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Сучасні підходи та перспективи генетичної оцінки молочної худоби
в селекційних програмах..... 9

О. Ситник, І. Кімейчук

Інноваційні підходи до стандартизації в лісовому товарознавстві: тенденції та перспективи.....24

О. Довгеля, О. Антоненко, О. Бачинський, Ф. Мельничук, С. Алексєєва

Вплив мінерального живлення та азотфіксатора на урожайність кукурудзи
в Центральному Лісостепу України 37

В. Кашпаров, С. Левчук, О. Швардак, Д. Кондратюк, М. Журба

Залежність абсорбції ¹³⁷Cs кормом для коропових риб в залежності
від вмісту в ньому різних видів Берлінської лазури 49

І. Смик, Л. Архипова

Дослідження забруднення повітря дрібнодисперсними частками
у туристичних локаціях Івано-Франківської області 62

Т. Чайка, І. Короткова, В. Ляшенко, Г. Поспелова, В. Оніпко

Абсцизова кислота та біопрепарати
як маркери адаптації органічної сої до гідротермального стресу.....76

В. Грубань, М. Храмов, О. Лимар, А. Пастушенко

Використання інноваційних технологій
для збільшення терміну придатності молочних продуктів.....96

В. Мартишко, Ю. Гуменюк, Р. Осадчий, О. Карлаш, Б. Горіславський

Обґрунтування типу підвіски одновісного причепа для транспортування плодів 114

Д. Жданов, Я. Васютинський, М. Гугляк, Ю. Засуха

Ефективність біотехнологічних інструментів
у покращенні здоров'я поголів'я свиней та екологізації виробництва..... 144

Contents

V. Danshyn, S. Ruban, V. Nazarenko

Modern approaches and prospects for the genetic evaluation of dairy cattle
in breeding programmes..... 9

O. Sytnyk, I. Kimeichuk

Innovative approaches to standardisation in forest commodity science: Trends and prospects24

O. Dovhelia, O. Antonenko, O. Bachinsky, F. Melnychuk, S. Alekseeva

Effect of mineral nutrition and nitrogen-fixing agent on maize yield
in the Central Forest-Steppe of Ukraine.....37

V. Kashparov, S. Levchuk, A. Shvardak, D. Kondratyuk, M. Zhurba

The dependence of ¹³⁷Cs absorption by carp fish feed on the content
of various types of Prussian Blue.....49

I. Smyk, L. Arkhypova

Research on air pollution by fine particles
in tourist locations in the Ivano-Frankivsk region.....62

T. Chaika, I. Korotkova, V. Liashenko, H. Pospelova, V. Onipko

Abscisic acid and biopreparations as markers
of organic soybean adaptation to hydrothermal stress76

V. Hruban, M. Khramov, O. Lymar, A. Pastushenko

Use of innovative technologies
to extend the shelf life of dairy products96

V. Martishko, Yu. Humeniuk, R. Osadchy, O. Karlash, B. Horislavsky

Justification of the suspension type of a single-axle fruit transport trailer 114

D. Zhdanov, Ya. Vasiutynskyi, M. Huhliak, Yu. Zasukha

Effectiveness of biotechnological tools in improving the health
of pigs and environmentalisation of production..... 144



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Modern approaches and prospects for the genetic evaluation of dairy cattle in breeding programmes

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Abstract. The evaluation of breeding value for economically important traits is a key tool in modern systems of genetic improvement in dairy cattle. This analytical study aimed to describe the current algorithm for genetic evaluation in dairy cattle and to identify promising directions for potential improvement in the near future. Genetic evaluation methods have undergone significant development, evolving from mass daughter-dam comparisons, direct and improved herd-mate comparisons, and modified contemporary comparison, to more complex approaches such as the Animal Model and genomic evaluation using mathematical techniques such as BLUP and REML. The implementation of modern genomic selection programmes required a substantial restructuring of the entire organisational system of breeding. The presence of reference populations, with ongoing monitoring of genetic and phenotypic traits, was a fundamental requirement. It was noted that a general trend in modern dairy farming was the increasing number of traits considered in selection to account for both observable traits (such as milk yield and composition) and “hidden” traits (such as health status, reproductive

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efficiency, productive longevity, and feed conversion efficiency), all of which significantly influence production economics. A comparative analysis of the monitored livestock population and productivity indicators in Ukraine and ICAR member countries was carried out, revealing key limitations within the national breeding system. It was established that genomic evaluation enables the shortening of generation intervals and the doubling of the rate of genetic progress in milk yield. The practical value of the study lies in providing scientifically grounded guidelines for developing an effective system of genetic evaluation and breeding resource management in Ukraine

Keywords: BLUP Animal Model; genomic selection; holo-omics; genome editing; biotechnology

Introduction

An important challenge in modern dairy farming is achieving a high level of genetic progress in economically significant traits through the implementation of effective breeding programmes. The genetic improvement of livestock, including dairy cattle, traditionally comprises the following stages: (1) defining the breeding objective; (2) constructing a selection index aimed at achieving the breeding objective with maximum profitability; (3) organising regular monitoring of traits included in the selection index; (4) conducting genetic evaluation of animals for selected traits; (5) developing and implementing a breeding programme based on specific selection criteria; and (6) selecting potential parents to produce the next generation. The genetic evaluation of animals involves the use of mathematical methods designed to minimise estimation error (Simm *et al.*, 2021). In the early 21st century, it was demonstrated that the breeding value of animals could be predicted at early stages of ontogeny by using a large number of genetic markers distributed across the entire genome. This led to the development of the approach now known as genomic selection (Misztal *et al.*, 2020).

In the monograph by R.A. Mrode *et al.* (2023), methods for using linear models to evaluate the genetic merit of animals are described. The publication outlines approaches to genetic evaluation using various sources of information, genetic covariance among relatives, best linear unbiased prediction of breeding value, models incorporating maternal effects, animal models, models

including social interactions between animals, methods for genomic prediction of genetic merit and forecasting selection response, as well as genetic and genomic models involving multiple breeds and crossbreeding analysis. It also presents methods for estimating genetic parameters.

The introduction of DNA microchip technology has led to the widespread use of genomic selection in livestock species, particularly in dairy cattle. Breeding values with higher reliability can now be estimated at early stages of life by combining DNA genotypes at tens of thousands of loci with existing databases on identity, pedigree, and phenotype for millions of animals. Genomic selection was initially applied to males to predict the performance of their offspring, but it is now widely used in females and even embryos to forecast their own future performance (VanRaden, 2020). Y.C.J. Wientjes *et al.* (2022) demonstrated that genomic selection outperforms pedigree-based selection in terms of long-term genetic progress, although it results in a comparable reduction in genetic variation. The genetic architecture of traits changed considerably over generations, particularly under selection pressure and in the presence of non-additive effects. In conclusion, non-additive effects had a significant influence on selection accuracy and the long-term response to selection, especially when selection was highly accurate. B.A. Scott *et al.* (2021) found that the implementation of genomic selection in Australian dairy cattle led to a 160% increase in genetic progress for national economic index values in

the Holstein breed and a 100% increase in the Jersey breed. According to K. Weigel *et al.* (2024), genomic testing of young bulls and heifers provides greater accuracy in selection decisions for traditional fertility traits such as daughter pregnancy rate, while also enabling improvement in emerging traits such as pregnancy loss.

The monograph by N. Ahmadi & J. Bartholomé (2022) focused on genomic prediction of quantitative traits. It covers the non-genetic foundations of quantitative traits – from quantitative trait loci to genomic prediction – along with methods for genomic prediction, underlying principles, an overview of factors affecting the reliability of genomic prediction, and the algebra of its accuracy. The authors provide a comprehensive review of genomic prediction methods and the associated assumptions concerning marker effect variance and the genetic architecture of quantitative traits. The text also explores approaches that account for non-additive genetic effects, the inclusion of trait correlations in genomic prediction, and the prediction of quantitative traits in animals with a long history of selection, particularly in dairy cattle. Furthermore, the monograph assesses the economic efficiency of genomic selection in comparison with traditional breeding approaches. S. Ruban & V. Danshin (2023) conducted a comparative analysis of selection response under traditional breeding programmes versus genomic selection. Their findings demonstrated that by reducing generation intervals in genetic improvement pathways – such as “sire of bulls,” “sire of cows,” and “dam of bulls” – genomic selection can accelerate genetic progress in milk yield by between 100.1 kg and 180.0 kg, representing an increase of up to 80%.

This study aimed to analyse the potential of modern methods for the genetic evaluation of dairy cattle and to outline prospects for their further development under conditions specific to Ukraine.

Materials and Methods

The research methodology was based on the principles of comparative analysis and systematic

synthesis of empirical statistical data on the genetic evaluation of cattle in Ukraine and ICAR (International Committee for Animal Recording) member countries. The primary data source was the 2023 State Register of Breeding Entities in Animal Husbandry (Zhukosky *et al.*, 2024), which included information on the number, breed composition, and productivity of the monitored livestock of dairy and dual-purpose cattle.

Additionally, data from the ICAR Annual Report (2023a) for the years 2022-2023 were analysed. These materials reflect the status of performance recording and breeding systems in over twenty European countries. Aggregated indicators from the official ICAR statistical section (2023b) were also used, including data on the number of monitored livestock, average lactation productivity, frequency of genomic evaluation use, and breed distribution by country. The ICAR reports made it possible to identify key trends in the development of breeding record-keeping in leading dairy-producing countries such as the USA, Germany, France, Canada, and Italy. To gain a deeper understanding of the context, a systematic review of scientific publications (Misztal *et al.*, 2020; VanRaden *et al.*, 2021) was conducted, with a focus on genomic selection practices, the use of Single Nucleotide Polymorphism (SNP) markers, Best Linear Unbiased Prediction (BLUP) models, and the application of the Net Merit Dollar Index (NM\$).

The comparative analysis included indicators such as milk yield, fat and protein content, the proportion of monitored livestock, the specific influence of individual breeds, and the degree of integration of genomic methods into breeding value assessment systems. Promising directions for the development of Ukraine's breeding system were identified through the extrapolation of effective international practices, particularly those from the USA, which was the first to introduce a genomic model for evaluating Predicted Transmitting Ability (PTA). Data processing methods involved elements of descriptive statistics, typological grouping, and interpretation of correlation relationships between traits (milk yield, fat,

protein, fertility, longevity), based on findings from previous studies (V.O.Danshin *et al.*, 2017). The study was analytical in nature and did not involve original experimental material. It aimed to synthesise existing practices and formulate scientifically grounded recommendations for improving the genetic evaluation of dairy cattle in Ukraine.

Results and Discussion

As of the end of 2024, the total number of dairy cows in Ukraine was 1.156 million head, with an annual milk production of 7.2 million tonnes, indicating a relatively intensive development of the sector despite the ongoing martial law. A key component of this industry remains the focus on producing economically viable animals through methods of genetic evaluation and the implementation of selection programmes for dairy cattle. Table 1

presents the breed composition and productivity indicators of the monitored (controlled) livestock, which accounted for approximately 9.7% of the total dairy cow population – significantly lower compared to the corresponding figures in most European countries (Table 2).

Market conditions in Ukraine's dairy sector impose strict requirements on cattle breeds, with the Holstein breed increasingly coming to dominate in recent years. According to analytical data, up to 68% of the national breeding livestock can be classified as Holstein. It is worth noting that the two most numerous Ukrainian dairy breeds – the Ukrainian Red-and-White Dairy and the Ukrainian Black-and-White Dairy – were developed in the late 20th century through combination crossbreeding schemes involving local breeds and partial use of Holsteins.

Table 1. Breed composition and productivity of the breeding (monitored) livestock of dairy and dual-purpose cattle in Ukraine

Breed	Livestock			Milk yield, kg	Percentage, %	
	total population, head	including cows, head	% of total cow livestock		fat	protein
Ayrshire	969	420	0.37	7,402	4.00	3.03
Holstein	175,412	51,429	45.76	9,810	3.84	3.32
Jersey	2,497	1,064	0.95	6,004	5.98	4.15
Lebedyn	766	329	0.29	6,035	4.14	3.31
Simmental	9,200	3,563	3.17	7,051	4.06	3.32
Ukrainian Brown Dairy	1,299	466	0.41	6,995	4.13	3.53
Ukrainian Red Dairy	3,733	1,213	1.08	7,310	3.83	3.52
Ukrainian Red-and-White Dairy	32,361	11,439	10.18	7,689	3.82	3.45
Ukrainian Black-and-White Dairy	118,599	41,094	36.56	8,218	3.74	3.22
Red Steppe	737	256	0.23	6,318	4.19	3.20
Brown Swiss	3,809	1,115	0.99	8,718	3.95	3.40
Total	349,382	112,388*	100	7,623	4.19	3.42

Source: developed by the authors based on O.M. Zhukosky *et al.* (2024)

These breeds have been genetically improved mainly through the use of Holstein sires, with semen imported from the USA. The milk production market in European countries is highly dynamic and varies

in terms of selection programme focus and the number of animals in the monitored (breeding) livestock (Table 2). This variation also contributes to a strong market for breeding resources (heifers, young bulls).

Table 2. Number and productivity of dairy cows in European countries, 2022-2023

Country	Total number of dairy cows	Monitored livestock	Proportion of monitored livestock, %	Milk yield, kg	Milk fat, %	Milk protein, %
Austria	546,035	437,712	80.2	7,918	4.16	3.40
Belgium	351,583	202,275	56.8	9,606	4.27	3.51
Croatia	96,477	68,384	70.1	4,975	4.07	3.49
Czech Republic	352,557	339,272	96.1	9,266	3.89	3.42
Denmark	557,831	498,644	91.1	10,571	4.33	3.62
Finland	242,017	189,148	78.2	9,332	4.46	3.56
France	3,429,000	1,968,566	56.2	7,516	–	–
Germany	3,712,815	3,251,621	87.6	8,547	4.12	3.46
Hungary	173,618	177,430	100.0	–	3.96	3.48
Ireland	1,643,470	25,309	100.0	5,301	4.30	3.52
Italy	2,009,834	1,407,368	70.0	–	–	–
Estonia	83,700	81,020	96.8	10,655	3.90	3.40
Latvia	127,803	106,175	83.0	7,748	–	–
Netherlands	1,576,925	1,455,909	92.3	9,346	4.45	3.61
Norway	203,327	178,897	97.7	7,955	4.30	3.55
Poland	2,203,900	807,719	38.0	7,596	–	–
Portugal	218,840	92,149	42.1	8,643		
Romania	133,438	133,438	100.0	7,149	3.83	3.45
Serbia	92,000	52,309	56.9	4,576		
Slovak Republic	114,410	101,392	89.9	8,362	3.79	3.37
Sweden	289,182	179,681	74.2	9,747	4.26	3.53
Switzerland	542,927	416,068	76.6	6,989	4.14	3.36
United Kingdom	265,801	267,322	100.0	8,699	4.34	3.38
Ukraine	115,600	112,388	9.7	7,623	4.19	3.42

Source: developed by the authors based on ICAR (2023a; 2023b)

The majority of stakeholders in Ukraine's dairy industry have adopted the North American model of cattle farming. However, under current conditions – including the limited size of the monitored livestock, the need to reduce dependence on expensive imports (semen, embryos, heifers), global risks of epizootic outbreaks, and the specific economic circumstances of Ukraine's agricultural sector – there is a pressing need to develop a domestic system of genetic evaluation. The direction of such efforts, as outlined in various scientific publications, is based on modern methodologies for breeding value assessment (Ruban *et al.*, 2021).

According to H.D. Norman *et al.* (2022), the historical development of genetic evaluation methods for dairy cattle can be divided into the following stages:

1) Daughter–Dam Comparison (DDC). The breeding value of a bull is estimated as the difference between the performance of his daughters and their dams.

2) Herdmate Comparison (HC). The performance of a bull's daughters is compared to that of daughters of other bulls raised under similar environmental conditions (e.g. herd, age, year and season of calving).

3) Improved Herdmate Comparison (IHC). This method includes adjustments to bull evaluations based on the number of herds in which the daughters were assessed, the distribution of daughters across those herds, and the number of lactations per daughter.

4) Modified Contemporary Comparison (MCC). In this method, a bull's evaluation incorporates information about his ancestors as well as the genetic merit of the parents of his contemporaries. This allows for a more accurate adjustment of the bull's breeding value in line with the genetic trend.

5) BLUP Animal Model (AM). A simultaneous evaluation of all animals (both bulls and cows), taking into account environmental effects and all genetic relationships among animals.

6) Genomic Evaluation (GE). Breeding value assessment based on SNP markers, which represent a substitution of one pair of nucleotides in the DNA sequence with another.

