduzzaman *et al.*, 2015).

Hatchability is one of the most important indicators for successful productive poultry farming. Factors that affect hatchability include: belonging of the duck to the breed or breed line, the weight of the laid egg, and the conditions of its incubation (Narahari *et al.*, 1991; Abd El-Hack & Hurtado, 2019). Incubation conditions can be called optimal if the hatchability is at the maximum level, this is ensured by regulating the microclimate indicators, such as temperature and humidity, sufficient air exchange (ventilation), and periodic changes in the slope of eggs. Before laying eggs for incubation, an ovoscopy is performed to extract unfertilised eggs. Disinfection of eggs and incubators has a positive effect on hatchability (Liptoi & Hidas, 2006).

The foetal mortality rate characterises the viability of embryos in fertilised duck eggs. Embryonic mortality is divided into early and late, depending on the incubation period at which the embryo died. The foetal mortality rate is influenced by several environmental, technological, and genetic factors. Thus, to date, there is data on the methods of creating transgenic poultry and the productive characteristics of ducks, however, the influence of the method of creating transgenic poultry on its productive characteristics has not been established.

Materials and Methods

The object of the study was the productivity of ducks (Anas platyrhynchos), which belonged to the Shan partridge (Shanma) and Shaoxing breeds, created using different biotechnological transgenesis procedures. The subject of the study was biotechnological methods for creating transgenic poultry. The study was conducted at the Department of Genetics, Breeding and Biotechnology of Animals of NUBIP of Ukraine and the Guowei research farm of the Zhejiang Academy of Agrarian Sciences. All studies were conducted in compliance with bioethical principles for vertebrate animals (Directive 2010/63/EU…2010).

In this study, 40 ducks were divided into 4 experimental groups and about 3,000 eggs obtained from the birds under study were investigated. The control group consisted of Shaoxing ducks. To establish a group of ducks created by busulfan injection, the Shaoxing breed was used as recipients and the Shanma breed as donors. The Shanma breed was used in studies of the productivity of ducks obtained by direct injection of a transgenic construct into the subgerminal cavity. Sperm transfection was performed with Shaoxing ducks. The control group consisted of 10 animals, a group of ducks created using busulfan – 5 animals, a group of individuals obtained using direct injections of transgenic constructs into the subgerminal cavity – 6 animals, a group of ducks hatched from eggs, whose mothers were artificially inseminated with sperm-mediated gene transfer

from donor ducks – 19 individuals. All the ducks under study were kept in the same conditions, using a cage type of housing with constant access to water and feed. The study was conducted for 142 days. Body weight was determined individually with an accuracy of 10 g for all ducks aged 41 to 61 weeks.

The average weight and size of eggs were determined daily. The length (L) and breadth (B) of the eggs are measured with an accuracy of 0.1 mm using a caliper.

Eggs were weighed using an electronic scale (JM-a 20001) with an accuracy of 0.1 g. The egg shape index was calculated using the equation:

$$SI = 100 \times d/D, \quad (1)$$

where d – transverse (small) diameter, mm; D – longitudinal (large) diameter, mm.

The egg productivity index was calculated using the equation:

$$EPI = D/AE \; x \; 100, \quad (2)$$

where D – number of days of the study period of the test duck, starting from the first day of egg production (laying the first egg); AE – total number of eggs obtained for the entire period of egg production from the duck under study, starting from the first day of egg production (laying the first egg).

The obtained data was statistically processed on a computer using the MS Excel 2016 spreadsheet processor using descriptive statistics and the F-criterion.

Results and Discussion

The results of the analysis of productive traits of the control group of ducks are presented in Table 1. The egg production of the control group, live weight of the birds, and the age of sexual maturity of the animals under study were consistent with the data obtained in previous studies of the Shaoxing breed (Chepiha *et al.,* 2017).

Table 1. *Duck performance in the control group*

Indicator	M±m	Cv±mCv
Egg productivity (during the week)	*6.01±0.209	29.9±0.054
Egg productivity index (142 days), %	87.5±4.53	16.4±0.090
Live weight, g	1,422.40±57.00	12.7±0.079
Age of sexual maturity, days	139.5±9.67	21.8±0.104
Egg weight, g	70.6±0.198	9.20±0.006
Egg length, cm	6.05±0.056	3.65±0.042
Egg breadth, cm	4.52±0.053	3.89±0.044
Egg shape index, %	*75.7%±0.3	0.7±0.018

Source: compiled by the authors

The data on the performance of ducks created by busulfan treatment of Shaoxing recipient embryos with Shanma donor embryos are shown in Table 2. According to Table 2, egg productivity of ducks (during the week) was within the same indicator of the Shaoxing breed. The egg productivity index was somewhat inferior to the control data. Live weight was within the limits typical of the Shaoxing breed. The age of sexual maturity was somewhat inferior to the control. By egg weight, the animals had indicators that are characterised by a larger range of variability than the control group. The egg productivity index (for 142 days) of the duck group created using busulfan was lower than that of the control group. This may indicate a negative impact of the procedure for sterilising donor embryos.

Table 2. *Productivity of a group of ducks created by busulfan injection*

Indicator	M±m	Cv±mCv
Egg productivity (during the week)	6.4±0.268	27.0±0.077
Egg productivity index (142 days), %	79.5±11.8	32.8±0.181
Live weight, g	*1,608.08±94.76	13.2±0.114
Age of sexual maturity, days	148.2±13.13	19.8±0.140
Egg weight, g	***71.4±0.157	5.07±0.071
Egg length, cm	6.26±0.134	4.93±0.070
Egg breadth, cm	4.53±0.041	2.09±0.045
Egg shape index, %	75.2%±0.3%	0.8±0.028

Note: statistical significance: *p<0.05; **p<0.01; ***p<0.001
Source: compiled by the authors

Table 3 shows the data obtained on the productivity of a group of ducks created by injecting DNA of the transgenic construct into the germinal cavity of a freshly laid egg. Egg production (during the week) in this group had no statistically significant differences from other groups. However, the egg productivity index (for 142 days) was statistically significantly different from the other groups. The eggs of this group weighed less than the group created by busulfan injections.

Table 3. *Productivity of a group of ducks created by direct injection of transgenic construct DNA into the subgerminal cavity of embryo*

Indicator	M±m	Cv±mCv
Egg productivity (during the week)	6.50±0.11	16.55±0.03
Egg productivity index (142 days), %	**89.0±2.0	9.22±0.022
Live weight, g	*1,480.42±35.01	10.03±0.023
Age of sexual maturity, days	143.16±7.25	21.47±0.049
Egg weight, g	***69.3±0.141	9.05±0.021
Egg length, cm	6.02±0.008	10.41±0.025
Egg breadth, cm	4.51±0.016	3.90±0.009
Egg shape index, %	***74.66±0.1	4.54±0.011

Note: statistical significance: *p<0.05; **p<0.01; ***p<0.001
Source: compiled by the authors

Table 4 shows the performance indicators of a group of ducks obtained by using sperm-mediated gene transfer. Egg production (during the week) in this group was the highest. The egg productivity index (for 142 days) of this group was the highest among all groups. The live weight of the group's poultry was slightly lower than that of other groups. A comparison of the data obtained shows that ducks in this group had the lowest age of onset of sexual maturity among the experimental groups. The egg weight of this group was inferior to other groups. Egg length in animals of this group was the smallest, egg breadth was also inferior to similar indicators of other groups.