In the study by V.O. Danshin *et al.* (2017), genetic correlations between economically important traits were assessed (Table 3).

Despite some breed-specific differences, the genetic correlations between traits generally indicate a significant unfavourable relationship between milk production and reproductive performance in cows. In contrast, productive longevity shows relatively weak correlations with both milk yield and calving interval. These findings highlight the need to include reproductive performance and productive longevity in the selection index used for choosing sire bulls.

Table 3. Estimates of genetic correlations between traits by breed

Trait	Genetic correlation				
	1	2	3	4	5
Holstein					
1. Milk yield	1				
2. Milk fat	0.95	1			
3. Milk protein	0.75	0.74	1		
4. Calving interval	0.30	0.29	0.19	1	
5. Productive longevity	0.01	0.05	0.06	-0.01	1
Ukrainian Black-and-White Dairy					
	1	2	3	4	5
1. Milk yield	1				
2. Milk fat	0.97	1			
3. Milk protein	0.81	0.80	1		
4. Calving interval	0.28	0.25	0.21	1	
5. Productive longevity	0.05	0.03	0.06	0.02	1
Ukrainian Red-and-White Dairy					
	1	2	3	4	5
1. Milk yield	1				
2. Milk fat	0.92	1			
3. Milk protein	0.77	0.79	1		
4. Calving interval	0.31	0.24	0.18	1	
5. Productive longevity	0.07	0.07	0.08	0.03	1
Ukrainian Red Dairy					
	1	2	3	4	5
1. Milk yield	1				
2. Milk fat	0.93	1			
3. Milk protein	0.79	0.75	1		
4. Calving interval	0.29	0.27	0.23	1	
5. Productive longevity	0.04	0.06	0.07	0.01	1

Source: V.O. Danshin *et al.* (2017)

Since 2007, countries with developed dairy industries – including Australia, Ireland, New Zealand, France, Germany, the Netherlands, Denmark, Sweden, Finland, the USA, and Canada – have adopted genomic selection based on genomic prediction methods (Simm *et al.*, 2021; Xu, 2022). This is based on the method of genomic prediction (Ahmadi & Bartholomé, 2022). The theoretical basis for genomic selection was established

in 2001 by T.H.E. Meuwissen *et al.* (2001). The most comprehensive system of genomic selection, as demonstrated in the U.S. dairy industry, is illustrated in Figure 1. This now-classic approach relies on a so-called reference (or training) population, consisting of animals with known phenotypes and genotypes for SNP markers, which is used to develop equations for predicting the genetic merit of each animal.

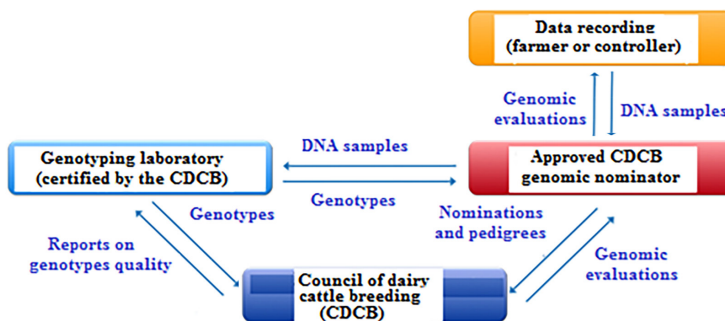


Figure 1. General scheme of genomic evaluation of dairy cattle in the USA

Source: Council on Dairy Cattle Breeding (n.d.)

The first genomic evaluations of PTA (Predicted Transmitting Ability; PTA equals half the breeding value of an animal) in the USA were published in April 2008, although they gained official status in January 2009. Since then, approximately 700,000 animals have been genotyped each year. Genotyping laboratories accredited by the Council on Dairy Cattle Breeding (CDCB), in cooperation with breed associations and artificial insemination organisations, collect DNA samples and pedigree data, and notify the CDCB of their intent to obtain a genomic evaluation for a specific animal – a process known as nomination. Most animals are genotyped using SNP chips (platforms containing probes for identifying SNPs across the genome), with densities ranging from 9,000 to 30,000 SNP markers. These chips provide information on the sequence of nucleotide pairs in the animal's DNA. High-density microchips are primarily used for research and bull evaluation. Each animal's genotype is verified by comparing it with those of its parents and grandparents (Pal, 2022).

Genotypes obtained from chips of varying densities are included in the evaluation process through imputation, which involves inferring missing SNP markers to expand genomic information while reducing costs. Imputation is primarily used for animals genotyped with low-density chips. In this process, genomic information is “borrowed” from animals in the reference population that have been genotyped using higher-density chips (Klímová *et al.*, 2020). For the genomic evaluation of dairy cattle in the USA, 79,294 SNP markers are used. The results of a bull's evaluation are presented in an electronic profile (Fig. 2) available on the ABS Genetics website, which includes the following information:

Since the development of the genomic selection system in the USA, efforts have been made to expand the size of the reference population. In genomic evaluations of Holstein cattle, genotype data are also used from animals in Canada, the United Kingdom, Switzerland, and Germany (Wiggans & Carrillo, 2022).

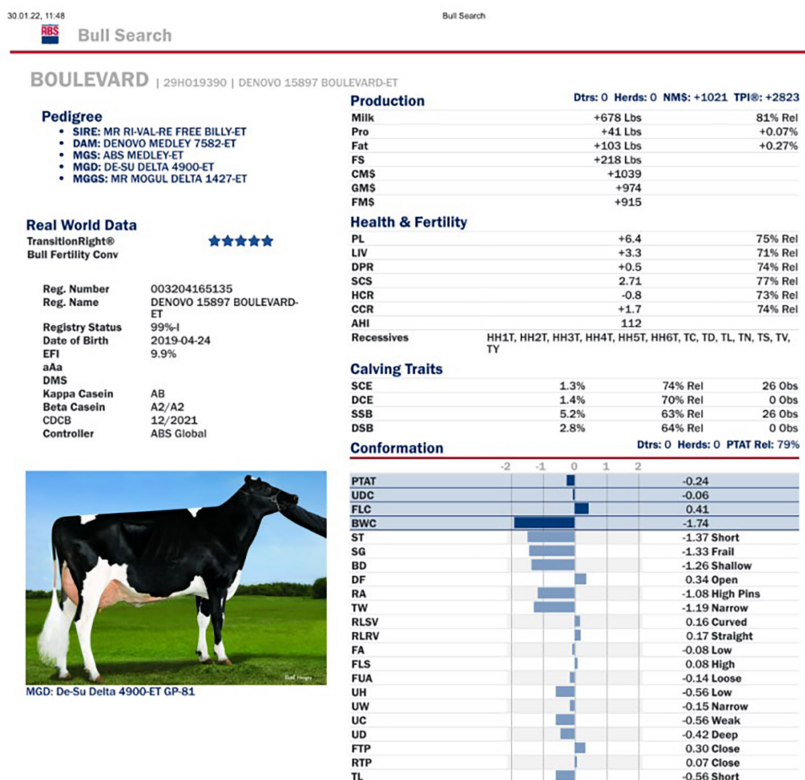


Figure 2. Example of a bull’s electronic profile from ABS Genetics

Source: ABS (n.d.)

As of June 2025, the number of genotyped Holstein animals totals approximately 9 million (Council on Dairy Cattle Breeding, n.d.). As a result of the implementation of genomic selection, the age of a sire at the time of a young bull’s birth has decreased to two years (Wiggans & Carrillo, 2022).

This reduction in generation interval has led to a twofold increase in the rate of genetic progress in the Holstein breed (Guinan *et al.*, 2023). A characteristic trend in dairy cattle breeding is the continuous expansion of selection traits, as illustrated by one of the selection programmes in the USA (Table 4).

Table 4. Composition of economic selection indices used by the United States Department of Agriculture for dairy cattle evaluation and selection, 1971-2022

Trait	Relative economic weight, %										
	PD\$ 1971	MFP\$ 1976	NM\$ 1994	NM\$ 2000	NM\$ 2003	NM\$ 2006	NM\$ 2014	NM\$ 2017	NM\$ 2018	NM\$ 2021	NM\$ 2025
Milk yield	52	27	6	5	0	0	-1	-1	-1	0	3.2
Milk fat	48	46	25	21	22	23	22	24	27	22	31.8
Milk protein		27	43	36	33	23	20	18	17	17	13.0

Table 4. Continued

Trait	Relative economic weight, %										
	PD\$ 1971	MFP\$ 1976	NM\$ 1994	NM\$ 2000	NM\$ 2003	NM\$ 2006	NM\$ 2014	NM\$ 2017	NM\$ 2018	NM\$ 2021	NM\$ 2025
Productive longevity			20	14	11	17	19	13	12	15	13.0
Somatic cell concentration in milk			-6	-9	-9	-9	-7	-7	-4	-3	-2.6
Body weight				-4	-3	-4	-5	-6	-5	-9	-11.0
Udder conformation				7	7	6	8	7	7	3	1.3
Leg and foot structure				4	4	3	3	3	3	1	0.4
Conception rate					7	9	7	7	7	5	2.1
Calving ability						6	5	5	5	3	3.3
Heifer conception rate							1	1	1	1	0.5
Cow conception rate							2	2	2	1	1.8
Cow livability								7	7	4	5.9
Health									2	2	1.5
Residual feed intake										-12	-6.8
Age at first calving										1	1.0
Heifers livability										1	0.8

Note: PD\$ – predicted difference in dollars; MFP\$ – milk, fat and protein combined value in dollars; NM\$ – Lifetime Net Merit in dollars

Source: P.M. VanRaden *et al.* (2021), Council on Dairy Cattle Breeding (n.d.)

In 1971, dairy cattle selection in the USA was based on just two traits – milk yield and milk fat content. Today, however, the NM\$ index (Lifetime Net Merit in dollars) includes twelve economically important selection traits. Similar developments have taken place in other countries. As a result of implementing genomic evaluation and enabling early selection of breeding bulls, the generation interval – the age of the sire at the time of a young bull's birth – has decreased to two years (Wiggans & Carrillo, 2022). This reduction has led to a twofold increase in the rate of

genetic progress in the Holstein breed (Guinan *et al.*, 2023). Furthermore, the availability of such detailed data enables the identification of new predictor traits (from the English predictor – a prognostic parameter or forecasting tool), which can simplify data collection procedures and improve the accuracy of genetic evaluations.

The study by S. Ruban & V. Danshin (2023) demonstrated that the use of genomic selection in dairy cattle breeding in Ukraine could significantly accelerate genetic progress by reducing generation intervals. Shortening generation

intervals along the breeding pathways – sires of bulls, sires of cows, and dams of bulls – would increase milk yield gains from 100.1 kg to 180.0 kg, representing an 80% improvement. In a related study, S.W. Alemu *et al.* (2025) investigated the impact of functional DNA variants on the accuracy of genomic prediction for milk fat content, protein content, milk yield, and total milk fat and protein in lactating dairy cows. This was compared with predictions based on the Illumina 50k SNP chip (covering approximately 50,000 nucleotide sequences). Functional variants were identified through Genome-Wide Association Studies (GWAS) – which examine the likelihood or extent of genomic regions influencing a quantitative trait – as well as RNA sequencing (RNA-seq) and histone modifications (ChIP-seq), and were found to be coding variants. The authors concluded that incorporating functional variants can improve the accuracy of genomic prediction compared to relying solely on SNP markers.

According to D. Lourenco *et al.* (2020), the single-step genomic Best Linear Unbiased Prediction method is gaining popularity. This approach allows for the application of the BLUP method using a mixed linear model, where the relationship matrix between animals consists of three submatrices: 1) a submatrix for genotyped animals; 2) a submatrix for non-genotyped animals; 3) a combined submatrix linking both genotyped and non-genotyped animals. These matrices contain probes that identify SNP genotypes across the entire genome. This method enables the optimal integration of data from both genotyped and non-genotyped animals, increasing the overall dataset size and thereby improving the accuracy of predicted breeding values. M. Bermann *et al.* (2022) found that the single-step genomic BLUP method is widely used to estimate genomic breeding values across various livestock species, as well as in Genome-Wide Association Studies. In such contexts, there arises a need to establish information databases that reflect the phenotypic and genotypic characteristics of specific breeds across multiple generations,

with verified pedigree information for each animal. Meeting these requirements is a key priority for Ukrainian breeders, particularly in the context of applying advanced genomic research methods, and depends heavily on the development of a robust reference population.

In the near future, advances in animal genetics and breeding will be closely linked to information and communication technologies. These encompass computer systems, software, programming languages, and data processing and storage – particularly within reference populations, which are increasingly integrated under the umbrella of omics technologies. The term omics derives from the suffix *-ome*, meaning whole or entire. In life sciences, omics typically refers to fields of study focused on large-scale data to understand biological processes, collectively referred to as the omes. One of the most promising directions for improving the genetic evaluation of dairy cattle is the application of holo-omics, which involves evaluating animals while accounting for the influence of the metagenome, or the genome of the microbial communities in specific organs. Key traits in this context include feed efficiency and methane emissions, both of which are significantly influenced by the rumen microbiota of ruminants (Ross & Hayes, 2022).

Another important area is the use of genome editing technologies in dairy cattle (Van Eenennaam, 2025). These technologies not only have the potential to enhance animal productivity but also to address diseases, including infectious ones. Below are examples of such applications:

1. Bovine Viral Diarrhoea (BVD) is one of the most significant diseases affecting the health and welfare of cattle worldwide. Using CRISPR/Cas9 technology combined with somatic cell nuclear transfer (SCNT), a live calf was produced with six altered amino acids in the BVDV-binding domain of the bovine CD46 gene. The calf exhibited markedly reduced susceptibility to infection, as evidenced by milder clinical symptoms and the absence of viral presence in its leukocytes (Workman *et al.*, 2023);

2. according to G.-M. Gim *et al.* (2023), the CRISPR-Cas9 technique was used to produce calves with edited PRNP genes (conferring resistance to bovine spongiform encephalopathy) and dairy calves with modified MSTN and BLG genes, resulting in the production of milk less likely to cause allergic reactions;

3. bovine herpesvirus type 1 (BHV-1), the causative agent of infectious bovine rhinotracheitis, has also been targeted. H. Dai *et al.* (2022) developed a line of cattle resistant to this virus using the CRISPR/Cas9 system;

4. M. Yuan *et al.* (2021) applied the CRISPR/Cas9 system to modify the ROSA26 locus, identified as a potential GSH in cattle, to develop animals with enhanced resistance to tuberculosis.

An important area in dairy cattle breeding is improving feed efficiency. There is growing scientific interest in the use of omics technologies in livestock selection. In addition to genomics, these include transcriptomics, proteomics, metabolomics, metagenomics, and epigenomics (Chakraborty *et al.*, 2022). Biotechnological methods of reproduction are also rapidly advancing, particularly in vitro fertilisation and the use of Sex-selected semen (Hopper, 2021). These approaches enable the evaluation of an embryo's genetic merit at early stages of development. Special attention is being paid to the application of artificial intelligence in dairy cattle breeding, which is now successfully used to address a wide range of tasks (De Vries *et al.*, 2023).

F. Monteiro *et al.* (2024) demonstrated, using artificial intelligence, that the composition of the rumen microbiome accounts for a significant portion of the variability in residual feed intake in dairy cows. Precision livestock farming, powered by artificial intelligence and sensor-based technologies, offers innovative solutions for advancing milk production. G. Koutouzidou *et al.* (2022) found that these technologies have enabled real-time monitoring and data-driven decision-making, resulting in improved animal welfare and increased productivity. A. De Vries *et al.* (2023) identified the main applications of ar-

tificial intelligence in dairy farming as follows: 1) development of expert systems for optimising feeding, culling, mastitis control, and individual sire bull selection; 2) meta-analysis for management strategies; 3) disease diagnosis; 4) detection of oestrus and prediction of successful insemination; 5) forecasting future milk yield; 5) estimation of individual feed intake based on productivity, behaviour, and metabolic indicators; 6) implementation of voluntary milking systems (robotic milking).

Thus, an analysis of the current state of genetic evaluation of dairy cattle in Ukraine has revealed positive trends in the adoption of advanced breeding approaches, particularly through the adaptation of genomic evaluation methods. At the same time, significant gaps remain in terms of the proportion of monitored livestock and the use of information technologies compared to leading countries. The findings confirm the effectiveness of reducing the generation interval and integrating SNP analysis to achieve significant genetic progress. Incorporating international experience and adapting promising tools – including artificial intelligence, omics technologies, and genome editing – will shape the future directions for improving breeding strategies in Ukrainian dairy farming.

Conclusions

A significant proportion of the dairy cattle livestock in Ukraine consists of Holstein animals or local crossbreeds, with Holstein ancestry accounting for approximately 75%-87.5% of pedigrees. A key limiting factor in the breeding system is the relatively small number of animals under monitored livestock. Genetic evaluation methods for dairy cattle have evolved considerably – from relatively simple approaches such as daughter-dam comparisons to more advanced techniques like the BLUP Animal Model and genomic evaluation – greatly enhancing the effectiveness of selection programmes. The implementation of genomic selection requires a comprehensive organisational overhaul of the entire breeding system, particularly in the Ukrainian context. Such a

system must not only include genetic laboratories and breeding information centres to maintain a continuous flow of data but also involve a revision of specialist training programmes at higher education institutions. A general trend in modern dairy farming is the increasing number of selection traits to better account for economic factors, with the effectiveness of breeding programmes being tested within a reference section of the population of a given breed.

Promising areas of research in this field include the use of genomic selection, holo-omics (evaluating genetic merit based on both the cow's genome and the associated metagenome), and genome editing techniques such as CRISPR-Cas9 in dairy cattle. These approaches, combined with extracorporeal fertilisation and sex-selected semen, offer new opportunities for advancing genetic evaluation methods and implementing effective breeding programmes. An important focus in dairy cattle breeding is the improvement of feed efficiency. The application of Omics technologies – such as transcriptomics, proteomics,

metabolomics, metagenomics, and epigenomics – in livestock selection is considered highly promising. The integration of artificial intelligence into these processes further enhances precision and decision-making in breeding strategies.

To support research in these areas, it would be advisable to establish a dedicated breeding and genetic centre in Ukraine, closely collaborating with reference herds of leading breeds. This would reduce reliance on costly imports of genetic materials such as semen, embryos, and live animals.

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Conflict of Interest

None.

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Сучасні підходи та перспективи генетичної оцінки молочної худоби в селекційних програмах

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Анотація. Оцінка племінної цінності за економічно важливими ознаками є основним інструментом в сучасних системах генетичного покращення молочної худоби. Мета наведених аналітичних досліджень пов'язана з описом сучасного алгоритму генетичної оцінки молочної худоби та визначення перспективних напрямів можливого удосконалення на найближче майбутнє. Встановлено, що методи генетичної оцінки пройшли довгий шлях розвитку від масового порівняння «дочка – мати», прямого та покращеного порівняння з одностадницями, модифікованого методу порівняння з ровесницями до таких комплексних методів як «Модель тварини» і геномна оцінка з використанням математичних підходів BLUP та REML. Впровадження сучасних геномних програм відбору потребує суттєвої перебудови організаційної системи всієї племінної роботи де наявність референтних популяцій з постійним моніторингом генетичних та фенотипових характеристик є основною задачею. Зазначено, що загальною тенденцією в сучасному молочному скотарстві є збільшення числа селекційних ознак для повного врахування реальних (кількість та склад молока), та «прихованих» ознак (стан здоров'я, рівень відтворення, продуктивне довголіття, ефективність використанні корму) які суттєво впливають на економіку виробництва. Проведено порівняльний аналіз підконтрольного поголів'я і показників продуктивності в Україні та країнах-членах ICAR, що дозволило виявити ключові обмеження національної селекційної системи. Встановлено, що використання геномної оцінки дозволяє скоротити генераційні інтервали та подвоїти темпи генетичного прогресу за надоєм. Практична цінність дослідження полягає у формуванні науково обґрунтованих орієнтирів для створення в Україні ефективної системи генетичної оцінки та управління племінними ресурсами

Ключові слова: BLUP «Модель тварини», геномна селекція, голо-оміка, редагування геному, біотехнологія



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Innovative approaches to standardisation in forest commodity science: Trends and prospects

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Abstract. It is important to create a regulatory environment for the integration of digital technologies into standardisation procedures. The aim of the study was to examine the possibilities for harmonising the Ukrainian regulatory framework with international requirements, identify barriers to the introduction of innovations, and outline the prospects for the development of the industry until 2030. The study used methods of comparative analysis of regulatory documents, content analysis of publications, analytical and descriptive methods. Both Ukrainian (DSTU) and international standards (ISO, FSC, PEFC) regulating the requirements for the quality, safety and environmental friendliness of forest products were considered. The study revealed modern innovative approaches to standardisation in forest commodity science, among which digital technologies, blockchain systems, artificial intelligence algorithms and remote monitoring tools played a key role. Based on the analysis of foreign experience (Germany, Canada, Sweden) and Ukrainian practice, the effectiveness of digital platforms for compliance control, online certification systems, the use of drones and satellite monitoring has been proven. It has been determined that leading countries have already implemented integrated ecosystems that combine standards, intelligent algorithms, and certification chains. In Ukraine, electronic product coding and the creation of databases of certified sites have been initiated, but further development is hampered by underfunding, fragmented policies and a

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lack of personnel. Areas for improvement have been proposed: attracting international technical assistance, developing public-private partnerships and updating educational programmes. Particular attention is paid to the need to form a single unified system for the evaluation of forest products in accordance with international trade requirements. The results of this study can serve as a basis for strategic decisions in the field of modernisation of the Ukrainian standardisation system

Keywords: forest products; FSC certification; digital technologies; sustainable development; quality control; innovations in forestry

Introduction

In the US (United States), the Forest Service, which is part of the Department of Agriculture (USDA Forest Service, n.d.), is rolling out artificial intelligence and digital monitoring technologies within its Forest Products Modernisation program. They're really focusing on automating timber quality assessment to cut down on subjective decision-making. On top of that, the system aims to ensure our regulations can be updated quickly via digital platforms. K. Klaric *et al.* (2023) showed that an increase in FSC certificates has a positive effect on Croatian timber exports, strengthening the industry's standing in the international market. The authors highlighted that having these certificates also builds trust with foreign partners, especially under free trade agreements. This research underscored certification's role in boosting competitiveness. However, as E. Lombardo *et al.* (2021) noted, the main drivers for adopting FSC and PEFC certification are often market pressure and companies wanting to be seen as environmentally responsible. Moral, legal, and institutional factors tend to be secondary, although they could play a significant role in further developing voluntary certification. J. Chen *et al.* (2020) argued in their study that it makes sense to harmonise FSC and PEFC standards at the ISO level, creating one global mechanism to simplify requirements and lower costs. They proved that synchronising certification procedures makes it easier for producers to access the market and reduces the expense of going through double procedures. The authors concluded that this kind of standard consolidation helps create a unified digital environment across supply chains.

Forest certification is increasingly seen not just as a quality control mechanism, but also as a tool for environmental marketing. A systematic review of blockchain technology use in the forestry sector by Z. He & P. Turner (2022) confirmed this innovative architecture's significant potential for improving the reliability and immutability of data in certification processes. Their proposed concepts have formed the basis for practical recommendations on how to integrate blockchain systems into Ukraine's electronic timber origin registries. K. Fernholz *et al.* (2021) pointed out that a significant portion of certified areas worldwide undergo dual certification (both FSC and PEFC simultaneously), which shows a drive for international recognition between systems. They noted that this duplication adds an extra burden on forestry enterprises but also opens up access to a wider range of markets. Their report also stressed the need for digitally integrating FSC and PEFC databases to avoid duplication.

In Ukraine, there's growing research on harmonising national standards with international ones. S.M. Bondarenko & A.V. Kopa (2017) highlighted the necessity of adapting FSC, PEFC, and ISO certification systems to the conditions of national forestry. The authors noted that successful implementation of international standards is only possible if the management and infrastructure base is modernised. They also pointed to the lack of trained personnel in certification auditing as a systemic problem. N. Marchenko *et al.* (2017) analysed the specifics of implementing harmonised DSTU EN standards for coniferous

timber, pointing out structural differences in classification approaches and quality requirements. Specifically, the authors emphasised the inconsistency between national technical specifications and European grading criteria. Their work highlighted the need to unify methods for assessing the physical and mechanical characteristics of timber.

P.H. Khomiuk *et al.* (2021) investigated the evolution of stand mensuration regulations and changes in roundwood grade classification according to new requirements. Their results indicate an improvement in the timber assessment system, particularly through its division into four quality classes and the differentiation of firewood by purpose. The researchers noted the need to implement digital tools for recording mensuration indicators to improve accounting accuracy. They propose updating methodological approaches in line with European practices of laser scanning and GIS technologies.

The aim of this study was to analyse contemporary innovative approaches to the standardisation of forest products, assess their effectiveness, and determine the possibilities for their implementation in Ukrainian practice. To achieve this aim, the following tasks were set:

- to analyse the current national and international standards in the field of forest commodity science;
- to identify the main problems that hinder effective standardisation in Ukraine;
- to formulate recommendations for improving the standardisation system in Ukraine, taking into account international requirements and industry specifics.

Materials and Methods

The study was conducted in 2023-2024, taking into account the experience of four countries: Ukraine, Canada, Germany, and Sweden. The geographical coverage was determined based on the relevance of digital technologies in the standardisation of forest products and the level of implementation of international certification systems.

The methodological basis was provided by analytical, descriptive and comparative methods.

The analysis covered State standards of Ukraine (DSTU 2152-93, 1993; DSTU 2980-95, 1995; DSTU 3404-96, 1996; DSTU EN 1309-1:2001, 2001; ISO 14001:2015, 2015; DSTU EN 1309-3, 2018), and international standards (FSC Standards, n.d.; ISO 38200:2018, 2018; Standards and implementation, 2023), in particular technical requirements for classification, measurement, certification and environmental management. The provisions of standards regarding the physical and mechanical characteristics of wood, criteria for sustainable forest management, requirements for transparency of product origin and conformity assessment procedures were evaluated. Particular attention was paid to the harmonisation of national standards with international ones, as well as to the assessment of the role of digital tools – blockchain technologies, remote sensing systems, drone monitoring and IoT solutions – in ensuring transparency and improving the effectiveness of standards.

The study used publications from scientific journal databases and reports of international organisations – The German National Forest Inventory (n.d.), FSC Standards (n.d.), FSC (n.d.), Forest Bioeconomy Cluster (n.d.), Precision forestry (n.d.), Law of Ukraine No. 1315-VII (2014), ISO 38200:2018 (2018) та Standards and implementation (2023).

Results and Discussion

In a general scientific context, the term “standardisation” is interpreted as activities aimed at establishing, implementing and applying rules for regulating various types of activities in specific areas that meet modern technical, economic and social requirements. According to Law of Ukraine No. 1315-VII (2014), standardisation is “an activity that consists of establishing provisions for general and repeated application in current or future activities in order to achieve an optimal degree of order in a particular field.” The main objectives of standardisation in the field of forest products

are to ensure stable quality, safety and compliance with environmental requirements. It allows for the unification of approaches to product evaluation, ensures traceability of origin and creates a unified system of technical requirements that are understandable to both the manufacturer and the consumer. In modern conditions, particular attention is paid to sustainable forest management standards, which, in addition to technical characteristics, also take into account social and environmental aspects.

In forest product commodity science, standardisation serves not only as a technical regulation, but also as a mechanism for ensuring the transparency of supply chains and the legality of wood origin. It creates a basis for objective evaluation of the quality of raw materials and finished products, determining their compliance with consumer and market requirements. According to the results of research by K. Klaric *et al.* (2023), certification based on international standards, in particular FSC, directly influences the competitiveness of forestry enterprises in the international market.

Standardisation also plays a decisive role in foreign trade, as compliance with international standards is often a prerequisite for entering European and global markets. In addition, standards ensure transparency and reliability in certification, allowing stakeholders – from consumers to

regulatory authorities – to obtain verified information about the quality, environmental friendliness and safety of products (Pavlishchuk *et al.*, 2022).

According to S. Pezdevšek Malovrh *et al.* (2019), FSC certification has a positive impact not only on export opportunities but also on domestic sustainable forest management practices, especially in the public sector. It contributes to the resolution of social and environmental issues, improves working conditions and the safety of loggers, and raises environmental awareness in the industry. At the global level, as noted by M. Bösch (2025), the spread of FSC and PEFC certification is closely linked to the quality of governance, the level of economic development and the foreign trade orientation of the forestry sector. The author also points out that countries with more active environmental organisations have higher levels of FSC certification, while PEFC is more common where this pressure is lower.

In the field of forest products in Ukraine, both national standards (DSTU) are applied, which regulate the requirements for the quality, safety and environmental friendliness of timber in accordance with the state regulatory framework, and international standards – ISO, FSC and PEFC, which ensure that products comply with global requirements for sustainable development, transparency of origin, environmental management and competitiveness in global markets (Table 1).

Table 1. Comparative characteristics of national and international standards in the forestry sector

Designation and title of the standard	Summary and characteristics	Geography of application	Functional purpose
DSTU (State standards of Ukraine)	National regulatory documents governing product quality, safety and environmental requirements. Harmonised with European EN standards	Ukraine, in particular state-owned and commercial forestry enterprises	Technical regulation of the quality of round timber, sawn timber, harvesting processes
ISO (International Organisation for Standardisation)	International standards for quality and environmental management; ISO 14001 – environmental management, ISO 38200 – timber supply chain	All countries involved in international trade, international companies, certification bodies	Environmental risk management, traceability of wood origin

Table 1. Continued

Designation and title of the standard	Summary and characteristics	Geography of application	Functional purpose
FSC (Forest Stewardship Council)	Voluntary international forest certification system. Defines the principles of sustainable forest management	Ukraine (over 4.7 million hectares of certified forests), Europe, North America, globally	Forest certification, supply chain control, compliance with environmental and social criteria
PEFC (Programme for the Endorsement of Forest Certification)	Global system for the recognition of national certification programmes. An alternative to FSC with a focus on local initiatives	In Ukraine – since 2021, also in over 50 countries worldwide	Certification of sustainable forest management, support for local standards, compliance with international environmental requirements

Source: developed by the authors based on FSC Standards (n.d.), FSC (n.d.), DSTU 2152-93 (1993), DSTU 2980-95 (1995), DSTU 3404-96 (1996), DSTU EN 1309-1:2001 (2001), ISO 14001:2015 (2015), DSTU EN 1309-3 (2018), ISO 38200:2018 (2018), Standards and implementation (2023)

The application of these standards promotes the integration of the Ukrainian forestry sector into international economic processes and improves the image of Ukrainian timber as a high-quality and responsible resource. The existence of a unified regulatory framework allows companies not only to meet the requirements of the domestic market, but also to enter foreign markets with products certified in accordance with international environmental and technical criteria, which, in turn, builds trust among international partners, promotes the

growth of the industry's investment attractiveness and provides competitive advantages for Ukrainian producers.

Within the framework of the state technical regulation system in Ukraine, there is a set of industry standards covering various stages of forest product circulation – from taxation and harvesting to classification, measurement and determination of wood quality characteristics. These include standards for terms and definitions, measurement methods, volume tables, and systems for classifying and identifying wood defects (Table 2).

Table 2. Classification of national standards regulating activities in the forestry sector

Standard designation	Name of standard	Purpose of the standard
DSTU 3404-96	Forestry. Terms and definitions	Terms and definitions of basic concepts in forestry
DSTU 2980-95	Forest cultures. Terms and definitions	Terms related to forest cultures, seed production, nurseries
DSTU 2152-93	Wood defects and processing defects. Terms and definitions	Terms related to wood defects and processing defects
DSTU EN 1309-1:2001	Method of measuring dimensions. Part 1. Sawn timber	Methods for measuring the dimensions of sawn timber
DSTU EN 1309-3	Measurement methods. Part 3. Biological damage	Methods for measuring the characteristics of biological damage

Source: developed by the authors based on DSTU 2152-93 (1993), DSTU 2980-95 (1995), DSTU 3404-96 (1996), DSTU EN 1309-1:2001 (2001), DSTU EN 1309-3 (2018)

Intensive globalisation and the growing role of international environmental initiatives are leading to the gradual alignment of forest products with global standards and sustainable development principles. International markets are increasingly demanding not only product quality and safety, but also transparency of origin, environmental responsibility of producers and compliance with social criteria. The Paris Climate Agreement and the European Green Deal, which set strategic guidelines for countries seeking to integrate into the European market, have a significant impact on environmental policy in the field of forest use (FSC, n.d.). In this context, there is a growing need not only for the implementation of internationally recognised standards (in particular FSC and ISO 14001), but also for the digital transformation of the forestry sector (Exploring the impact of the Paris Agreement..., 2024). At the same time, a study by J. Stubenrauch *et al.* (2022) emphasised that large-scale forest restoration in line with climate goals could have a reverse effect on forest ecosystems, in particular due to the threat to biodiversity posed by the creation of homogeneous forest plantations. Rapid growth in production volumes, the need to ensure timber traceability and minimise the human factor in inspections are driving the active use of automated solutions. Modern tools – in particular GPS monitoring systems, drone photography, satellite sensing and blockchain – enable operational control, accurate recording of raw material quality parameters and ensure the consistency and reliability of data in supply chains (Stopfer *et al.*, 2024). All this not only increases management efficiency, but also ensures that forest products comply with certification requirements, transparency of inspections and access to markets with high environmental standards.