Table 4. *Productivity of a group of ducks obtained by sperm-mediated gene transfer*

Indicator	M±m	Cv±mCv
Egg productivity (during the week)	*6.61±0.15	12.12±0.029
Egg productivity index (142 days), %	91.8±2.3	5.57±0.013
Live weight, g	*1,323.50±65.36	11.04±0.025
Age of sexual maturity, days	140.67±13.13	20.87±0.048
Egg weight, g	67.0±1.990	8.08±0.019
Egg length, cm	5.93±0.11	5.10±0.012
Egg breadth, cm	4.44±0.04	2.40±0.006
Egg shape index, %	*74.91±0.1	5.03±0.012

Note. Statistical significance: *p<0.05; **p<0.01; ***p<0.001
Source: obtained by the authors

Based on the data obtained by the authors of the current study and the calculations made, the influence of each of the methods for obtaining experimental animals on the performance indicators of their groups separately and on productivity in general was compared. The number of eggs laid by a female over a certain period of time is an indicator of her egg productivity, or egg production, and is the main feature, valuable for breeding selection and a decisive indicator, not only of the poultry egg, but also of the meat productivity. This is due to the fact that egg productivity determines the productive and reproductive qualities of the female, that is, ultimately, the amount of product received from one individual. This indicator is influenced by factors such as the age of ducks, breed, feeding and temperature conditions in the premises where animals are kept, etc., which is consistent with the results obtained by A.M. King'Ori (2011). In the current study, all experimental animals were kept in the same conditions. Egg production is genetically determined, as a high level of polymorphism of genes that affect poultry egg production is currently known (Huang & Lin, 2011). The influence of the genotype of animals on their egg productivity cannot be excluded, which may have caused fluctuations in the coefficients of variation obtained in this study in some groups, which actually reached 30% of the maximum level of biological variability characteristic of samples of unrelated individuals.

The data of the control group of ducks correspond to the performance indicators of their breed (Shaoxing) and the results of previous studies by the authors (Chepiha *et al*., 2019; Konoval *et al*., 2019; 2021*)*. Comparison of the results of the analysis of the control group and ducks obtained by busulfan injection indicates that there are no statistically significant differences between the groups in egg productivity (within a week). Table 3 shows data on the productivity of a group of ducks created by direct injection of a transgenic construct into the subgerminal cavity. Comparison with the control group showed differences in egg productivity index and egg weight. In terms of egg productivity during the week and egg production index, the group created by sperm-mediated gene transfer was better compared to other experimental groups. However, statistically significant differences were obtained only between the control group and the group obtained by using sperm-mediated gene transfer.

One of the most significant indicators that characterise trends in the growth and development of poultry is live weight. Live weight refers to quantitative characteristics and is determined by hereditary characteristics; balanced feeding and keeping conditions of poultry play an important role in the severity of hereditary

potential. Poultry with normal body weight have fewer problems with productive and reproductive functions than poultry with insufficient or excessive body weight for their age. Poultry growth is a complex biological process that occurs due to a combination of the interaction of genotype and environmental conditions. Normal growth is characterised by the formation of body weight, typical in size and shape for a given species, breed, line, and cross.

The results of the current study were as follows: for the control group – 1,422.40±57.00 g; for the group created with busulfan – 1,608.08±94.76 g; for the direct injection group – 1,480.42±35.01 g; for the group obtained by sperm-mediated gene transfer – 1,323.50±65.36 g. Evaluating the productivity of this indicator, it can be concluded that the negative effect of busulfan in the corresponding group is not observed in comparison with the indicators of egg productivity. In contrast, this group of ducks had a higher live weight than animals obtained by sperm-mediated gene transfer. It can be assumed that the live weight of ducks obtained by using busulfan was higher, since they had a lower egg productivity index. The animals obtained by DNA transfection of the construct and injections also statistically significantly differed in live weight. For the groups created by other methods, there was also no significant deviation of this indicator compared to the control group, which means that the biotechnological processes used do not have a significant impact on this indicator. The growth and weight gain of poultry have a complex structure and depend on genetic and environmental factors. These factors are species, sex, breed, care, and feeding, which is confirmed by R.G. Campbell *et al*. (1985). While growth is characterised by weight gain, development is characterised by changes in the functions, structure, and shape of tissues and organs in the body. The sex of birds also affects live weight. The effect of sex on poultry growth becomes more apparent with age. Although the growth of waterfowl varies by species, it is usually faster in males than in females. Providing poultry with nutrients and minerals, the cost and type of feed, bird health, well-being and environmental issues are important factors for ensuring normal growth and development of animals (Darmani Kuhi *et al.,* 2010). Another important aspect is the duck housing system, the impact of which is highlighted in the study by K. Önk *et al.* (2018)

The mean age of sexual maturity was 139.5±9.67 days for the control group, 148.2±13.13 days for the busulfan-treated group, 143.16±7.25 days for the direct injection group, and 140.67±13.13 days for the sperm-mediated gene transfer group. Again, a negative effect of busulfan on the reproductive ability of ducks can be observed, which is expressed in the later onset of sexual maturity of ducks compared to the control and other groups. Normal indicators of the age of sexual maturity in other groups indicate that there is no significant effect of transgenesis procedures on the physiological development and reproductive ability of the duck's body. Egg weight is the second most important breeding trait, which is of the greatest economic importance in the production of egg products. Weight is one of the indicators of egg quality, can indicate the suitability of eggs for incubation and has an impact on hatchability. This indicator is influenced by: duck breed, temperature conditions, and feeding (Okruszek *et al.,* 2006).

The average egg weight index is within the breed standard for all groups except the group created by sperm-mediated gene transfer, but there is no direct relationship between weight loss and the biotechnological procedure used. This indicator may be influenced by the age of ducks (Chepiha *et al.,* 2017). It is also important to keep in mind that when comparing the data obtained in this study regarding the average egg weight with the data obtained in other studies, it is necessary to consider the interbreeding difference. The difference in this

indicator between different breeds is described in the study by A. Okruszek *et al.* (2006). Also in this study, the following was given: the difference between ducks in different weeks of egg production. Egg quality indicator, which is determined by the ratio of the transverse diameter to the longitudinal one and expressed as a percentage – the egg shape index. The egg shape index score was normal in all the groups studied, regardless of the method of their creation. Despite large fluctuations in the average annual index of egg shape from individual laying hens (from 67 to 83%), the average coefficient of variability of the trait is small and varies mainly in the limits of 3.6-5.4%.

Normal indicators of the shape index for light cross ducks are 70%, heavy – 71%; for musk ducks – 72%. The egg productivity index is the optimal indicator for characterising the egg production of the ducks under study. The best result, as noted above, was obtained from the group created by sperm-mediated gene transfer (91.8±2.3%), the direct injection group of the transgenic construct also showed a fairly high performance indicator (89.0±2.0%), which indicates that this biotechnological method of introducing the transgenic construct did not have a clear effect on this indicator. The lowest value of the egg productivity index was obtained in the group created by busulfan injection (79.5±11.8%), so it can be assumed that the data obtained are related to the effect of the 4-butandiol dimethanesulfonate (busulfan), which inhibits the division of primary germ cells, sterilising the recipients' organisms before the injection of donor blastoderm cells and, thus, affecting the subsequent egg productivity of poultry.

The shape of eggs is practically not related to the features of feeding and keeping poultry. However, this trait is influenced by the age of ducks, their breed, and the week of the cycle when the egg-laying is examined. Special attention should be paid to reducing the shape variability that was characteristic of large eggs at the end of the egg-laying cycle, which have a variable shape and are prone to breaking. The hatchability of duck eggs within the normal shape index is almost identical, so selection for a certain standard egg shape should be introduced. Notably, this indicator may be influenced by other factors (Abd El-Hack *et al.,* 2019*)*. For example and analysis, data obtained in studies that examined this criterion of economic productivity of ducks and the factors that affect it can be cited. Thus, the study by I. Ismoyowati *et al.* (2020) examined the expression of growth hormone, the genes responsible for follicle-stimulating hormone receptors, the genes of prolactin, ovoinhibitor, melatonin receptor, and insulin-like growth factor-2 in the ovary, and found polymorphisms associated with egg-laying traits in the Jinding and Youxian duck breeds. This study confirms the genetic determinism in the manifestation of this trait. The groups of animals investigated by the researchers could be polymorphic in genes whose polymorphism is associated with the egg production of ducks. Other factors that have been studied can also be attributed to the influence of egg productivity and overall productivity factors, namely: breed, age of ducks, and temperature conditions in the room where the ducks are kept. In the current study, all animals were in the same room, were the same age, belonged to two breeds at the same time, or were chimeras of these two breeds, which could also affect their productive traits.

Summarising, it is worth noting that the results of the study of 4 groups of animals, one of which was control, and 3 experimental, indicate a high level of polymorphism within each of the groups, which may be conditioned by the influence of genes associated with the productivity of ducks. All the animals under study were equally descended from groups of animals belonging to two breeds that were polymorphic in their quantitative trait genes. Therefore, it is possible that the influence of this particular polymorphism was higher than the influence of the factor of the method of obtaining the

animals under study in terms of their productivity. Thus, the results confirm the need for careful individual selection of source material at the stage of preparing experiments that are complex in execution and long in duration of monitoring. Therefore, the question of creating duck lines with an increased frequency of homozygous loci (as is customary in studies, for example, with mouse and chicken lines) with a low level of internal linear polymorphism remains relevant. The creation of lines with a high frequency of homozygous loci can be time-consuming and unexpected in terms of results, given the presence of genetic burden and the effect of multiple allelism by genes of quantitative traits and the interaction of non-allelic genes on animal productivity.

All the animals under study were F_0 (in case of creating chimeras) or F_1 (when using sperm-mediated gene transfer) and descended from siblings, descendants of groups of ducks of the same origin. The starting material was egg embryos obtained from a group of ducks (P) that produced 50 eggs in one day, which were used in experiments to create transgenic ducks. In total, more than 2,000 newly laid eggs were used in the previous experiment, which ultimately produced only 36 female ducks and 19 male ducks, some of which were infertile (mostly males) and did not produce offspring. The main experimental losses were associated with embryonic mortality due to infection of embryos after violating the integrity of the eggshell of the recipient embryo when creating a window in a duck egg that has broad spores. The simplest and most successful method for obtaining transgenic offspring was the method of sperm-mediated gene transfer. It allowed the DNA of the construct to enter the oocyte together with the sperm, which helped to avoid experimental interference in the development of the embryo, and also gave hope that the organisms obtained would not be mosaics due to the presence of a transgenic construct in all their cells. This expectation was not met, possibly due to the rapid fragmentation of blastomeres and cell division during embryonic development, when embedding the construct in the duck genome was carried out for some time only in some blastomeres. All this indicates that there are a number of questions that should be addressed in the future when using transgenic constructs as a tool for creating animals with unique properties.

Conclusions

Data analysis and comparison of performance indicators of different groups of ducks indicate a high efficiency of the sperm-mediated gene transfer method compared to other transgenesis methods used on ducks. This method has demonstrated advantages in efficiency, ease of use, and non-traumatic effects on the embryo. The lowest value of the egg productivity index was obtained in the group created by busulfan injection (79.5±11.8%), the highest – in the group created by sperm-mediated gene transfer (91.8±2.3%). The direct injection group of the transgenic design has an egg productivity index of 89.0±2.0%, which indicates that this biotechnological method of introducing the transgenic design did not have a clear effect on this indicator. The live weight of ducks of different groups ranged from 1,323.50±65.36 g (sperm-mediated gene transfer) to 1,608.08±94.76 g for the group created using busulfan. Ducks in the direct injection group had an average weight of 1,480.42±35.01 g. The control group, on average, acquired sexual maturity at the age of 139.5±9.67 days, the busulfan group – 148.2±13.13 days, the direct injection group – 143.16±7.25 days, the sperm-mediated gene transfer group – 140.67±13.13 days. The biotechnological process using direct injections of transgenic constructs does not affect the productive capacity of the duck, but the procedure itself is associated with high risks of infection in the embryo, and, as a result, with low rates of embryonic survival. Studies of ducks obtained from exposure of recipient embryos to busulfan followed by injection of cells from intact donor

embryos revealed the most severe damage to the reproductive system of ducks, in particular egg production and reproductive capacity, which could be the result of sterilisation of the recipients, namely, the action of an alkylating agent on their primary germ cells for the purpose of sterilisation by affecting the progenitor cells of the recipient's future germ cells before the introduction of donor blastodermal cells. However, no significant effect on the physical fitness of the duck was found. In the future, it is advisable to investigate the impact of biotechnological procedures for obtaining transgenic ducks on their offspring in subsequent generations, considering the presence or absence of a transgenic design and its intergenerational transmission.

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Conflict of Interest

None.

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Вплив біотехнологічних процедур трансгенозу на продуктивність качок

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Анотація. Використання птиці як унікальної моделі біологічних досліджень відзначилось високим рівнем ефективності, однак, методи створення трансгенних качок, ускладнені структурою шкаралупи яйця водоплавних птахів, мають низьку ефективність. Метою роботи було визначення впливу різних біотехнологічних процедур створення трансгенних качок на їх продуктивні якості та репродуктивну здатність для виділення оптимального методу створення трансгенної птиці для подальшого використання в наукових, дослідних або господарських цілях. Під час проведення дослідження використовували зважування, морфометричний та статистичний аналіз продуктивних ознак. Досліджено 40 качок (4 дослідні групи тварин та близько 3000 їх яєць). Найнижче значення індексу яєчної

продуктивності отримано в групі, створеній за допомогою ін'єкції бусульфану (79,5 11,8 %), найвище – у групі, створеній методом трансфекції сперми (91,8±2,3 %), група прямої ін'єкції трансгенної конструкції – 89,0±2,0 %, що свідчить про те, що даний біотехнологічний метод введення трансгенної конструкції не мав явного впливу на даний показник. Вага качок в різних експериментальних групах варіювалася від 1323,50±65,36 г (з використанням методу трансфекції) до 1,608.08±94,76 г (в групі, створеній за допомогою бусульфану). Качки, які отримували прямі ін'єкції, мали середню вагу 1,480.42±35,01 г. У контрольній групі середня вага при досягненні статевої зрілості становила 139,5±9,67 днів, в групі бусульфану –148,2±13,13 днів, в групі прямих ін'єкцій – 143,16±7,25 днів, а в групі трансфекції – 140,67±13,13 днів. Встановлено, що метод ін'єкції в ембріон реципієнта, стерилізованого бусульфаном, та введення донорських бластодермальних клітин негативно впливає на репродуктивні якості качок. Практичне значення дослідження полягає в тому, що в результаті аналізу продуктивності качок, отриманих різними методами трансгенозу, визначено те, що найефективнішим з оцінених є трансфекція ДНК трансгенної конструкції зі спермою (Sperm-mediated gene transfer, SMGT)