However, despite the active development of international standards, the lack of uniform technical requirements for forest products in different countries remains a significant problem. Discrepancies in wood grade classification, documentation requirements, environmental criteria,

or certification procedures complicate export and import operations and create an additional burden on producers. This is particularly relevant for countries with economies in transition, which are forced to adapt their national standards to ISO, FSC or PEFC standards, requiring significant financial, technical and organisational resources. The lack of a harmonised evaluation system complicates the process of international recognition of certificates, reducing the competitiveness of products and slowing down their promotion on world markets. In this regard, there is a growing need to unify approaches to standardisation both at the level of regional unions (EU) and within bilateral trade agreements.

Traditional regulatory approaches are being replaced by digital solutions, including electronic certification, remote monitoring, blockchain systems and artificial intelligence. This approach ensures comprehensive traceability of raw materials, increases the accuracy of inspections, reduces the risk of fraud and optimises management decisions. The use of digital platforms in the forestry sector is already actively practised in many countries, including Germany and Canada, where electronic certification systems are integrated into national databases for managing timber supply chains (Chain of custody certification, n.d.). Ukraine is also implementing similar solutions through initiatives of the State Forest Resources Agency, including the use of QR coding of products and the creation of electronic registers of certified areas (State Forest Resources Agency of Ukraine, 2024).

Blockchain plays a special role in strengthening trust in certified products, as it allows for the storage of an unalterable chronology of data on the origin of timber, logistics, processing and final circulation. As part of the FSC Blockchain Pilot project, as well as through independent initiatives in EU countries, blockchain is being used as a technology that eliminates gaps in supply chain transparency and guarantees the accuracy of information at every stage (FSC, n.d.). At the same time, remote monitoring based on satellite images and drones is becoming increasingly

widespread in forestry. Systems such as Starling were already being used around the world to monitor deforestation and forest inventory (Monitor deforestation..., n.d.), while in Ukraine, drones were helping to record changes in areas where ground monitoring was difficult.

Improvements in quality control also become possible through the introduction of intelligent systems based on artificial intelligence and the Internet of Things (IoT). Sensors and cameras connected to analytical algorithms automatically monitor the moisture, density and geometric characteristics of wood, enabling efficient sorting and minimising resource losses. In Sweden, Canada and a number of other countries, AI is already being used in the harvesting and processing

stages of wood production to create digital product passports (The future of forestry: High-tech tools leading the way, 2024). The introduction of such systems in Ukraine is a promising step towards modernising standards and improving the quality and market attractiveness of domestic forest products. In the context of the global digitalisation of forestry, countries such as Germany, Canada and Sweden are demonstrating best practices in integrating innovative technologies into the standardisation and management of forest resources. The experience of these countries includes digital platforms, automated control systems and artificial intelligence, which ensure transparency, efficiency and compliance with international standards (Table 3).

Table 3. International practices of digital transformation of the standardisation system in the forest industry

Country	Digital technologies used	Objective/results of implementation
Germany	Remote sensing systems, PEFC digital audit, national digital databases	Ecosystem assessment, environmental audits, certification transparency
Canada	Online courses on forest management, professional educational platforms	Professional development, implementation of sustainable practices
Sweden	Digital learning platforms, AI forest analysis systems, digital twins	Landscape management, effective training, accurate planning
Ukraine	QR coding, drone monitoring, electronic registers	Transparency of origin, logging control, compliance with standards

Source: developed by the authors based on The German National Forest Inventory (n.d.), Online micro-certificate: Forest management planning (n.d.), Precision forestry (n.d.), Forest Bioeconomy Cluster (n.d.), V. Myroniuk *et al.* (2024)

In Germany, PEFC Germany (n.d.) certifies forest management in accordance with sustainable management principles, including regular environmental audits. In addition, the Thünen Institute implements a national forest inventory using remote sensing and mathematical modelling, which allows for a comprehensive evaluation of the state of forest ecosystems (The German National Forest Inventory, n.d.).

Canada is notable for its developed system of professional training in forestry. For example, the University of British Columbia offers online courses in forest management planning, which allow specialists to gain up-to-date knowledge

on sustainable development in the forestry sector (Online micro-certificate: Forest management planning, n.d.).

Sweden is actively implementing digital solutions for professional training and upgrading the skills of forestry specialists. Biometria, in collaboration with Xtractor, has developed a digital learning platform that provides effective training for employees in line with modern industry requirements. As part of the Precision Forestry project, which is being implemented jointly with AI Sweden, technologies are being developed for analysing forest areas using artificial intelligence, which contributes to the creation of

digital twins of landscapes for management decisions (Precision forestry, n.d.).

The effective implementation of innovations in the forestry sector largely depends on cooperation between government agencies, businesses and scientific institutions. In Canada, there is the Forest Bioeconomy Cluster (n.d.), which aims to develop innovations in the bioeconomy with the active participation of industry, government and research centres. In Sweden, a similar role is played by the Paper Province association (n.d.), which coordinates joint projects between businesses, universities and regulators to improve the competitiveness and environmental performance of the pulp and paper industry.

Ukraine has the potential to implement modern digital technologies in forestry. In particular, the use of remote sensing and satellite data allows for effective monitoring of forest conditions, detection of illegal logging, and evaluation of the impact of military operations on forest ecosystems. For example, a study published in the journal *Forest Ecology and Management* proposes a national framework for monitoring Ukraine's forest resources using remote sensing, which is an effective tool for spatial assessment of changes in forests (Myroniuk *et al.*, 2024).

The main obstacles to innovation in Ukraine's forestry sector are underfunding, bureaucratic procedures and a shortage of qualified specialists. To overcome these problems, it is necessary to intensify state support programmes, attract international grants and develop partnerships between state institutions, scientific institutions and the private sector. In particular, international initiatives such as the Forest Recovery project (Updates on Ukrainian forest status, n.d.), aimed at the restoration and sustainable development of Ukrainian forests, can serve as a platform for the introduction of modern technologies and the exchange of experience.

By 2030, Ukraine is expected to make significant progress in implementing innovative standards in forestry, with the widespread use of artificial intelligence and the Internet of Things

technologies for monitoring and managing forest resources. Another important area will be the integration of blockchain technologies to ensure transparency and traceability of wood origin, which is a key factor for entering international markets with high environmental standards. Participation in international initiatives and adaptation to global environmental requirements will help strengthen Ukraine's position in the global forest products market.

The results of the study confirm the high relevance of introducing innovative approaches to the standardisation of forest products, particularly in terms of harmonising national technical regulations with international standards. This approach is in line with the current scientific paradigm and finds conceptual support in the research of other experts. In the Swedish experience presented by E. Mattsson *et al.* (2024), justifies the introduction of methodological indicators of sustainable forest management that take into account not only technical but also environmental and social criteria. A similar logic can be seen in the current approach, which provides for a comprehensive evaluation of the compliance of forest products with the requirements of sustainable development.

The digital transformation of the forestry industry is seen as a systemic response to the need for greater transparency, efficiency and trust in certification procedures. In this context, Y. Mendel's (2022) conclusions are particularly relevant, as they highlight the expediency of widespread implementation of information technologies to modernise Ukraine's timber industry complex. The results obtained in the current study not only confirm this thesis but also develop it by emphasising the importance of using blockchain platforms, remote monitoring systems and intelligent accounting tools. The study by O.Z. Mykytyn (2015) analyses the state of adaptation of the national certification system to the requirements of the European Union. The author argues that there are significant barriers to harmonisation, particularly in the areas of classification, sorting and methods of assessing wood quality, which are fully

consistent with the analytical conclusions of the current study regarding the need to unify technical procedures and conformity assessment criteria.

V. Makarova *et al.* (2023) examined the impact of digital tools on the effectiveness of foreign economic activity of forestry enterprises. The positive effect of the introduction of ERP systems, electronic auctions and automated supply chains, as documented by the authors, complements the vision in the current study of digital transformation as a strategic vector for the development of the industry. This approach not only increases transparency but also reduces transaction costs and contributes to the formation of a favourable investment climate. Y.I. Hayda (2015) interpreted it as a factor in the formation of the environmental reputation of enterprises, which opens up additional opportunities for integration into international markets. The current study specifies this idea through an empirical analysis of the impact of FSC and PEFC certificates on increasing export potential and trust on the part of international counterparties. The study by F. Ehrlich-Sommer *et al.* (2024) focuses on the development of sensor technologies and artificial intelligence in forest management systems. The current analysis is fully consistent with these provisions: the use of IoT devices and automatic visual control systems is considered an effective tool for improving measurement accuracy, optimising sorting processes and preventing resource losses.

Within the Ukrainian context, A. Deyneka's (2013) analysis is worth noting, as the author identifies critical limitations to implementing FSC certification in Eastern Ukraine. These include staff shortages, low levels of internal control and fragmented regulatory policy. The proposals identified in the current study – the development of electronic registers, strengthening institutional capacity and digital integration of certification data – can be considered practical steps towards overcoming these barriers. During the study of the digital transformation of the forest product standardisation system, the above scientific provisions on the harmonisation

of standards, the introduction of innovations and the improvement of transparency in certification procedures were taken into account.

Conclusions

Standardisation in forestry is transforming from a technical regulation tool into a comprehensive mechanism for ensuring quality, traceability of origin and environmental responsibility of products. The adaptation of national standards to international FSC, PEFC and ISO requirements contributes to the integration of the Ukrainian forestry sector into global markets and meets the challenges of sustainable development. Digital transformation tools are particularly important, as they enable real-time compliance monitoring and increase transparency and trust in Ukrainian timber.

The study found that leading countries, including Sweden, Canada and Germany, have already implemented digital forest resource management models based on remote monitoring, artificial intelligence and blockchain technologies. The use of such tools allows for effective control of timber quality, minimisation of the human factor and increased environmental responsibility of producers. At the same time, initiatives on QR coding and the creation of electronic registers have only recently begun in Ukraine, which indicates existing potential but also significant lag. An analysis of the regulatory framework revealed significant gaps, including the non-compliance of national standards with international technical requirements, fragmented assessment methods, and weak integration of environmental criteria. Problems of insufficient funding, a shortage of qualified personnel, and imperfect certification procedures are also confirmed by other studies.

Despite positive examples, such as the introduction of satellite monitoring, QR coding and e-certification, the development of innovation is hampered by limited funding, bureaucracy and a shortage of qualified specialists. To overcome these barriers, it is necessary to expand public-private partnerships, attract international

grants and intensify scientific research in the field of digital standardisation. The use of artificial intelligence, IoT and blockchain to create fully transparent supply chains that meet EU requirements is promising.

The results of the study demonstrate the feasibility of a comprehensive approach to modernising the standardisation of forest products, with an emphasis on digital infrastructure, international cooperation and regulatory harmonisation. Further research in the direction of standard unification, evaluation of the effectiveness of digital tools, and adaptation of international practices to the Ukrainian context is promising. Such a

scientific and practical approach will strengthen Ukraine's position in the certified forest products market by 2030 and ensure the sustainable development of the industry in line with global environmental guidelines.

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Інноваційні підходи до стандартизації в лісовому товарознавстві: тенденції та перспективи

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Анотація. Актуальним є створення нормативного середовища для інтеграції цифрових технологій у стандартизаційні процедури. Мета дослідження полягала у вивченні можливостей гармонізації української нормативної бази з міжнародними вимогами, виявленні бар'єрів впровадження інновацій та окресленні перспектив розвитку галузі до 2030 року. У межах дослідження застосовано методи порівняльного аналізу нормативних документів, контент-аналіз публікацій, аналітичні й описові методи. Розглянуто як українські – ДСТУ, так і міжнародні стандарти – ISO, FSC, PEFC, що регламентують вимоги до якості, безпеки та екологічності лісової продукції. У результаті проведеного дослідження виявлено сучасні інноваційні підходи до стандартизації в лісовому товарознавстві, серед яких ключове значення мають цифрові технології, блокчейн-системи, алгоритми штучного інтелекту та засоби дистанційного моніторингу. На основі опрацювання зарубіжного досвіду (Німеччина, Канада, Швеція) і української практики доведено ефективність цифрових платформ для контролю відповідності, систем онлайн-сертифікації, використання дронів і супутникового моніторингу. Визначено, що провідні країни вже впровадили інтегровані екосистеми, які поєднують стандарти, інтелектуальні алгоритми та сертифікаційні ланцюги. В Україні ініційовано електронне кодування продукції, створення баз сертифікованих ділянок, але подальший розвиток стримується через недофінансування, фрагментарність політики та нестачу кадрів. Запропоновано напрями удосконалення: залучення міжнародної технічної допомоги, розвиток державно-приватного партнерства, оновлення освітніх програм. Особливу увагу приділено необхідності формування єдиної уніфікованої системи оцінювання лісопродукції відповідно до вимог міжнародної торгівлі. Результати цього дослідження можуть слугувати основою для формування стратегічних рішень у сфері модернізації української системи стандартизації

Ключові слова: лісова продукція; сертифікація FSC; цифрові технології; сталий розвиток; контроль якості; інновації в лісовому господарстві



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Effect of mineral nutrition and nitrogen-fixing agent on maize yield in the Central Forest-Steppe of Ukraine

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Abstract. In modern agricultural production, there is an increasing demand for the use of biopreparations, particularly nitrogen-fixing agents, which can reduce the need for mineral fertilisers while enhancing maize yields. This study aimed to determine the effects of different fertilisation

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strategies and plant densities on the morphological characteristics and yield of maize under the agroecological conditions of the Central Forest-Steppe of Ukraine. Field experiments were conducted during 2023-2024 under the soil and climatic conditions of a farm in the Kyiv Region, using the maize hybrid P9255 (FAO 330). The area of each experimental plot was 33 m², with four replications and a sequential multilevel arrangement. The application of a nitrogen-fixing biological agent in combination with mineral fertilisers had a positive effect on plant growth. In particular, plant height was 1.7%-5.1% greater than in the treatments where only mineral fertilisers were applied. An evaluation was carried out to determine the effectiveness and impact of optimising the fertilisation system on the morphological characteristics and productivity of maize plants through the use of a biological preparation in combination with mineral fertilisers at various application rates. Overall, the application of mineral fertilisers alone resulted in a 14.5%-22.5% increase in grain yield compared to the control. The highest yield, reaching 12.94 t/ha, was recorded under the N₁₅₀P₁₂₀K₁₂₀ treatment at a plant density of 70,000 plants/ha. In treatments combining mineral fertilisers with the biological agent, grain yield was 12.2%–21.2% higher than in the corresponding control variants. The highest average grain yield, amounting to 13.38 t/ha, was achieved with the N₁₅₀P₁₂₀K₁₂₀ + nitrogen-fixing agent treatment at a plant density of 70,000 plants/ha. The application of the biological preparation in combination with mineral fertilisers produced a 3.6% (or 0.43 t/ha) higher grain yield compared to treatments without the biological agent

Keywords: biological preparation; plant density; plant height; mineral fertilisers; grain yield

Introduction

Improving the efficiency of resource use in crop production is one of the key objectives of modern agriculture. In the context of climate change, the limited availability of mineral fertilisers, and the growing need for environmentally sustainable technologies, the adoption of alternative or combined approaches to crop nutrition, including the use of biological agents, is becoming increasingly important. Maize (*Zea mays* L.) is one of the leading food and fodder crops, occupying a prominent position in the structure of grain production in Ukraine. Its high sensitivity to mineral nutrition makes it a suitable model crop for evaluating fertiliser efficiency. The combination of mineral and biological nitrogen sources may serve as a tool for increasing yields while reducing dependence on conventional agrochemicals (Mokrienko & Kornienko, 2024).

Maize is cultivated in most countries around the world. According to N. Tanklevska *et al.* (2020), approximately half of global maize production is concentrated in the USA and China,

which are also the largest consumers and processors of maize products. Ukraine accounts for around 3.2% of global maize grain production. At the same time, maize represents nearly 50% of Ukraine's total grain output, making it a strategically important crop for ensuring the sustainable development of the national agricultural sector. According to B. Kamenshchuk (2020), maize yield and production volumes can be increased by improving cultivation technologies through the realisation of genetic potential, the introduction of new high-yielding hybrids, the use of modern plant protection products, and the optimisation of mineral nutrition.