Ключові слова: розмір яйця; маса яйця; яєчна продуктивність; ін'єкції; бластодермальна химера; бусульфан

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Effect of feeding *Artemisia capillaris* on egg production and egg quality in quail

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Abstract. The use of safe, natural feed additives in animal feeding is becoming increasingly important to provide animals with biologically active substances, reduce the risk of chemicals being transferred to the end consumer through products, their accumulation, and increase productivity. The purpose of the study was to determine the effect of Artemisia capillaris on egg productivity of quail hens. To conduct the experiment, 4 groups were established – a control group and three experimental groups, 72 animals (♀60+♂12) in each. The experiment, which lasted 210 days, involved feeding poultry with complete balanced feeds that had the same chemical composition and differed only in the content of the test additive, which was introduced into the feeds of poultry of experimental groups 2,3, and 4 in the amount of 0.5%, 1.0%, and 1.5% by weight of the feed, respectively. The introduction of dry wormwood powder in mixed feed of quail hens in the amount of 0.5-1.5% has a positive effect on such indicators of egg productivity as the gross yield of eggs, which increased in the experimental groups by 2.6-5.6%, the egg weight increased by 5.6-12.9%, while feed costs decreased by 2.8%. According to the qualitative indicators of eggs, there was an increase in the absolute weight of yolk by 3.0-8.3%, albumen by 2.6-6.5%, shell by 0.6-1.2%, and a decrease in the egg index by 0.9-4.1%. Feeding A. Capillaris to poultry leads to an increase in the content of palmitic, stearic, oleic, linolenic, eicosanoic, and arachidonic fatty acids in egg yolk by 0.01-6.07%. The optimal level of A. Capillaris in the diet was determined to be 1.0% of the total weight of the feed; this amount had a positive effect on the above indicators, increasing them, which can be used in the industrial technology of quail eggs production, and in organic production

Keywords: wormwood; quail hens; safety; egg weight; fatty acids; feed conversion

Introduction

Due to the ever-increasing requirements for the quality of livestock products, researchers focus their efforts on the search, study, and implementation of safe, biologically active feed additives that improve the use of feed nutrients by activating enzyme systems, reducing stress in animals, improving metabolic processes, improving immunity and productivity, while not being toxic to the body and not accumulating in tissues, organs, or livestock products.

Consumption of foods with chemical residues can cause allergic reactions, alter the gut microbiota, and most importantly, promote the spread of drug-resistant microorganisms in the population, which ultimately leads to therapeutic failures (Dos Santos *et al*., 2020). According to Z.P. Liu *et al*. (2023), one of the ways to solve this problem was the use of medicinal plants that have been known since ancient times, becoming increasingly popular today, in particular, due to the decline in the effectiveness of synthetic substances and public concern about the side effects and drug interactions of synthetic drugs.

The livestock industry is facing an increase in public demand for organic products, as a result, producers are forced to meet these needs, while maintaining high standards of food safety and quality. That is why more and more research is aimed at investigating the effects of natural feed additives on the animal body, such as probiotics, organic acids, and phytogenic additives. The use of phytogenic feed additives in animal nutrition is gaining increasing interest, especially for use in the pig and poultry industries, as evidenced by a significant increase in scientific research on these issues since 2000 (Abdelli *et al*., 2021).

Phytogenic feed additives are products of plant origin (herbs, seeds, fruits, and other parts of plants) that are used in the development of diets for animals in order to improve

their productivity (El-Sabrout *et al*., 2023). These feed additives contain various bioactive components such as polyphenols, alkaloids, flavonoids, and have antimicrobial, antioxidant, growth-stimulating and immunoregulatory effects (Alagawany *et al*., 2021). However, as noted by L. Zhang *et al*. (2022) and J. Chen *et al*. (2022), the effects of phytobiotics on the animal body are not limited to the listed properties, they also have a positive effect on digestion, increasing the secretion of enzymes and the absorption of nutrients. In addition, phytobiotics are natural flavourings that stimulate feed intake and improve the body's performance.

In the context of growing demand for phytogenic feed additives in animal husbandry and limited available resources, the search for new feed additives of plant origin is becoming very important. One of the typical representatives of phytogenic feed additives is wormwood (*Artemisia*), belonging to the Asteraceae family (*Compositae*) or asters *(Asteraceae*). Plants of this species can be found in the natural flora of many countries, such as China, Argentina, France, Bulgaria, Hungary, Romania, Spain, Italy, and the United States. This herbaceous plant is well known in traditional medicine since ancient times, where it is used in the treatment of diseases of the digestive, respiratory, and reproductive systems. Native North Americans used crushed leaves of *A. absinthium, A. biennis, A. frigida* and *A. ludoviciana* for the treatment of ulcers and wounds (Miao *et al*., 2022). The most famous are such types of wormwood as: *Artemisia Cina*, *Artemisia Annua*, *Artemisia Absiuthium*, *Artemisia Abrucanum*, *Artemisia Vulgaris*, *Artemisia Capillaris*; all these types contain aromatic oils, which include a biologically active compound – artemisinin. According to G. Batiha *et al.* (2020), artemisinin in combination with other synthetic drugs has proven to be the most effective treatment for a number of diseases of parasitic aetiology, both in humans and in poultry farming. To date, it has been proven that the synergy between plant components and synthetic substances not only contributes to a better therapeutic effect, but even enhances the mutual influence, which is conditioned by biologically active substances contained in plants. It is proved that in comparison with pure artemisinin, the leaves of *Artemisia* can release 40 times more artemisinin into the body's bloodstream.

Numerous studies of the effectiveness of extracts have been described in the literature *Artemisia* and its essential oils, their antibacterial, antifungal and antiparasitic effects on the bird's body (Batiha *et al*., 2020). The presence of bitterness in *Artemisia* stimulates the secretion of salivary glands and digestive organs, which improves digestion and further absorption of feed nutrients (Zhang *et al.*, 2022). Wormwood also contains antioxidant compounds such as vitamin E and other phenolic compounds that have a positive effect on product quality (Chen *et al*., 2022). The hepatoprotective effect of *Artemisia absinthium* and *Artemisia vulgaris* on the body has also been proven, and the product has been found to be non-toxic and can be safely used in animal feeding (Hrytsyk *et al.*, 2019). There is limited information on the use of *Artemisia capillaris* in quail feeding. Considering the above, the purpose of the scientific and economic experiment was to investigate the effect of feeding wormwood powder (*A. capillaris*) on egg productivity and quality of quail eggs.

Materials and Methods

The study was conducted in the laboratory of the Department of Animal Nutrition and Feed Technology of the National University of Life and Environmental Sciences of Ukraine. For this purpose, 288 birds of 42 day-old Japanese quails were selected, from which 1 control and 3 experimental groups were established on the principle of analogues, 72 birds (♀60+♂12) in each (Table 1). When forming groups, the following factors were considered: live weight, age, and sex of the bird. The experiment lasted 210 days and was divided into 7 sub-periods of 30 days each for ease of recording.

Table 1. *Scheme of the scientific and economic experiment*

Group	Number of birds, units	Content of wormwood powder (*Artemisia capillaris*) in mixed feed, %
Control 1	72 (♀60 + ♂12)	0
Experimental: 2	72 (♀60 + ♂12)	0.5
3	72 (♀60 + ♂12)	1.0
4	72 (♀60 + ♂12)	1.5

Source: developed by the authors

During the study period, the experimental quail population was kept in a six-tiered battery cage of 18 animals (15 females and 3 males), the area per quail was 133 cm^2, feeding front – 2 cm and watering front – 1 cm. In the room, the microclimate indicators corresponded to the established standards. Quail hens of all experimental groups were fed complete mixed feeds developed in accordance with the established standards, considering the age of the bird. Feeding standards for quail hens were developed by the Research Institute of Poultry Farming of the Ukrainian Academy of Agrarian Sciences (Ryabokon *et al.*, 2005) and other Ukrainian researchers (Ibatullin *et al.*, 2022). Mixed feed for all groups was manufactured from the same ingredients. However, due to the introduction of 0.5-1.5% of wormwood powder to obtain mixed feed of the same nutritional value in the control and experimental groups, the proportions of individual ingredients were changed using mathematical methods for optimising mixed feed formulations (Table 2).

Table 2. *Composition of complete mixed feed for quail hens, % by weight*

Ingredient	Group			
	1	2	3	4
	Content, %			
Soy cake	30.5	30.6	30.8	31.0
Corn	54.5	53.9	53.3	52.6
Fish flour	5.9	5.9	5.9	5.9
Premix KN 2.5%	2.5	2.5	2.5	2.5
Sunflower oil	0.6	0.6	0.6	0.6
Limestone flour	6.0	6.0	5.9	5.9
Wormwood powder	0	0.5	1.0	1.5

Source: developed by the authors

The chemical composition of the mixed feed used for feeding poultry was identical and differed only in the content of wormwood powder (Table 3). The addition of dry wormwood powder *(Artemisia capillaris*) to mixed feed was performed by weight dosing and multi-stage mixing according to the experimental scheme (Table 1). The birds were fed twice a day – in the morning and in the evening. Throughout the experiment, accurate records were kept of the feed consumed and the residues that remained uneaten. In terms of chemical composition, all diets for poultry in all groups were identical and differed only in the content of wormwood (Table 3). Introduction of wormwood *(A. capillaris)* to the feed was carried out by weight dosing and multi-stage mixing, according to the experimental scheme (Table 1). Ready-made feed was distributed twice a day (morning and evening). Throughout the experiment, accurate records were kept of the feed consumed and the residues that remained uneaten.

Table 3. *Energy and nutrient content in 1 kg of mixed feed for quail hens*

Indicator	Content	Indicator	Content
Metabolic energy, kcal	2,850.0	Tryptophan, g	2.4
Crude fat, g	50.0	Arginine, g	12.2
Raw fibre, g	34.0	Calcium, g	28.0
Crude protein, g	210.0	Total phosphorus, g	8.0
Linoleic acid, g	21.7	Available phosphorus, g	5.2
Methionine, g	5.0	Sodium, g	1.8
Methionine+cystine, g	7.4	Vitamin A, MU	15,000
Lysine, g	10.9	Vitamin E, mg	20.0
Threonine, g	7.8	Vitamin D_3, MU	3,000

Source: developed by the authors

During the experiment, the safety of livestock was monitored. Feed consumption was recorded: daily, weekly, and for the entire period of the experiment. Feed costs per 10 eggs and 1 kg of egg weight were calculated weekly and for the entire period of the experiment. The conditions of feeding, watering, housing, care, and disease prevention in the animals in the experiment were in accordance with European legislation on animal welfare and comfort (Council Directive 1999/74/EC; Council Directive 1998/58/EU; Council Directive 2010/63/EU).

Based on the daily accounting of egg production, the egg productivity of quails for the initial and average laying hens, and the intensity of egg production were calculated monthly and for the entire period of the experiment. The eggs were weighed on a Radwag PS 600.R2 scale with an accuracy of ±0.01 g, in the last five days of each sub-period of the experiment. At the end of each month of the experiment, eggs were randomly selected to assess their quality by morphological indicators.

The egg shape index was defined as the ratio of the horizontal egg diameter to the vertical one (expressed in %), and the egg diameter was measured using a caliper. Shell thickness was measured with a micrometer in the following areas: in the equatorial part, blunt end, and tip with an accuracy of 0.01 mm, the relative weight of albumen, yolk, and shell was expressed as a percentage of egg weight. The ratio of albumen weight to yolk weight was also calculated.

The chemical composition of the feed was determined by generally accepted methods:

- crude protein – according to the Kjeldahl method;
- raw fat – according to the S.V. Rushkovsky method;
- raw fibre – according to the Henneberg and Stoman method;
- amino acid content – using an automatic TTT 339 analyser with LG ANB cationite with SO_3 active groups
- mineral element content – using spectral analysis on an "ElvaX" X-ray fluorescence spectrometer.
- in egg yolks, the content of fatty acids was determined by lipid extraction using the Folch method (Folch *et al.*, 1957), followed by their hydrolysis to fatty acids and methylation of the latter. Fatty acid methyl esters were separated by gas chromatography on a Trace GC Ultra gas chromatograph (USA) with a flame ionisation detector. Identification of fatty acid methyl esters was performed by comparing the retention time of test samples with the retention time of a mixture of standard fatty acid methyl esters (FAME) Mix C14-C22 (Supelco).

Biometric processing of the data obtained in this study was performed using MS Excel 2016 using the built-in statistical functions. In

the process of processing experimental data, the following were calculated: M (arithmetic mean) and ±m (its error), P (significance level). The following symbols were used in the tables to indicate the significance level of the probability criterion (P): *P<0.05, **P<0.01, ***P<0.001 compared to control group 1.

Results and Discussion

According to the results obtained, it was found that different levels of *A. capillaris* in the diet of quails affected their egg productivity (Table 4). Thus, the gross egg harvest for 7 months of the experiment on average for groups ranged from 8,563 to 9,038 eggs. The experimental groups outperformed the control group – by 2.6% (group 2), by 5.5% (group 3), and by 3.0% (group 4). Thus, an increase in the content of wormwood powder in the diet had a positive effect on productivity, and the gross egg yield was highest when 1.0% of the supplement was introduced into the diet. An increase in the amount of supplements in the diet of quails of group 4 to 1.5% resulted in a slight decrease in productivity compared to group 3, but was higher than in the control.

Table 4. *Indicators of egg productivity of quails for 7 months of experiment*

Indicators	Group			
	1	2	3	4
Gross egg collection, units:				
– for the entire experiment	8,563	8,785	9,038	8,823
– for a 30-day period	1,223.3±16.16	1,255.0±20.21	1,291.1±25.49*	1,260.4±20.84
Egg production for the initial quail hen, units:				
– total	142.7	146.4	150.6	147.1
– for a 30-day period	20.4±0.27	20.9±0.34	21.5±0.42*	21.0±0.35
Egg production per average quail hen, units:				
– total	164.9	167.7	171.0	168.9
– for a 30-day period	23.6±0.84	24.0±0.86	24.4±0.91	24.1±0.85
Egg production rate, %	78.5±2.81	79.9±2.88	81.4±3.04	80.4±2.83
Egg weight, g	11.06±0.080	11.39±0.119*	11.81±0.189**	11.80±0.162
Amount of egg mass, g				
– total	94,702.3	100,006.3	106,870.6	104,191.1
– for a 30-day period	13,528.9±168.80	14,286.6±216.36*	15,241.1±322.94**	14,884.4±213.71

Notes: *p<0.05; **p<0.01; ***p<0.001 compared to group 1
Source: developed by the authors

The gross egg harvest indicator averaged over the 30-day experiment period also shows the probability of changes obtained. Thus, the experimental groups outperformed the control by 2.6-5.5%. However, the highest rate was in poultry of experimental group 3, which outperformed the control by 5.5% (p<0.05). Egg production for the initial quail for the entire period of the experiment was the highest in poultry of experimental group 3 – by 8 eggs, groups 2 and 4 also outperformed the control, but less significantly – by 4 eggs in group 2 and by 4 – in group 4. This indicator, calculated for a 30-day experiment period, shows that an increase in wormwood powder in mixed feed to 1.0% leads to an increase in egg production by 5.5% (p<0.05). Groups 2 and 4 also outperformed the control by 2.5 and 2.9%, respectively, but they lagged behind the indicator of group 3.