Maize has the potential to produce high grain and green mass yields on all soil types suitable for the cultivation of major agricultural crops. Optimal conditions for maize growth and development are found on fertile soils that are well-supplied with moisture and free from waterlogging. According to O.I. Zinchenko *et al.* (2001), the presence of mineral nutrients in the soil, along with a near-neutral

or slightly acidic pH level, is an important factor for the robust development of the root system and the overall health of the crop. For grain maize production, chernozem and dark chestnut soils with high fertility are considered optimal.

At present, agricultural production in Ukraine is experiencing rising costs for various technological inputs, including fuel and lubricants, seeds, pesticides, and particularly mineral fertilisers. This situation necessitates the development of improved cultivation and fertilisation technologies for maize. The combined use of mineral fertilisers and biological agents has a beneficial effect on soil properties, helping to maintain soil fertility and facilitate the mobilisation of phosphorus and potassium from insoluble compounds into plant-available forms. This enhances the efficiency of mineral fertilisers, improves nutrient uptake, and increases the resilience of crops to adverse environmental conditions, ultimately contributing to higher yields. Research on the application of biological preparations, particularly nitrogen-fixing agents, is of increasing relevance. These agents positively influence crop productivity and can reduce the need for nitrogen fertilisers (Hovenko, 2022).

Researchers in various countries are studying biological preparations based on the nitrogen-fixing endophytic bacterium *Methylobacterium symbioticum*. According to R. Torres Vera et al. (2024), this bacterium interacts with the host plant by supplying it with nitrogen from the atmosphere. When applied to the plant in the form of an aqueous solution, colonisation by naturally occurring bacteria takes place, and they continue to coexist with the plant throughout the growing season. As a result, crops may receive an additional nitrogen supply of 20–30 kg/ha or more. In particular, M.Â. Rodrigues et al. (2024) evaluated nitrogen fixation in maize plants treated with *Methylobacterium symbioticum* during 2021 and 2022. The additional nitrogen provided reached 58.8 kg/ha and 14.5 kg/ha, respectively. According to their findings, further research should focus on identifying which crops and growing conditions

allow nitrogen-fixing agents to be most effective in enhancing nitrogen fixation and improving plant productivity.

One of the key morphobiological traits influencing plant growth and development is stem height. This characteristic is particularly important in maize, where it has both biological and technological significance and a considerable impact on crop productivity and yield. Based on three years of research, I.I. Senyk et al. (2023) established a direct correlation between plant height in maize and the rate of mineral nitrogen application. The optimisation of nutritional conditions through balanced rates of mineral fertilisers combined with biological preparations leads to more intensive stem growth and enhanced leaf area development, which positively affects overall crop yield.

Therefore, investigating the effectiveness of combining mineral fertilisers with biological preparations in maize fertilisation systems is a crucial research objective. Such approaches create favourable conditions for plant growth and development, ultimately contributing to higher productivity, which remains a highly relevant goal.

This study aimed to examine the effects of fertilisation system optimisation and plant density on the morphological characteristics and productivity of maize crops under the conditions of the Central Forest-Steppe of Ukraine.

Materials and Methods

Field research was carried out during 2023–2024 following standard methodologies developed by A.P. Lisoval (2001), S.O. Trybel et al. (2001), and V.O. Ushkarenko et al. (2019). The study was conducted under the soil and climatic conditions of the Liubaretske agricultural limited liability company (ALLC) in the Boryspil District of Kyiv Region (coordinates: 50°13'19.8"N, 31°09'49.6"E), using the maize hybrid P9255 (FAO 330). The experimental field is characterised by leached, low-humus chernozem soil with a humus content of 3.8%. The content of available nutrients in the soil was as follows: NO₃ –

2.05 mg/100 g of soil, P_2O_5 – 7.3 mg/100 g, K_2O – 14.0 mg/100 g. The soil pH was 6.2, and the bulk density was 1.2 g/cm³. Each experimental plot had an area of 33 m². The experiment was arranged in four replications using a sequential multi-tier layout. The field trial design included variants with different application rates of

mineral fertilisers, as well as treatments with the biological preparation–Blue N, based on the nitrogen-fixing endophytic bacterium *Methylobacterium symbioticum*. Control treatments included unfertilised plots and plots treated with the biological preparation alone, all tested under three different plant densities (Table 1).

Table 1. Field experiment design

Variant	Mineral fertiliser application rate, kg/ha active ingredient	Biopreparation rate, kg/ha	Plant density, thousand plants/ha		
Control (no fertiliser)	–	–	60	70	80
NPK fertiliser + urea	$N_{60}P_{45}K_{45}$	–	60	70	80
NPK fertiliser + urea	$N_{90}P_{60}K_{60}$	–	60	70	80
NPK fertiliser + urea	$N_{120}P_{90}K_{90}$	–	60	70	80
NPK fertiliser + urea	$N_{150}P_{120}K_{120}$	–	60	70	80
Control (no fertiliser) + nitrogen fixer	–	0.333	60	70	80
NPK fertiliser + urea + nitrogen fixer	$N_{60}P_{45}K_{45}$	0.333	60	70	80
NPK fertiliser + urea + nitrogen fixer	$N_{90}P_{60}K_{60}$	0.333	60	70	80
NPK fertiliser + urea + nitrogen fixer	$N_{120}P_{90}K_{90}$	0.333	60	70	80
NPK fertiliser + urea + nitrogen fixer	$N_{150}P_{120}K_{120}$	0.333	60	70	80

Source: developed by the authors

Mineral fertilisers were applied to the soil at rates specified in the experimental design. NPK fertiliser ($N_{15}P_{15}K_{15}$) was used as the base fertiliser, supplemented with urea (N_{46}). The fertilisers were broadcast and incorporated into the soil using a cultivator to a depth of 10-12 cm. After emergence, when the plants reached the 5-6 leaf stage, plots in the relevant treatments were sprayed with the biological preparation at the recommended application rate of 333 g/ha. The working solution volume used was 250 L/ha.

The study was conducted in compliance with the Convention on Biological Diversity (1992).

Results

The research area is characterised by unstable and insufficient precipitation. The average annual relative humidity is 76%, with fluctuations ranging from 36% to 99%. In 2023, total precipitation during the growing season reached 375.4 mm, exceeding the long-term average by 9.4%. The hydrothermal coefficient (HTC) for that year was 1.23 (Table 2).

Table 2. Meteorological conditions during the growing season, Boryspil District

Indicator	2023	2024	Long-term average
Sum of active temperatures (April-September), °C	3,063.6	3,412.5	2,825.0
Sum of effective temperatures (> 10°C), April-September	1,463.6	1,715.4	1,300.0
Total precipitation, April-September, mm	375.4	329.2	343.0
HTC	1.23	0.96	1.21

Source: compiled by the authors based on measurements from the i-Metos online weather station

In 2024, the total precipitation was only 329.2 mm, amounting to 95.9% of the long-term norm. The HTC for that year was 0.96, indicating mild drought conditions. In 2023, between June and the first ten-day period of July, 116.8 mm of rainfall (31.1% of total growing season precipitation), while in 2024, this figure was 102.6 mm (31.2%), suggesting that maize plants received adequate moisture during the pre-flowering period. Overall, an analysis of weather data for 2023-2024, compared with long-term averages, indicates that the amount of rainfall during the growing season is generally sufficient to support high crop yields, particularly for maize.

In 2023 and 2024, a gradual increase in the sums of active (SAT) and effective temperatures (SET) was observed. The effective temperature sums exceeded the long-term average by

163.6°C-415.4°C. Such an increase in heat accumulation may accelerate the growth and development of agricultural crops, leading to earlier maturation. This was evident in 2024, when maize harvesting began in late August – approximately two weeks earlier than usual – at grain moisture levels ranging from 14% to 25%, depending on the maturity group of the hybrids.

The application of mineral fertilisers improved nitrogen nutrition in maize plants, resulting in more vigorous growth and development. Plant height was measured during the flowering stage, which occurred in the first and second ten-day periods of July, allowing for the assessment of the influence of the studied factors on this trait. It was found that applying the lowest rate of mineral fertilisers increased the height of hybrid P9255 plants by an average of 3.7% compared to the control (Fig. 1).

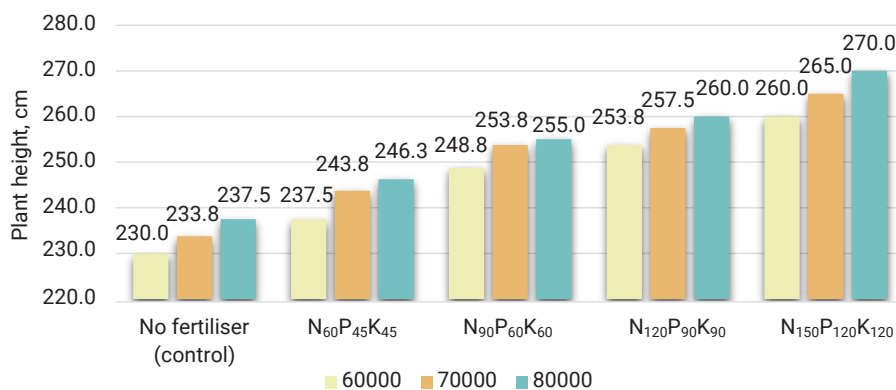


Figure 1. Maize plant height during the flowering stage depending on plant density and fertilisation background (mineral fertilisers)

Source: developed by the authors based on research results

The application of the highest rates of mineral fertilisers resulted in an average increase in plant height of 13.4% compared to the control. As plant density increased, a gradual rise in height was observed due to intensified competition between plants, provided that there was no moisture deficit. Specifically, at a plant density of 70,000 plants/ha, the average plant height exceeded that at 60,000 plants/ha by 1.9%, and at 80,000 plants/ha by 3.1%. Measurements of plant height in treatments

where mineral fertilisers were used in combination with foliar application of a biopreparation revealed that, under minimum fertiliser application rates, plant height on average exceeded the control by 3.1% (Fig. 2). The use of maximum fertiliser rates led to an average increase in plant height of 15.5% compared to the control. The highest average plant height values were recorded in the treatments N₁₂₀P₉₀K₉₀ + nitrogen fixer (255-266 cm) and N₁₅₀P₁₂₀K₁₂₀ + nitrogen fixer (265-284 cm).

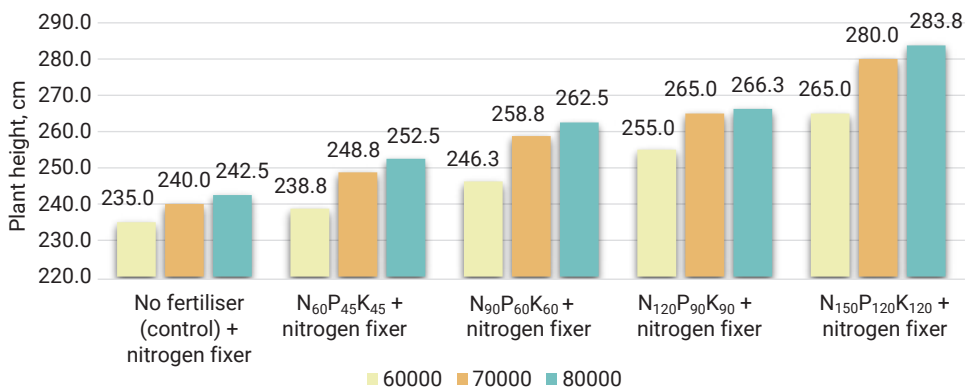


Figure 2. Maize plant height during the flowering stage depending on plant density and fertilisation background (mineral fertilisers + biopreparation)

Source: developed by the authors based on research results

The average height of hybrid P9255 plants was 4.2% greater at a plant density of 70,000 plants/ha and 5.4% greater at 80,000 plants/ha compared to the respective values recorded at 60,000 plants/ha. Following the foliar application of the nitrogen-fixing agent, the average plant height exceeded that of treatments with mineral fertilisers alone by 1.7%-5.1%. This outcome is primarily attributed to the improved uptake of mineral nutrients by the plants, particularly nitrogen. A strong positive correlation ($r = 0.98$) was observed between the rate of nitrogen application and plant height.

Harvesting of maize grain from the experimental plots was carried out in mid-September

2023 and in late August 2024. Enhanced nitrogen availability contributed to more vigorous plant growth and development, which in turn promoted higher productivity compared to the control. The highest grain yields were recorded in the N₁₂₀P₉₀K₉₀ and N₁₅₀P₁₂₀K₁₂₀ treatments at a plant density of 70,000 plants/ha, reaching 12.71 and 12.94 t/ha, respectively (Fig. 3). A strong positive correlation ($r = 0.92$) was identified between nitrogen application rates and yield across different plant densities. Overall, treatments with mineral fertilisers produced maize yields 1.56-2.38 t/ha (14.5%-22.5%) higher than the control.

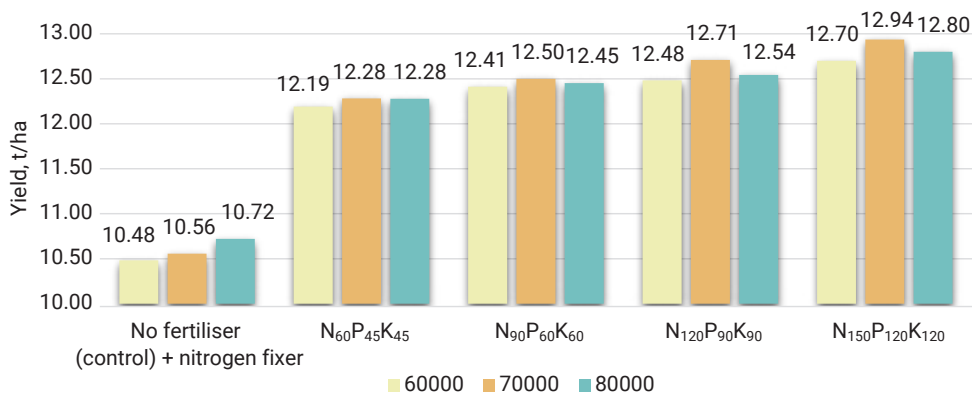


Figure 3. Maize yield depending on plant density and mineral fertiliser application rates

Source: developed by the authors based on research results

Treatments combining mineral fertilisers with foliar application of a nitrogen-fixing agent supported vigorous plant growth and development, resulting in high productivity (Fig. 4). In these treatments, maize grain yields were

1.38-2.30 t/ha (12.2%-21.2%) higher than in the control. The highest average grain yield, reaching 13.38 t/ha, was recorded in the $N_{150}P_{120}K_{120}$ + nitrogen fixer treatment at a plant density of 70,000 plants/ha.

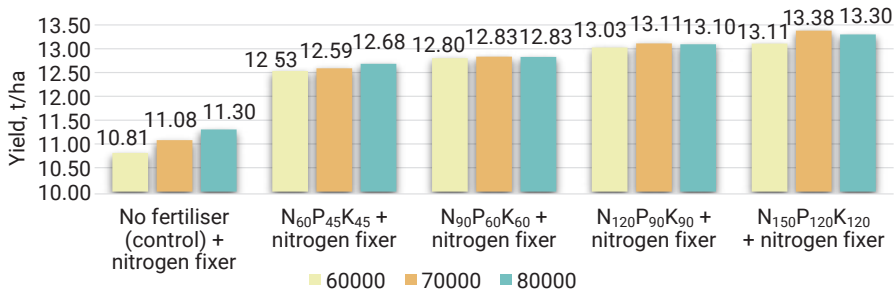


Figure 4. Maize yield depending on plant density and mineral fertiliser application rates with nitrogenfixing agent

Source: developed by the authors based on research results

Maize responded positively to the inclusion of the nitrogen-fixing agent in the cultivation technology, which enhanced nitrogen uptake. A strong positive correlation ($r = 0.95$) was observed between nitrogen application rates and crop yield across different plant densities. On average, grain yield was 3.6% or 0.43 t/ha higher compared to treatments without foliar application of the biopreparation.

Similar studies have been conducted by other researchers. Polish authors A. Zapałowska & W. Jarecki (2024) reported a significant effect of mineral fertilisation on maize growth parameters, including plant height, ear length, number of kernels per ear, and 1,000-grain weight. Ukrainian researchers I.I. Senyk *et al.* (2023) established a direct correlation between maize plant height and the application rate and form of mineral nitrogen fertilisers. Under an identical fertiliser rate of $N_{210}P_{60}K_{60}$, the tallest plants were observed with anhydrous ammonia (268.9 cm at the milk-wax ripeness stage), followed by urea (266.0 cm), and UAN-32 (251.5 cm). M.I. Dudka *et al.* (2020) demonstrated that foliar feeding with a mixture of urea and chelated micronutrients increased plant height by 6-7 cm, and by up to 10 cm when

applied twice – during the 5-6 and 8-9 leaf stages. In terms of yield, a single application increased maize grain output by 0.30-0.34 t/ha, while two applications raised it by 0.41 t/ha.

According to O.I. Tsyliuryk *et al.* (2024), under the conditions of the Northern Steppe of Ukraine, the maximum height of maize plants, regardless of the hybrid maturity group, was observed at a plant density of 30,000 plants/ha. A gradual increase in plant density to 60,000 plants/ha led to a decrease in plant height from 210-253 cm to 202-236 cm. This reduction was attributed to competitive interactions among plants for essential resources such as water, nutrients, and light. The application of mineral fertilisers, particularly at a rate of $N_{60}P_{60}K_{60}$, enabled an increase in plant height by 6-25 cm compared with unfertilised treatments. Research by O.V. Sydyakina & M.O. Ivaniv (2020) found that the application of growth stimulants resulted in increased plant height by 3-6 cm at harvest, depending on the mineral nutrition background. The use of mineral fertilisers at a rate of $N_{180}P_{90}$, combined with foliar application of a growth stimulant, resulted in a grain yield increase of 3.8 t/ha. Y.I. Tkalič *et al.* (2023) treated maize crops with plant growth

regulators at the 3-5 leaf stage, which contributed to a 5-7 cm (2.1%-2.8%) increase in plant height and a 1.23 t/ha (18.7%) increase in grain yield. O.I. Len *et al.* (2021) studied the combined effect of applying mineral fertilisers at a rate of $N_{45}K_{40}K_{60}$ and foliar feeding at the 5-6 leaf stage with urea (N_{15}) and a water-soluble multiminer- al micronutrient fertiliser. The resulting yield increase in hybrid grain compared with the control ranged from 1.06 to 1.20 t/ha, or 18.9%-19.2%.

Researchers I.M. Didur & V. Tsyhansky (2017), as well as V. Tsyhansky (2017), established that foliar feeding with biological preparations creates optimal conditions for the uptake of mineral nutrients and maximises the biological potential of maize plants. Experiments conducted by R. Singh *et al.* (2024) focused on the interaction between nitrogen, phosphorus, and potassium fertilisers, which significantly influence the absorption of micronutrients by maize plants and their availability in the soil. The application of increased nitrogen rates raises the concentration of micronutrients in maize grain.

T. Marchenko (2019) reported a strong correlation between plant height, ear placement height, leaf surface area, and grain yield in hybrids. According to L. Shevchenko & L. Tokmakova (2018), the use of growth stimulants through seed bacterisation and surface treatment of vegetative plants at the 7-9 leaf stage nearly doubles the net photosynthetic productivity and prolongs the photosynthetic activity of maize plants. V. Kaminskyi & N. Asanishvili (2020) highlighted the potential and experimentally confirmed the effectiveness of improving photosynthetic performance and the overall productivity of maize through the development of agrocenoses with optimal morphostructural parameters and functional characteristics, achieved by implementing advanced cultivation technologies.

According to D.A. López-Carmona *et al.* (2019), the application of biological preparations promotes the development of the root system and enhances symbiosis with nitrogen-fixing bacteria and mycorrhizae. This, in turn, improves maize plants'

utilisation of soil moisture, leading to increased crop productivity and enhanced grain quality, particularly with higher levels of protein, starch and fat. Polish researchers M. Niewęglowski *et al.* (2024) and A. Zapałowska & W. Jarecki (2024) found that the application of mineral fertilisers and biological stimulants increased the protein content in the grain of the maize hybrids studied, compared with the control. An increase in starch and fat content in the grain was also observed, although the differences were not statistically significant. At the same time, the influence of moisture availability must be taken into account. Research by N. Wang *et al.* (2025) showed that limited water supply reduced grain yield, dry matter accumulation, nitrogen and water uptake and use efficiency, and negatively affected most soil properties compared with plots supplied with sufficient water.

Thus, determining the effectiveness of mineral fertilisers in combination with modern biological preparations in maize nutrition systems remains a complex and highly relevant task. The effectiveness of nitrogen-fixing agents in increasing maize grain yield is a key component of cultivation technologies in the context of climate change and rising mineral fertiliser costs. The conducted studies have demonstrated the impact of optimising fertiliser regimes on the formation of optimal morphostructural plant characteristics and maize yield under varying plant densities in conditions of unstable and limited moisture availability.

Conclusions

It was established that optimising maize nutrition – specifically, combining mineral fertilisers with the biopreparation Blue N – had a positive effect on crop functioning. This approach contributed to an increase in plant height, enhanced accumulation of organic matter, and, consequently, higher yield levels. The height of P9255 maize plants under the application of the biological preparation was, on average, 1.7%-5.1% greater than in treatments where only mineral fertilisers were used.

With increasing plant density, a gradual rise in maize height was observed, attributed to

intensified competition among plants. In treatments with mineral fertilisers alone, plant height at a density of 70,000 plants/ha was 1.9% higher, and at 80,000 plants/ha – 3.0% higher than at 60,000 plants/ha. When mineral fertilisers were combined with foliar application of a nitrogen-fixing agent, plant height at a density of 70,000 plants/ha exceeded that at 60,000 plants/ha by 4.7%, and at 80,000 plants/ha – by 6.0%

On average, over the period 2023-2024, the application of mineral fertilisers alone resulted in a 14.5%-22.5% increase in maize grain yield compared with the control. The highest yield, reaching 12.94 t/ha, was recorded under $N_{150}P_{120}K_{120}$ treatment with a plant density of 70,000 plants/ha. In treatments where mineral fertilisers were combined with a biological agent, grain yield increased by 12.2%-21.2% compared with the corresponding control plus nitrogen-fixing agent. The treatment $N_{150}P_{120}K_{120}$ + nitrogen fixer at a

plant density of 70,000 plants/ha provided the highest average grain yield, reaching 13.38 t/ha.

Future research should focus on identifying agricultural crops that, in association with *Methylobacterium symbioticum*, will facilitate efficient atmospheric nitrogen assimilation and contribute to improved crop productivity. Additionally, it is necessary to assess the economic and energy efficiency of optimising nitrogen nutrition in agricultural crops through the use of nitrogen-fixing biopreparations.

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Conflict of Interest

None.

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Вплив мінерального живлення та азотфіксатора на урожайність кукурудзи в Центральному Лісостепу України

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Анотація. У сучасному аграрному виробництві зростає потреба у застосуванні біопрепаратів, зокрема азотфіксаторів, які дозволяють зменшити використання мінеральних добрив і водночас

підвищити урожайність кукурудзи. Метою дослідження було встановлення впливу різних варіантів оптимізації удобрення та густоти стояння рослин на морфологічні характеристики та урожайність кукурудзи в агроекологічних умовах Центрального Лісостепу України. Польові дослідження проведено впродовж 2023-2024 рр. в ґрунтово-кліматичних умовах господарства в Київській області на посівах кукурудзи гібриду Р9255 (ФАО 330). Розмір дослідної ділянки був 33 м², повторність 4-кратна, розміщення ділянок послідовне багаторядне. Використання біологічного препарату азотфіксатора в системі удобрення кукурудзи у поєднанні з мінеральними добривами позитивно впливало на ріст рослин. При цьому висота рослин кукурудзи на 1,7-5,1 % перевищувала відповідний показник варіантів із внесенням лише мінеральних добрив. Проведено оцінку ефективності та визначено вплив оптимізації системи удобрення на морфологічні ознаки та продуктивність рослин кукурудзи за рахунок застосування біопрепарату у поєднанні із мінеральними добривами за різних норм їх внесення. Загалом, за застосування лише мінеральних добрив одержано урожайність зерна кукурудзи на 14,5-22,5 % вищу, порівняно з контролем. Найбільшу урожайність, яка сягала 12,94 т/га, отримано у варіанті N₁₅₀P₁₂₀K₁₂₀ за густоти стояння 70 тис./га. У варіантах із застосуванням мінеральних добрив у поєднанні з біопрепаратом одержано урожайність зерна кукурудзи на 12,2-21,2 % вищу, ніж на відповідному контролі. Найбільший середній показник урожайності зерна, який сягав 13,38 т/га, забезпечив варіант N₁₅₀P₁₂₀K₁₂₀ + азотфіксатор за густоти стояння рослин на рівні 70 тис./га. При застосуванні біопрепарату у поєднанні з мінеральними добривами одержано на 3,6 % або 0,43 т/га вищу урожайність зерна культури, ніж у варіантах без його внесення

Ключові слова: біопрепарат; густина стояння; висота рослин; мінеральні добрива; урожайність зерна



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The dependence of ^{137}Cs absorption by carp fish feed on the content of various types of Prussian Blue

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Abstract. In the context of increased radioactive contamination of aquatic ecosystems following the Chernobyl accident, there is a clear need for a scientifically sound selection of feed additives to reduce ^{137}Cs accumulation in fish. This study aimed to determine the optimal concentrations and types of Prussian Blue (PB)/hexacyanoferrates in feed, considering both their radiological effectiveness and economic viability. The research employed a wet granulation method for feeds, with the addition of ferrocin, potassium ferric hexacyanoferrate (KFCE), and ammonium ferric hexacyanoferrate (AFCE) at concentrations of 0.1% and 1%. This was followed by a series of batch experiments to measure the activity concentration of ^{137}Cs and calculate the distribution coefficients over time. The results indicated that moistening the feed in an aqueous medium led to a three-fold increase in its mass within the first hour of contact, after which a steady state was achieved. For all types of PB, the ^{137}Cs distribution coefficients consistently increased during the first 120 minutes, reaching 3.5-4.5 L/kg at 0.1% PB content and 5.6-6.2 L/kg at 1%. No statistically significant difference was recorded between the types of hexacyanoferrates, suggesting similar effectiveness for ferrocin, KFCE, and AFCE at the same concentration. However, it was established that increasing the concentration from 0.1% to 1% only doubled the effectiveness, which raises questions about the economic viability of such a step. The practical significance of the data obtained lies in the potential for using feeds supplemented with the most accessible forms of Prussian Blue to reduce the radioactive burden in fish farming, particularly in radioactively contaminated water bodies

Keywords: radiocaesium; hexacyanoferrate; countermeasure; radioactive contamination; freshwater fish; reduction factor

Introduction

Various types of Prussian Blue (PB)/hexacyanoferrates very effectively absorb caesium from solutions, which allowed their use as a primary countermeasure in animal husbandry after the Chernobyl accident. A report by the expert group of the Chernobyl Forum of the International Atomic Energy Agency (IAEA) (Anspaugh & Balonov, 2006) states that in some settlements, fish are the main source of radiocaesium intake into the body and internal radiation dose for the population. In this regard, it is relevant to assess the radiological effectiveness of using PB to reduce radioactive contamination in fish based on the dynamics of ^{137}Cs absorption by fish feed depending on the content of different types of hexacyanoferrates.

The IAEA recommendations conclude that the use of protective measures/countermeasures is the most effective way to protect the population from radiation after the Chernobyl NPP accident. The national report of Ukraine compiled by I.V. Abramenko *et al.* (2022) states that the main

part of $^{134}, ^{137}\text{Cs}$ enters the human body through livestock products. Numerous experimental studies by A. Karpenko *et al.* (2023) and O.N. Antipenko (2023) showed that the most effective and inexpensive countermeasures aimed at reducing radiocaesium intake into the human body and livestock products are the use of various types of Prussian Blue as feed additives. Based on the summarised data, A. Nisbet & J. Sherwood (2024) concluded that the radiological effectiveness of using PB to reduce $^{134}, ^{137}\text{Cs}$ content in the meat and milk of cattle, as well as the meat of sheep, deer, and pigs, is 80-90%.

In European countries, including Austria, Germany, Norway, France and the United Kingdom, ammonium ferric hexacyanoferrate (AFCE) – $\text{NH}_4\text{Fe}[\text{Fe}(\text{CN})_6]$ was used as an animal feed additive after the Chernobyl accident, the price of which currently reaches 1 Euro per 1 g. Due to the high cost of AFCE in Ukraine after the Chernobyl accident, ferrocin (a mixture of 95%

ferric hexacyanoferrate (FCF – $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$) and 5% potassium ferric hexacyanoferrate (KFCF – $\text{KFe}[\text{Fe}(\text{CN})_6]$), was used, the price of which is significantly lower (0.05 Euro per 1 g) (Thermo Scientific Chemicals, 2025).

PB has found wide application not only in radiation protection as an antidote for internal intake of thallium and caesium radioisotopes due to its exceptional adsorption properties, but also in nanomedicine, chemical sensing, catalysis, spectroscopy, food production, and ion batteries. C. Rodwihok *et al.* (2024) showed that hexacyanoferrate forms insoluble colloids and its ability to adsorb Cs^+ reaches 200 g/g, which is more than 1000 times more effective compared to other alkali metals, such as potassium and sodium ions. Therefore, PB prevents caesium from being absorbed in the gastrointestinal tract (GIT) of animals and entering the blood, as a result of which caesium radioisotopes are transited out of the body with faeces. N. Chakraborty *et al.* (2024) investigated nanoscale forms of Prussian Blue as promising materials for medical applications, particularly for combating infections, controlling wound processes, and drug delivery. The authors emphasise that the ability for selective adsorption of cations, including caesium, opens up prospects for interdisciplinary use of these compounds. The work by D. Parajuli *et al.* (2025) is devoted to developing a protocol for the long-term storage of radioactive caesium in the form of stable complexes with metal hexacyanoferrates. The research results confirmed the exceptional stability of such compounds, allowing them to be considered a safe form of radionuclide isolation in industry and ecology. Researchers S. Khandaker *et al.* (2025) created an innovative adsorbent in which potassium hexacyanoferrate is embedded in the structure of layered hydroxides, ensuring high efficiency of ^{137}Cs extraction from seawater. The authors demonstrated that the obtained material retains sorption properties even in complex conditions with a high content of competing ions.

Employees of NULES (National University of Life and Environmental Sciences) of Ukraine,

P. Pavlenko *et al.* (2023), in natural conditions of the Chernobyl exclusion zone, and V. Kashparov *et al.* (2024), in controlled laboratory experiments, showed the high radiological effectiveness of using one type of PB, namely potassium ferric hexacyanoferrate (KFCF), to reduce radiocaesium activity in fish. In the study by P. Pavlenko *et al.* (2023), the activity concentration of ^{137}Cs in silver carp was reduced by 7-16 times and 12-27 times when the feed contained 0.1% and 1% KFCF, respectively. At the same time, the dynamics of ^{137}Cs sorption from water by feed containing 1% and 0.1% KFCF, which was manufactured under industrial conditions by extrusion – dry granulation – was studied. In farm animals, KFCF additives in feed affected ^{137}Cs absorption in the GIT, but did not affect the rate of excretion of caesium already deposited in the body. For fish, in the work of V. Kashparov *et al.* (2024), the effect of KFCF on the rate of radiocaesium excretion from the body of silver carp was statistically significantly established for the first time, which was almost twice the excretion rate when only pure feed without KFCF was used. Based on the results obtained, it can be concluded that the use of KFCF in fish feed is a highly effective and inexpensive countermeasure. The use of other types of PB (hexacyanoferrates), such as AFCF or ferrocyanin, may also be effective, but requires further study. The aim of this work was to evaluate the dynamics of ^{137}Cs sorption by feed containing 0%, 0.1%, and 1% AFCF, KFCF, and ferrocyanin from water under controlled laboratory conditions, to provide a scientific basis for using optimal concentrations of various types of PB in feeds, in terms of radiological effectiveness and price, to reduce ^{137}Cs radioactive contamination in fish.