Egg production on an average quail is an indicator of egg productivity of poultry, considering its integrity. The data obtained indicate

that egg production per average quail increased in proportion to the increase in the content of wormwood powder in feed. However, this indicator was highest in poultry of experimental group 3. They outperformed the control by 6 eggs, or 3.7%. Quails of groups 2 and 4 outperformed the control group less significantly – by 3 and 4 eggs, respectively, or by 1.7 and 2.4%. This figure calculated over a 30-day period shows that this advantage was statistically unlikely in all study groups. Poultry of experimental group 3 outperformed the control by 3.4%. Egg production for the average quail was higher than the control in quails of groups 2 and 4, respectively, by 1.7 and 2.1%.

According to the degree of productivity of quails that consumed mixed feed with the addition of wormwood powder, an excess of 1.4-2.9% over the control group was found. In the group 3, this indicator was the highest among all groups. The effect of introducing wormwood powder into mixed feed also affected the weight of eggs, where an increase in this indicator was observed with an increase in the content of the drug in the feed. For example, quails that consumed mixed feed with 0.5% of wormwood powder showed a higher egg weight of 0.33 g or 3.0% compared to the control group ($p<0.05$). An increase in the drug content to 1.0% led to a further increase in egg weight in group 3 by 0.75 g or 6.8% ($p<0.01$). Although an increase in the content of the drug in mixed feed to 1.5% led to a slight decrease in egg weight compared to group 3, and the advantage over the control was 0.74 g or 6.7%.

The amount of egg mass obtained from experimental quails is the most objective indicator of their productivity. This figure was the highest in experimental group 3 – 106.871 kg. They outperformed the control by 12,168 kg, or 12.8%. Quails in groups 2 and 4 outperformed the control group by 5.3 and 9.5 kg or 5.6 and 10.0%, respectively. This indicator calculated for the 30-day period of the experiment showed the advantage of quails of experimental group 3 over the control by 12.8% ($p<0.01$). Poultry of experimental group 2 also significantly exceeded the control – by 5.6% ($p<0.05$). The advantage of quails of experimental group 4 was no less substantial, but not statistically significant, they exceeded the control by 10.0%.

Analysis of the morphological composition of experimental quail eggs is shown in Table 5. The absolute weight of egg yolk significantly increased in all experimental groups. Thus, quails of experimental groups 2,3, and 4 outperformed the control, respectively, by 4.8% ($p<0.05$); 10.2 ($p<0.001$), and 9.9% ($p<0.001$). The weight of egg albumen in experimental group 3 increased most significantly – this correlates with the highest egg weight in poultry of this group.

Table 5. *Morphological composition of experimental quail eggs*

Group	Absolute weight, g			Shell thickness, mm	Form index, %
	Yolk	Albumen	Shell		
1	3.32±0.030	6.12±0.016	1.62±0.032	26.3±0.06	80.9±0.20
2	3.48±0.046*	6.28±0.050*	1.63±0.027	26.4±0.15	80.0±0.08*
3	3.66±0.058***	6.51±0.111*	1.64±0.033	26.0±0.16	75.9±0.09***
4	3.65±0.069***	6.52±0.113*	1.64±0.030	26.0±0.06***	76.1±0.18***

Notes: *p<0.05; **p<0.01; ***p<0.001 compared to group 1
Source: developed by the authors

The absolute weight of egg albumen also increased, but less significantly. It was highest among quails of experimental group 4. They outperformed the control by 6.5% ($p<0.05$). The absolute weight of egg albumen in experimental group 2 was also 2.6% higher than the control ($p<0.05$). This indicator in experimental group 3 was higher than the control – by 6.4%

(p<0.05). The absolute weight of eggshells was inversely proportional to their total. This indicator was lowest in quails of the control group, by 0.6% compared to group 2 and by 1.2% compared to groups 3 and 4. Eggshell thickness was lower in quails of experimental groups 3 and 4 – by 1.1% (p<0.001). This indicator in experimental group 2 was higher than the control by 0.4%. Usually, the shape index increases with increasing egg weight and increasing their transverse diameter (rounding), but in this case, the opposite phenomenon of decreasing egg shape index can be observed. Thus, in quails of experimental groups, it decreased by 0.9-5.0% (p<0.001).

The fatty acid content of quail egg yolk is shown in Table 6. In terms of palmitic acid content, poultry of experimental groups 3 and 4 were 1.51-1.59% superior (p<0.05) to the control group. In the yolks of poultry of experimental group 3, the content of stearic acid was higher (p<0.05) by 0.35%. As for the content of oleic acid, poultry of experimental groups 2, 3, and 4 were 3.38-6.07% superior (p<0.05) to analogues of the control group. According to the content of linoleic acid in the yolks of poultry of experimental group 4, the control group prevailed by 0.48% (p<0.05). In the yolks of poultry of experimental groups 3 and 4, the content of linolenic acid was higher by 0.06-0.08% (p<0.05). In terms of eicosanoic acid content, poultry of experimental groups 3 and 4 were 0.01-0.02% superior (p<0.05) to the control group. Regarding the content of arachidonic acid in egg yolks, group 3 poultry was 0.24% (p<0.05) and group 4 poultry was 0.50% (p<0.01) higher than the control analogues. There was no statistically significant difference in the content of other fatty acids.

Table 6. *Fatty acid content in quail egg yolk, %*

Acid	Group			
	1	2	3	4
Myristic ($C_{14:0}$)	0.38±0.009	0.38±0.008	0.38±0.008	0.37±0.007
Myristoleic ($C_{14:1}$)	0.04±0.012	0.05±0.013	0.05±0.010	0.06±0.011
Pentadecylic ($C_{15:0}$)	0.10±0.013	0.10±0.012	0.09±0.013	0.09±0.011
Palmitic ($C_{16:0}$)	20.64±0.533	20.71±0.475	22.15±0.528*	22.23±0.478*
Palmitoleic ($C_{16:1}$)	3.26±0.095	3.31±0.089	3.46±0.086	3.42±0.088
Heptadecanoic ($C_{17:0}$)	0.15±0.025	0.18±0.033	0.20±0.033	0.12±0.025
Stearic ($C_{18:0}$)	9.88±0.124	9.84±0.183	10.23±0.116*	9.67±0.205
Oleic ($C_{18:1}$)	43.54±1.110	46.92±1.122*	47.12±1.177*	49.61±1.012*
Linoleic ($C_{18:2}$)	11.92±0.253	11.56±0.235	11.66±0.213	12.40±0.350*
Linolenic ($C_{18:3}$)	0.46±0.012	0.48±0.010	0.52±0.019*	0.54±0.015*
Eicosanoic ($C_{20:0}$)	0.03±0.001	0.03±0.002	0.04±0.003*	0.05±0.003*
Icosanoic ($C_{20:2}$)	0.02±0.007	0.02±0.006	0.02±0.008	0.02±0.006
Arachidonic ($C_{20:4}$)	2.11±0.054	2.20±0.041	2.35±0.051*	2.61±0.046**
Docosapentaenoic ($C_{22:5}$)	0.15±0.008	0.16±0.009	0.14±0.012	0.15±0.010
Docosahexaenoic ($C_{22:6}$)	1.21±0.574	1.26±0.445	1.24±0.754	1.28±0.647