Materials and Methods

The experiment was conducted at the Ukrainian Institute of Agricultural Radiology of the National University of Life and Environmental Sciences of Ukraine in 2024. Industrial feed for carp fish produced by “Skalaria” (Ukraine) with a pellet size of 3 mm, containing 30% protein, 10% fat,

3% fibre and vitamins A, D₃, E and C, was used as a control and basis for the preparation of feed with different contents of various types of Prussian blue (PB). Feed with different PB contents was produced in laboratory conditions using the wet granulation method. For this purpose, industrial feed for carp fish from the company "Skalaria" (Ukraine) was ground for 100 seconds in an IKA Model M 20 S000 crusher (Germany) to obtain a control batch of feed that did not contain PB and a base for preparing feed with a content of 0.1% and 1% PB (Fig. 1): ferrocin – a mixture of 95% ferric hexacyanoferrate (FCF) – $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$ and 5% (KFCF) (Ukraine), potassium ferric hexacyanoferrate (KFCF) – $\text{KFe}[\text{Fe}(\text{CN})_6]$ and ammonium ferric hexacyanoferrate (AFCF, Giese salt) – $\text{NH}_4\text{Fe}[\text{Fe}(\text{CN})_6]$ (Thermo Scientific Chemicals, 2025). To prepare feed with a 1% PB content, 1 g of PB (Fig. 1a) was added to 99 g of

crushed basic feed (Fig. 1b) and thoroughly mixed again for 100 seconds in an IKA Model M 20 S000 crusher (Fig. 1c). After that, 10 g of feed containing 1% PB was added to 90 g of mixed basic feed and thoroughly mixed again for 100 seconds in a crusher to prepare feed with a concentration of 0.1% PB. All equipment was thoroughly cleaned after each stage of feed preparation to prevent cross-contamination of hexacyanoferrate in the feed. For wet granulation, 60% distilled water was added to a 100 g mixture of feed with different PB contents and thoroughly mixed until a homogeneous plastic mass was obtained (Fig. 1d). Wet granulation of the feed was carried out using a manual meat grinder. The feed was then dried in an oven at 105°C for 1 hour (Fig. 1d).

As a result of the work done, feed containing 0%, 0.1% and 1% ferrocin, KFCF and AFCF was prepared (Fig. 2).

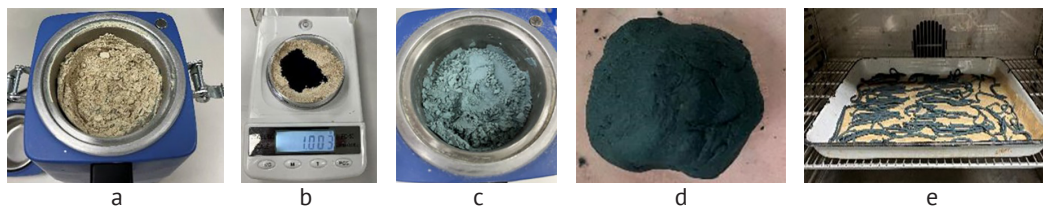


Figure 1. Stages of feed preparation with different PB content: grinding of the main feed

Note: a – does not contain PB; b – addition of 1 g of PB to the main feed; c – homogenisation of feed with 1% PB; d – moistening of feed for wet granulation; e – drying of feed at 105 °C

Source: developed by the authors

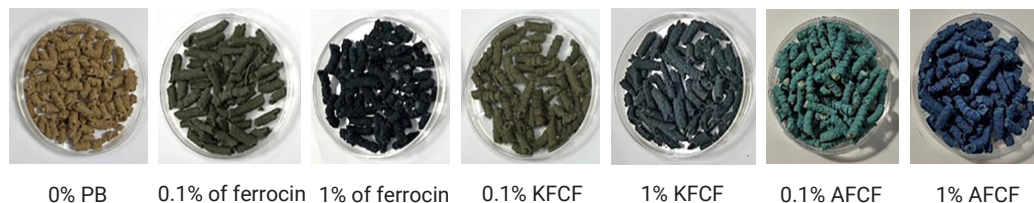


Figure 2. Feed containing 0%, 0.1% and 1% ferrocin, KFCF and AFCF

Source: developed by the authors

The dynamics of ^{137}Cs sorption from water by feed with different contents and types of selective cesium sorbent containing 0%, 0.1% and 1% ferrocin, KFCF and AFCF after wet granulation were determined in 33 ml plastic vessels with holes in the

bottom. The tare weight (wet beaker with holes and Petri dish lining) was 9 g. Feed samples weighing 5.0 ± 0.1 g (DM) with different hexacyanoferrate contents were used. The feed samples were kept for different periods of time (1 minute, 5 minutes,

15 minutes, 30 minutes, 60 minutes and 120 minutes) in 800 ml glasses with distilled H₂O, to which

a solution of ¹³⁷Cs (3 ml with activity concentration of 0.62 ± 0.05 kBq ml⁻¹) was added (Fig. 3).



Figure 3. Study of ¹³⁷Cs sorption by feed with different hexacyanoferrate contents from an aqueous solution

Source: developed by the authors

After each soaking stage in the solution, the wet mass (WM) was measured using a KERN balance (Germany) with an accuracy of 0.01 g. The ¹³⁷Cs activity in the feed was measured in a weighing bottle for 300 seconds using a scintillation gamma spectrometer (SEG-05, Ukraine; LSRP software) with a 63 × 63 mm NaI(Tl) detector within a passive shield (5 cm) (Fig. 4). As the sample volumes often didn't match the calibration geometry, correction factors were applied. Geometric correction was based on determining

the mass/volume of the sample in each container. The correction factors were calculated using the relationship between efficiency and sample volume for each container with known solution activity. The correction factors (CF) for the 15 ml H₂O weighing bottle geometry were 0.6 (Fig. 4a). After each stage of ¹³⁷Cs absorption by the feed from the solution, its activity concentration was monitored in 120 ml plastic containers. The correction factors (CF) for the “100 ml H₂O powder” geometry were 0.8 (Fig. 4).

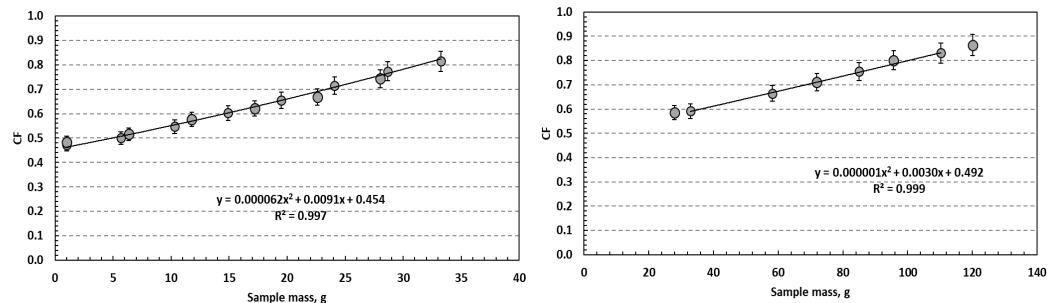


Figure 4. Correction factors (CF) for measuring ¹³⁷Cs activity in water in plastic vessels

Note: a – 33 ml beakers, b – 120 ml “powder”

Source: developed by the authors

A standard set of MS Excel tools was used to analyse the experimental data, namely to obtain mean values and standard deviations. The

reliability of the differences between samples was analysed using the non-parametric Kruskal-Wallis test. Statistical significance was set at $p < 0.05$.

Results and Discussion

The results obtained show the change in feed weight and ^{137}Cs distribution coefficients (solid-liquid distribution coefficient (K_d) – the ratio of the activity concentration of ^{137}Cs in the feed (C_p , Bq/kg) to the activity concentration of ^{137}Cs in the solution (C_w , Bq/L) depending on the contact time with water (Fig. 5). From the results, it's clear that the activity concentration of ^{137}Cs was highest in the feed prepared by wet granulation that contained 1% ferrocin, KFCF, or AFCF, after 120 minutes of contact with the solution (Table 1). At this point, the activity concentration of ^{137}Cs in the feed at natural moisture content (WM, wet mass) was $K_d = 5.6$ - 6.2 times higher than its activity concentration in water (2.3 ± 0.2 kBq/L), and 3.7 – 4.2 times higher compared to feed that did not contain hexacyanoferrates (Fig. 5). When the feed contained 0.1% of different PB types, radiocaesium was absorbed only 1.4 – 1.6 times less effectively compared to feed containing 1% hexacyanoferrates (Table 1). No statistically

significant differences ($p > 0.05$) were observed in the absorption of ^{137}Cs from water by feed with different types of caesium-selective sorbent, whether it contained 0.1% or 1% of ferrocin, KFCF, or AFCF (Fig. 5, Table 1).

The activity concentration of ^{137}Cs in feed with 1% and 0.1% hexacyanoferrates, after 120 minutes of contact with the solution, was approximately 4 times higher compared to feed that contained no hexacyanoferrates at all (Table 1). This was partly due to its wetting by the solution (the mass increased threefold from 5 g to 15 g after being kept in water (Fig. 5a, 5b, and 5c)). In feed with 1% KFCF, which was prepared by dry granulation and used in the work of P. Pavlenko *et al.* (2023) and V. Kashparov *et al.* (2024), the ^{137}Cs distribution coefficients were lower ($K_d = 3.6 \pm 0.2$ (Bq/kg)/(Bq/L)) compared to feed prepared by wet granulation ($K_d = 5.9 \pm 0.2$ (Bq/kg)/(Bq/L), Fig. 5b). This is attributed to its higher density and less wetting by the solution (mass increased only twofold when kept in water).

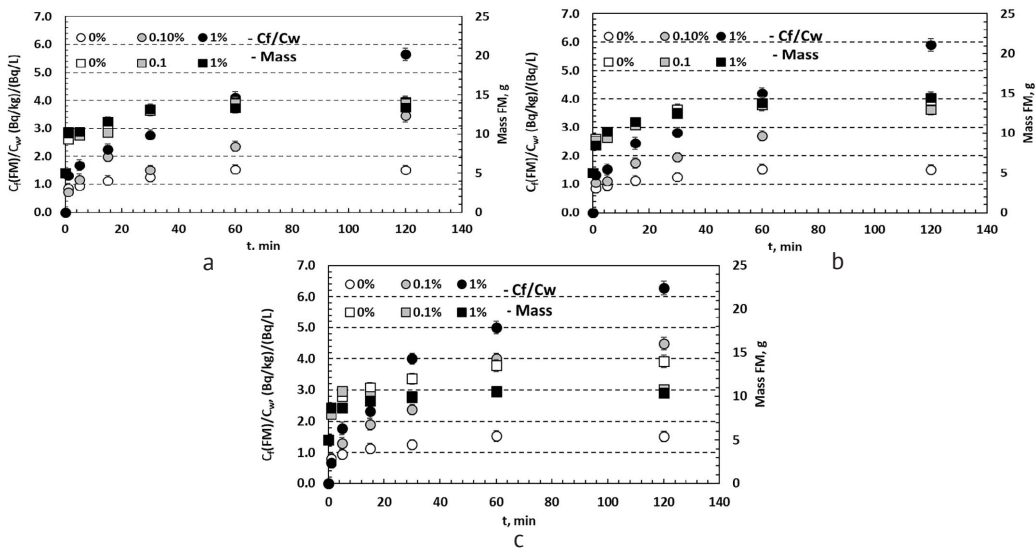


Figure 5. Dynamics of feed weight (Mass FM, g) and ^{137}Cs distribution coefficients at different iron hexacyanoferrate contents

Note: K_d – ratio of activity concentration of ^{137}Cs in feed (C_p , Bq/kg) to the activity concentration of ^{137}Cs in solution (C_w , Bq/L); a – Ferrocin, a mixture of 95% ferric hexacyanoferrate (FCF) – $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$ and 5% (KFCF) – $\text{KFe}[\text{Fe}(\text{CN})_6]$; b – potassium ferric hexacyanoferrate (KFCF) – $\text{KFe}[\text{Fe}(\text{CN})_6]$; c – ammonium ferric hexacyanoferrate (AFCF) – ($\text{NH}_4\text{Fe}[\text{Fe}(\text{CN})_6]$)

Source: developed by the authors

Table 1. Activity concentration of ^{137}Cs in feed (C_p , Bq/kg WM) at different iron ferric hexacyanoferrate contents

Feed composition, %	Hexacyanoferrate	Activity concentration of ^{137}Cs in feed, Bq/kg		
		After 30 minutes of contact with the solution	After 60 minutes of contact with the solution	After 120 minutes of contact with the solution
1	Ferrocin, a mixture of 95% FCF and 5% KFCF	6.4±0.6	9.6±1.0	13.1±1.3
	Potassium ferric hexacyanoferrate (KFCF)	6.6±0.7	9.8±1.0	13.7±1.4
	Ammonium ferric hexacyanoferrate (AFCF)	9.3±0.9	11.6±1.2	14.6±1.5
0.1	Ferrocin, a mixture of 95% FCF and 5% KFCF	3.5±0.4	5.5±0.6	8.0±0.8
	Potassium ferric hexacyanoferrate (KFCF)	4.6±0.5	6.3±0.6	9.4±0.9
	Ammonium ferric hexacyanoferrate (AFCF)	5.5±0.6	9.3±0.9	10.4±1.0
0	FCF, KFCF i AFCF	2.9±0.5	3.6±0.5	3.5±0.5

Source: developed by the authors

Based on the experimental results (Fig. 5), the parameters of approximation of the kinetics of cesium sorption by feed with different hexacyanoferrate contents upon contact with the solution $t = 0.25$ -2 hours were obtained by a linear dependence of the type $K_d(t) = a + bt$

(Table 2). The results show that the rate of increase in the distribution coefficient of ^{137}Cs in feed containing 1% PB (2.0 ± 0.1 L/kg/hour) was 1.3-2 (1-1.5 L/kg/hour) and 10 (0.2 ± 0.1 L/kg/hour) times higher compared to feed containing 0.1% and 0% hexacyanoferrates, respectively.

Table 2. Time dependencies of ^{137}Cs distribution coefficients in feed ($K_d(t) = a + bt$), L/kg

Feed composition, %	Hexacyanoferrate	a, (Bq/kg)/(Bq/l)	b, L/kg/ hour	R ²
1	Ferrocin, a mixture of 95% FCF and 5% KFCF	1.9±0.2	2.0±0.1	0.98
	Potassium ferric hexacyanoferrate (KFCF)	1.9±0.2	2.0±0.1	0.99
	Ammonium ferric hexacyanoferrate (AFCF)	2.5±0.3	2.0±0.1	0.88
0.1	Ferrocin, a mixture of 95% FCF and 5% KFCF	1.4±0.2	1.0±0.2	0.87
	Potassium ferric hexacyanoferrate (KFCF)	1.4±0.2	1.3±0.1	0.998
	Ammonium ferric hexacyanoferrate (AFCF)	1.8±0.3	1.5±0.2	0.85
0	FCF, KFCF i AFCF	1.2±0.2	0.2±0.1	0.70

Source: developed by the authors

K. Niizeki et al. (2020) in experiments with the cultivation of Japanese dace (*Tribolodon hakonensis*) and K. Matsuda et al. (2020) in five species of salmonids showed that the main activity of ^{137}Cs enters fish orally along with radioactively contaminated feed (over 99%) and a small part (less than 1%), mainly in winter, enters directly

from water. The rate of ^{137}Cs entering fish directly from water is $k_w = 0.03$ -0.07 1/day, which was obtained in experiments at different temperatures and for different fish species in experiments by V. Kashparov et al. (2024) and T. Wada et al. (2023). Thus, with a ^{137}Cs content in water (C_w) at the level of units Bq/l and an excretion rate $k_b = 0.007$ 1/day,

the equilibrium activity concentration of ^{137}Cs in fish ($C_{\text{fish}} = C_w * k_w / k_b$, Bq/kg) due to water contamination alone will not exceed tens of Bq/kg. At the same time, due to the contamination of natural fish feed in real natural conditions, the activity concentration of ^{137}Cs in fish is hundreds of times higher and reaches thousands of Bq/kg when the content of radiocaesium in water is at the level of units Bq/l (Man & Kwok, 2000; Kaglyan *et al.*, 2024). Considering that fish usually consume artificial feed in less than 1 hour, the contamination of feed with ^{137}Cs from hexacyanoferrate due to sorption will be insignificant and less than 4 times different from traditional fish feed without PB (Fig. 5). Therefore, the activity concentration of ^{137}Cs in artificial feed with PB will slightly exceed the radioactive contamination of water in the reservoir.

Depending on the size of the fish and the water temperature, no more than 5-8% of artificial feed per live weight of carp fish is used daily (Shipton, 2021; CARP 2022-2023, 2023; Huang *et al.*, 2025). With a radio-caesium content in water at a level of units Bq/l, the intake of firmly fixed ^{137}Cs per 1 kg of live weight of fish per day will be less than 1 Bq/day together with artificial feed with PB. This will lead to a steady-state activity concentration in fish of less than 100 Bq/kg, which is an order of magnitude lower than the contamination of fish in similar natural conditions (Man & Kwok, 2000; Wada *et al.*, 2023).