Notes: *p<0.05; **p<0.01 compared to group 1
Source: developed by the authors

Relative weight indicators of egg components are shown in Table 7. The relative weight of egg yolk in quails from experimental groups increased by 0.6-1.0% (p<0.01). The relative weight of albumen practically did not change and was slightly lower in quails of the experimental groups by 0.4-0.6% compared to the control.

Table 7. *Relative weight of egg components*

Group	Relative weight, %			Yolk to albumen (weight ratio)
	Yolk	Albumen	Shell	
1 (n=20)	30.1±0.10	55.4±0.27	14.6±0.19	0.55±0.005
2 (n=20)	30.7±0.13*	54.9±0.20	14.4±0.08	0.57±0.006**
3 (n=20)	31.1±0.24**	54.9±0.31	14.0±0.15*	0.58±0.010*
4 (n=20)	31.1±0.28**	55.00±0.40	13.9±0.13**	0.57±0.011*

Notes: *p<0.05; **p<0.01; ***p<0.001 compared to group 1
Source: developed by the authors

The relative weight of the shell decreased in the experimental groups. This indicator was lowest in poultry of experimental group 4. Compared to the control, it was lower by 0.7% (p<0.01). In addition, the difference in this indicator was significant in experimental group 3 – 0.6% (p<0.05) below the control. In experimental group 2, the relative weight of eggshells was slightly and not significantly lower than the control group – by 0.2%. The ratio of yolk and albumen weight increased in quails of the experimental groups. It was higher than the control by 0.2-0.3% (p<0.01), which correlates with absolute yolk and albumen weight values. Thus, according to the complex of indicators of egg productivity and egg quality, there is positive effect of introducing dry wormwood powder into the mixed feed of quail hens in the amount of 0.5-1.5%. However, these indicators were highest for the introduction of 1.0% of wormwood. Feed consumption per unit of production is the most important indicator that determines production efficiency (Table 8), especially in poultry farming, where feed costs can reach 90% of the production cost.

Table 8. *Feed consumption per 10 eggs, kg*

Month of experiment	Group			
	1	2	3	4
1	0.43	0.42	0.42	0.42
2	0.39	0.38	0.37	0.37
3	0.35	0.34	0.33	0.34
4	0.33	0.32	0.31	0.33
5	0.33	0.33	0.32	0.32
6	0.34	0.33	0.32	0.33
7	0.35	0.35	0.35	0.34
Average	0.36±0.014	0.35±0.014	0.35±0.014	0.35±0.014

Source: developed by the authors

Feed consumption decreased in quails of experimental groups along with an increase in the content of dry wormwood powder to 1.5%. Thus, during the 1st month of the experiment, this indicator in quails of experimental groups decreased by 2.3% in relation to the control. In the 2nd month of the experiment, feed consumption in quails of experimental group 2 decreased by 2.6%, and in poultry of experimental groups 3 and 4 – by 5.1%. In the following months, a similar trend continued – a decrease in feed consumption in the groups of poultry that consumed the drug under study. In the 3rd month of the experiment, the lowest feed consumption was in poultry of experimental group 3. This figure was 5.7% lower than the

control. In poultry of experimental groups 2 and 4, this indicator decreased by 2.9%.

At the 4th month of research, the indicative feed consumption per 10 eggs in quails of experimental group 3 was again the lowest – 0.31 kg. This figure was 6.1% lower than the control. In quails of experimental group 2, feed consumption was 3.0% lower. In poultry of experimental group 4, this indicator was at the control level. At the 5th month of research, the feed consumption rate decreased in poultry of experimental groups 3 and 4 – by 3.0%. Within 6 months, feed costs decreased in quails of all experimental groups, but they were the lowest in poultry of experimental group 3 – 5.9% less than the control. In poultry of groups 2 and 4, this indicator was 2.9% lower than the control. At the 7th month of research, feed consumption in quails of experimental groups 2 and 3 was at the control level and amounted to 0.35 kg/10 eggs. However, in quails of experimental group 4, this indicator was lower than the control by 2.9%. Analysis of the average feed costs for 10 eggs during the entire experiment shows that this indicator was equally lower in quails in all experimental groups, compared to the control group, by 2.9%.

The results of the conducted studies proved the positive effect of feeding quail hens with different contents of *Artemisia capillaris* on the indicators of their egg production, morphological composition of eggs, the content of fatty acids in the yolk, and feed consumption per unit of production. According to the conducted experiments, the poultry of the experimental groups in terms of egg productivity exceeded the control group by 2.6-5.5%, while the highest indicator was in the poultry of experimental group 3, which was fed 1.0% of wormwood grass powder. Recently, phytogenic substances, which are biologically active compounds, are increasingly attracting interest as feed additives, in particular in poultry farming, due to their ability to increase the efficiency of feed use by increasing the production of digestive secretions and improving the absorption of nutrients, while reducing the pathogenic load in the intestines and reducing the load on the immune status of animals (Adbelli *et al*., 2021). The literature contains data on the use of herbs and other components from wormwood *(Artemisia)* as phytogenic feed products in poultry feeding. The results obtained are consistent with the data of P. Remberg *et al*. (2004), according to which the use of oil from *Artemisia Herba* improved the productive qualities of chicken hens by increasing the secretory activity of the gastrointestinal tract. Similar data on increasing egg productivity of chickens, by almost 5.0%, was obtained when 2.0-3.0% of *Artemisia argyi* were included in their diet (Chen *et al*., 2022; Zhou *et al*., 2023). In addition, M.A. Al-Nuri (2012) also reported a positive effect of adding 0.25-0.50% of Artemisia powder on the productivity of broiler chickens.

Feeding quail with *Artemisia capillaris* led to an increase in the absolute mass of the shell. With an increase in the amount of wormwood in the diet, the shell weight increased in poultry of the experimental groups by 0.62-1.23% compared to the control, while quails of group 2 showed an increase in the shell thickness by 0.1 mm. This is consistent with the results of P. Baghban-Kanani *et al*. (2019) who report that feeding laying hens 5.0-7.5% of *Artemisia annua* increased the thickness of the shell and reduced the cholesterol content in the egg yolk. In additon, according to G. Cherian *et al*. (2013), E.A. Brisibe *et al*. (2009) *Artemisia annua* leaves have high concentrations of Ca, Mg, P, and S, which have a positive effect on the development of eggshells.

Feeding quail hens with different amounts of *Artemisia capillaris* affected the fatty acid content of egg yolk. The introduction of wormwood powder into the feed of quail hens led to an increase in the content of palmitic acid by 1.51-1.59%, stearic acid by 0.35%, oleic acid by 3.38-6.07%, linoleic acid by 0.48%, linolenic acid by 0.06-0.08%, eicosanoic acid by 0.01-0.02% and arachidonic acid by 0.24-0.50% compared to

the control a group. This is consistent with the findings of J. Chen *et al.* (2022*),* where is the introduction of 2.0% of *Artemisia argyi* in the diet of chicken hens caused a significant increase in the content of heptadecanic acid ($C_{17:0}$), eicosadienoic acid ($C_{20:2}$), docosahexaenoic acid ($C_{22:6}$), linolenic acid ($C_{18:3}$), linoleic acid ($C_{18:2}$) in the yolk, and when the level of *Artemisia argyi* increases up to 3%, the content of these acids no longer increased, but rather decreased. The obtained indicators are consistent with the findings of I.S. Carvalho *et al*. (2011), who examined the content of fatty acids in 13 species of *Artemisia,* in particular, *Artemisia capillaris* , and it was found that it contains palmitic ($C_{16:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and linolenic ($C_{18:3}$) fatty acids and traces of palmitoleic ($C_{16:1}$), stearic ($C_{18:0}$) and eicosanoic ($C_{20:0}$) fatty acids. As a result, an increase in the intake of these fatty acids in the body of quails with mixed feeds contributes to their direct transition to production, and to the egg yolk in particular. According to the data obtained by A. Saleh *et al*. (2021), the inclusion of paprika in the diet alone or in combination with carmoisin increased ($p<0.05$) the content of linolenic and oleic fatty acids in the egg yolk of laying hens.

Feeding mixed feed with different levels of *Artemisia capillaris* to quail hens resulted in a reduction in feed consumption per 10 eggs during the entire experiment period. In general, for the entire period of the experiment, which lasted 210 days, the lowest feed consumption per 10 eggs was in quails that consumed mixed feed from 1.0% of *Artemisia capillaries*. Feed costs in this group were 2.9% lower than in the control analogues. Similar data was obtained on laying hens by N.H. Mansoub (2011) when feeding them *Artemisia annua*, explaining this fact by its antibacterial and antifungal action, which as a result leads to a decrease in the number of pathogenic microbes in the digestive system, an increase in immunity and productivity of chickens. Reducing feed consumption per unit of production depends on the type of phytobiotics that are included in the poultry diet. Thus, in experiments on laying hens by N. Puvača *et al*. (2020), when introducing essential oil of *M. Alternifolia* into diets, there was a decrease in the feed conversion rate by 2.4-3.2%, while M. Bozkurt *et al*. (2014) did not notice differences in feed intake when feeding laying hens herbs thyme, oregano, rosemary, and turmeric. The same data was obtained S.R. Chowdhury *et al*. (2002) when feeding different doses of garlic. Moreover, the positive effect on productivity is conditioned by the chemical composition and nutritional value of wormwood, so in particular, the leaves of *Artemisia anna* contains more than 4,271 kcal/kg of gross energy, 27.0% crude protein, and >50% essential fatty acids.

Thus, the use of phytobiotics as feed agents depends on the type and form of the additive, and therefore, the study of the impact on the productive qualities of a certain group or species of animals is relevant, in particular today, when the requirements for the quality of final products have significantly increased. The data obtained as a result of the experiment proved a positive impact of *Artemisia capillaris* on egg productivity of quail hens, while improving the quantitative and qualitative indicators of products, and a decrease in feed conversion rates was proved.

Conclusions

The study found that the introduction of *Artemisia capillaris* dry powder into the composition of mixed feed of quail hens in the amount of 0.5-1.5% has a positive effect on their egg productivity, egg quality, and feed consumption per unit of production. It was revealed that the optimal level of wormwood powder in quail feed is 1.0%, although the results obtained for the introduction of 0.5 and 1.5% of the additive to complete poultry feed also had a positive effect on productivity and exceeded the indicators of the control group. When 1% of *Artemisia capillaris* was introduced into the quail diet, an increase in gross egg production was observed –

by 5.5% ($p<0.05$), egg production per average and initial quail – by 5.5 and 3.7% ($p<0.05$), egg production rate – by 2.9%, egg weight – by 6.8% ($p<0.01$), and egg weight for the 30-day period of the experiment – by 12.8% ($p<0.01$). Feed costs per 10 eggs at this level of wormwood in the feed are reduced by 2.9%. These facts on increasing productivity and reducing feed costs per unit of production are obtained due to the presence of biologically active substances in wormwood, which contribute to the release of digestive enzymes, activate digestion, and increase the digestibility of nutrients. There was also an increase in the relative weight of yolk by 3.3% ($p<0.01$) in poultry that received 1.0% of *A. capillaris*, which increased its weight relative to albumen by 5.5% ($p<0.01$). The presence of fatty acids in the composition of the supplement under study directly proportional to the increase in their content in egg yolks, where an increase in the content of palmitic, stearic, oleic, linolenic, eicosanoic, and arachidonic fatty acids in egg yolk was noted by 0.01-6.07%. Therefore, given the positive effect of *Artemisia capillaris* powder on egg production and product quality of quails, it is advisable to conduct further research on the effect of wormwood on productivity and product quality in other species of animals and poultry.

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Conflict of Interest

None.

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Вплив згодовування *Artemisia capillaris* на яєчну продуктивність та якість яєць перепелів

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Анотація. Все частіше постає питання використання в годівлі тварин безпечних, натуральних кормових добавок з метою забезпечення тварин біологічно-активними речовинами, зменшенням ризиків передачі хімічних речовин до кінцевого споживача через продукцію, їх накопиченням, а також для підвищення рівня продуктивності. Метою дослідження було визначення впливу Artemisia capillaris на яєчну продуктивність перепілок-несучок. Для проведення досліду за принципом аналогів було сформовано 4 групи – контрольну та три дослідні, по 72 голови (♀60 + ♂12) у кожній. Впродовж досліду, який тривав 210 діб, птицю годували повнораціонними збалансованими комбікормами, які мали однаковий хімічний склад і різнилися лише за вмістом досліджуваної добавки, яку вводили в комбікорми птиці 2-ї, 3-ї та 4-ї дослідних груп в кількості відповідно 0,5 %, 1,0 та 1,5 % від маси комбікорму. Введення сухого порошку полину у комбікорми перепілок-несучок в

кількості 0,5-1,5 % позитивно впливає на такі показники яєчної продуктивності як валовий збір яєць, який зростав в дослідних групах на 2,6-5,6 %, кількість яєчної маси збільшувалася на 5,6-12,9 %, при цьому витрати кормів зменшилися на 2,8 %. За якісними показниками яєць відмічали зростання абсолютної маси жовтка на 3,0-8,3 %, білка на 2,6-6,5 %, шкаралупи на 0,6-1,2 %, а також зменшення індексу яйця на 0,9-4,1 %. Згодовування птиці A. Capillaris призводить до підвищення вмісту пальмітинової, стеаринової, олеїнової, ліноленової, ейкозанової та арахідонової жирних кислот у яєчному жовтку на 0,01-6,07 %. Оптимальним рівнем введення A. Capillaris в раціон було визначено 1,0 % від загальної маси комбікорму, дана кількість позитивно впливала на вищеперераховані показники збільшуючи їх, що може бути використано за промислової технології виробництва яєць перепілок-несучок, а також при органічному виробництві продукції

Ключові слова: полин; перепілки-несучки; збереженість; маса яйця; жирні кислоти; конверсія корму

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