Since the sorption properties of feed with different types of Prussian blue did not differ statistically significantly, it can be concluded that their radiological effectiveness will be the same and will correspond to the radiological effectiveness of using similar feed for cyprinid fish with KFCF additives. As shown in the works of P. Pavlenko *et al.* (2023) and V. Kashparov *et al.* (2024), the radiological effectiveness (reduction factor) of using similar feed for cyprinid fish from the "Skalaria" company, both in laboratory and real conditions in the Chernobyl exclusion zone, was 7-16 and 12-27 times higher with a feed content of 0.1% and 1% KFCF. Thus, when the concentration of KFCF

in the feed was increased 10 times from 0.1% to 1%, the radiological effectiveness increased only 2 times, which is consistent with the results of changes in the sorption capacity of various types of hexacyanoferrates obtained in this work (Tables 1 and 2). Even with a feed content of 0.1% PB, its radiological effectiveness remains extremely high (about 10 times) and exceeds the effectiveness of countermeasures previously proposed to reduce cesium radioisotope contamination of fish in freshwater reservoirs many times over. Researchers J.T. Smith *et al.* (2003) proposed increasing the concentration of the chemical analogue of caesium, potassium, in water by adding large amounts of KCl to reservoirs. In real conditions, when 15 tonnes of potassium chloride fertiliser were added to Lake Svyatoye (0.05 kg per m² of water surface), the resulting desorption of previously fixed caesium from bottom sediments resulted in short-term and insignificant radiological effectiveness (less than 40%). A.Ye. Kaglyan *et al.* (2018) patented a method for cleaning crucian carp from ^{137}Cs by transferring the fish to clean water and using clean feed. This method requires labour-intensive fishing and keeping the fish in "clean" conditions, which only reduces the activity of ^{137}Cs in fish by half after 100 days. In contrast, as shown by V. Kashparov *et al.* (2024), the use of feed containing 0.1% KFCF allows the activity concentration of ^{137}Cs in crucian carp to be reduced by half in just 30 days without catching fish in "dirty water".

The results obtained indicate that the radiological effectiveness of using different types of Prussian blue in feed for carp fish will be the same, and the main factor determining the feasibility of using one type of hexacyanoferrate or another will be its price and availability. It has been established that with the cost of feed for carp fish in Ukraine at around 1-2 euros per kilogram, adding 0.1% ferrocyanide or KFCF (0.05 euros per 1 g) to it will increase the cost of feed with radioprotective properties by less than 5%. A similar application of AFCF can double the cost of feed (Thermo Scientific Chemicals, 2025). The

results of the study indicate the potential for creating inexpensive and effective feed additives to reduce radioactive contamination of fish, which may be of practical importance in restoring safe fish farming in post-radiation regions.

Summarising the results obtained, it should be noted that all the studied types of Prussian blue – ferrocin, potassium ferric hexacyanoferrate (KFCH) and ammonium ferric hexacyanoferrate (AFCH) -demonstrate similar sorption capacity for ^{137}Cs at the same concentrations in feed. Although increasing the content of hexacyanoferrates in feed from 0.1% to 1% increases radiological efficiency, this increase is not proportional and is accompanied by a significant increase in the cost of the product. Given the lack of statistically significant differences in sorption properties between types of hexacyanoferrates, the choice of a specific compound is determined primarily by its cost and availability. Therefore, it is advisable to continue the practical implementation of the most cost-effective forms of PB in fish feed in post-radiation regions.

Conclusions

As a result of the wet granulation process, feed for carp fish was produced with 0%, 0.1% and 1% ferrocin (a mixture of 95% ferric hexacyanoferrate) – $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$ and 5% potassium ferric hexacyanoferrate – $\text{KFe}[\text{Fe}(\text{CN})_6]$; potassium ferric hexacyanoferrate – $\text{KFe}[\text{Fe}(\text{CN})_6]$ and ammonium ferric hexacyanoferrate – $(\text{NH}_4)_3\text{Fe}[\text{Fe}(\text{CN})_6]$. In laboratory batch experiments, the dynamics of changes in feed mass in water and the distribution coefficients of ^{137}Cs in feed with different contents and types of Prussian Blue (PB) were

measured. For this purpose, 5 g of each type of feed was placed in 800 mg of a solution with a activity concentration of ^{137}Cs of 2.3 Bq/mL.

The results showed that the weight of the feed increased due to wetting with water. The distribution coefficients of ^{137}Cs in the feed also increased with increasing contact time of the feed with the solution and reached a maximum value after 120 minutes. At the same time, no statistically significant differences were observed in the sorption of ^{137}Cs by feed containing 0.1% or 1% ferrocin, KFCH or AFCH. The absorption of radiocaesium by feed from water containing various types of PB was insignificant compared to feed without hexacyanoferrate additives. Therefore, given the strong fixation of ^{137}Cs by various types of Prussian Blue, it can be concluded that this will not lead to an increase in the intake of radiocaesium into the fish organism.

The results obtained earlier and in this work may indicate the high radiological efficiency and low cost of using fish feed with additives of various types of hexacyanoferrates, which requires direct verification of the use of these feeds in experiments with fish in laboratory and real production conditions.

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Conflict of Interest

None.

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Залежність абсорбції ^{137}Cs кормом для коропових риб в залежності від вмісту в ньому різних видів Берлінської лазури

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Анотація. У контексті підвищеного радіоактивного забруднення водних екосистем після Чорнобильської аварії постає необхідність у науково обґрунтованому виборі кормових добавок для зменшення накопичення ^{137}Cs у рибі. Метою дослідження було визначення оптимальних концентрацій та видів гексаціанофератів/Берлінської лазури (БЛ) в кормах з огляду на їхню радіологічну ефективність і економічну доцільність. У дослідженні застосовано метод мокрого гранулювання кормів з додаванням фероцину, залізо-гексаціаноферату калію (KFCF) та амонію (AFCF) у концентраціях 0.1 % та 1 %, з подальшим проведенням серії batch-дослідів із заміром питомої активності ^{137}Cs та обчисленням коефіцієнтів розподілу у часі. Результати показали, що зволоження корму у водному середовищі призводило до трикратного збільшення його маси протягом першої години контакту, після чого досягався стаціонарний рівень. Для всіх типів БЛ коефіцієнти розподілу ^{137}Cs стабільно зростали протягом перших 120 хвилин і досягали 3.5-4.5 л/кг при вмісті 0.1 % БЛ та 5.6-6.2 л/кг при 1 %. Статистично достовірної різниці між типами гексаціанофератів не зафіксовано, що свідчить про подібну ефективність фероцину, KFCF і AFCF

при однаковій концентрації. Водночас встановлено, що підвищення концентрації з 0.1 % до 1 % збільшує ефективність лише вдвічі, що ставить питання про економічну доцільність такого кроку. Практичне значення отриманих даних полягає у можливості використання кормів із додаванням найдоступніших форм Берлінської лазури для зниження радіоактивного навантаження у рибництві, зокрема в умовах радіаційно забруднених водойм

Ключові слова: радіоцезій; гексаціаноферат; контрзахід; радіоактивне забруднення; прісноводна риба; фактор зменшення



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Research on air pollution by fine particles in tourist locations in the Ivano-Frankivsk region

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Abstract. The revitalisation of tourism in the Ivano-Frankivsk region highlights the need for systematic monitoring of atmospheric air, particularly in terms of the content of fine aerosol particles, in order to assess the level of environmental safety in popular tourist destinations. The aim of the study was to comprehensively assess atmospheric air pollution by PM_{2.5} and PM₁₀ particles in locations with varying degrees of anthropogenic load, as well as to identify the factors that determine the spatial variability of their concentrations. During the empirical stage, a series of instrumental field measurements were carried out using portable monitoring equipment. The locations were classified into three groups: recreational areas (low load), tourist and recreational areas (medium load) and transport and trade hubs (high load). Multivariate analysis methods were used for statistical data processing, in particular fourth-order polynomial regression and regression modelling of the relationship between PM₁₀ and PM_{2.5} levels, taking into account the load typology. It was found that the average concentrations of PM_{2.5} and PM₁₀ in high-load zones were 14.63 µg/m³ and 34.63 µg/m³, respectively, which does not exceed the maximum permissible values set by national and international standards. The lowest levels were recorded in natural recreational areas, due to the natural filtration capacity of green spaces and the absence of traffic. In areas with medium load, intermediate levels of pollution were found, indicating the influence of both stationary and mobile sources of emissions. Regression analysis

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showed a nonlinear relationship between PM_{2.5} and PM₁₀ ($R^2 = 0.87$), indicating the dominance of the fine fraction in the total mass of aerosol dust. In addition, it was found that an increase in relative air humidity correlates with a decrease in the concentration of fine particles, which is important for predicting air pollution levels depending on weather conditions

Keywords: environmental monitoring; tourist load; atmospheric aerosols; spatial analysis; PM_{2.5}; PM₁₀; regression modelling

Introduction

The issue of the impact of tourism on the natural environment is becoming increasingly relevant in light of global climate change, intensification of anthropogenic processes and rapid growth in tourist flows. In modern conditions, the tourism industry, on the one hand, is an important factor in the economic development of territories, and on the other hand, significantly increases the load on ecosystems, changing natural balances and threatening biodiversity (Stryzhak, 2022).

A. Dias *et al.* (2024) paid special attention in their scientific research to the impact of tourism on the quality of atmospheric air as one of the basic components of the environment that directly affects human health and ecosystem stability. According to modern approaches, the indicators of air quality in tourist locations are recognised as concentrations of fine particles (PM_{2.5}, PM₁₀), levels of carbon dioxide (CO₂), formaldehyde (HCHO) and basic meteorological parameters (temperature, relative humidity). Research into the impact of tourist flows on air quality is important for assessing the environmental sustainability of regions and developing strategies for their adaptation to new challenges.

The relationship between tourism and the state of natural resources is the focus of current scientific research at both the global and regional levels. In particular, G. Miller & A. Torres-Delgado (2023) argued that the rapid and unregulated growth of tourist flows causes systematic depletion of the natural environment. The authors emphasised that the degradation of ecosystems, the deterioration of air, water and soil quality, and the overloading of infrastructure directly affect the

tourist attractiveness of territories and the capacity of regions for sustainable development.

In recent scientific research, A. Dias *et al.* (2024) paid particular attention to the impact of tourist activity on air quality as one of the key elements of environmental stability in a region. Atmospheric air not only serves as a physical environment, but also plays a critical role in maintaining biotic balance, ensuring comfort and forming the health-preserving potential of recreational spaces. The deterioration of air quality due to increased tourist flows is accompanied by a local increase in anthropogenic emissions, in particular from transport infrastructure, accommodation facilities and entertainment establishments. Within the framework of environmental monitoring of tourist locations, the main indicators of air quality are the concentrations of fine aerosol particles PM_{2.5} and PM₁₀, which have the ability to penetrate deep into the respiratory system, carbon dioxide (CO₂) levels as a marker of ventilation activity and traffic density, formaldehyde (HCHO), which is formed as a result of incomplete combustion of organic substances, as well as basic meteorological parameters – air temperature, relative humidity, atmospheric pressure.

D. Yıldırım *et al.* (2023) emphasised the need for regular and spatially adapted monitoring of atmospheric air quality, especially in regions with high tourist traffic density. The authors argued that global warming and climate change are causing transformations in the distribution, duration and concentration of harmful substances in the air, which poses additional risks to public health, reduces the recreational potential

of territories and disrupts the ecosystem balance. In this context, atmospheric air monitoring within tourist destinations is seen as one of the key tools for ensuring environmental safety, adaptive management and forecasting the sustainability of ecological systems under the influence of intensive anthropogenic activity. At the same time, the environmental impact of tourism should be considered not only through the prism of air pollution, but in a broader ecosystem context, which includes the impact on marine and coastal environments, the state of flora and fauna, hydrological balances and landscape structure. Tourism, especially in regions specialising in coastal recreation, exerts multidirectional pressure on natural complexes through the generation of large amounts of solid waste, disruption of the natural rhythms of wild species, degradation of soil cover, erosion processes and transformation of biotopes.

A study by S. Barik *et al.* (2024) demonstrated that unregulated growth in tourist flows leads to significant pollution of coastal areas with household waste, in particular plastic products, packaging, textile residues and organic matter. Such pollution negatively affects the state of biodiversity, both marine and coastal species, disrupts the functionality of ecosystems, and reduces the attractiveness of beaches for recreational use. In addition, the accumulation of waste in the marine environment leads to the formation of secondary microplastics, which threaten food chains and the health of marine organisms.

In turn, the study by M. Ben-Haddad *et al.* (2024) focused on another specific manifestation of anthropogenic pressure - the blooming of macroalgae, which, in a changed marine environment, trap large amounts of plastic on the coast, creating an additional environmental burden and complicating the operation of tourist areas. The massive accumulation of algae enriched with plastic not only causes aesthetic and sanitary discomfort, but also increases the risk of pathogenic environments, which complicates the maintenance of infrastructure in proper sanitary

and hygienic condition. Such processes require the implementation of integrated coastal management models that synchronise environmental monitoring, spatial planning and the participation of local communities in maintaining ecosystem balance. Thus, to ensure the environmental sustainability of tourist regions, especially in coastal areas, it is necessary to comprehensively rethink approaches to managing tourist flows, taking into account the impact on all components of the environment - from air and water to biodiversity and soil and vegetation cover.

The issue of integrating tourism into the paradigm of sustainable development was analysed in detail in the work of S.M. Rasoolimanesh *et al.* (2023). The researchers emphasised that declarative support for environmental safety is not enough to ensure the sustainability of tourist areas - it is necessary to apply a system of quantitative indicators that allow the social, economic and environmental consequences of tourist activity to be verified. The authors also developed an evaluation structure based on a multidimensional analysis of the impact of tourism on the achievement of the Sustainable Development Goals.

Of considerable interest in the context of the greening of tourist areas is the study by A.I. Osman *et al.* (2023), which examined the prospects for the use of renewable energy in tourist infrastructure. The authors argued that the introduction of solar, wind and hybrid energy systems not only reduces overall greenhouse gas emissions but also improves the environmental image of destinations. At the same time, they drew attention to the need for economic justification of such decisions to increase their adaptability at different levels of management.

At the regional level, especially in Ukraine, the issue of sustainable tourism development and environmental protection is becoming particularly relevant. The work of B.O. Shulika *et al.* (2023) analyses the potential of ecotourism as an effective tool for biodiversity conservation and local community activation. The authors showed that the development of ecotourism

practices aimed at preserving landscape diversity also contributes to the formation of an environmentally-oriented worldview and improves the environmental literacy of tourists.

At the same time, O. Tabenska & A. Prylut-skyi (2023) emphasised the regulatory and institutional dimension of sustainable tourism. In their work, they proved the need to form a national strategy for the development of eco-tourism, which should include components of environmental education, active community participation in decision-making, and the adaptation of international experience in managing tourist flows, taking into account local environmental conditions. Thus, the analysis of the literature indicates a systematic awareness by the scientific community of the challenges associated with the ecological transformation of tourist areas, as well as the relevance of developing tools for monitoring, spatial planning and evaluation of the load on natural components of the environment. In this context, the applied analysis of air quality under conditions of varying intensity of tourist activity becomes particularly important, which determines the relevance of the research presented in this article.

The issue of studying the relationship between the level of tourist load and the concentrations of fine particles in the air is becoming increasingly important. PM_{2.5} and PM₁₀ are recognised by the World Health Organisation as some of the most dangerous pollutants for human health due to their ability to penetrate deep into the respiratory system, causing serious diseases. In view of this, a quantitative assessment of PM_{2.5} and PM₁₀ concentrations in different types of tourist locations (from natural and recreational to transport and commercial areas) allows not only to identify risk areas, but also to justify measures to optimise tourist activities.

In this context, instrumental monitoring of air quality in tourist locations in the Ivano-Frankivsk region, followed by their differentiation according to levels of anthropogenic load and the application of mathematical methods for analysing

pollution factors, is of particular scientific and practical importance. The aim of the study was to comprehensively model the relationship between the level of tourist load and the concentrations of PM_{2.5} and PM₁₀ particulate matter in the air to identify the leading factors in the formation of dust load in recreational areas.

Materials and Methods

The study was conducted using a comprehensive approach that combined theoretical analysis of contemporary scientific literature and field measurements of atmospheric air quality in tourist locations in the Ivano-Frankivsk region. The methodology ensured comprehensive coverage of the subject matter and representativeness of the data obtained. Field measurements were taken on 30 August 2024 within tourist locations in the Ivano-Frankivsk region. The selection of sites was based on the need to cover a variety of environmental conditions and levels of anthropogenic load, from natural recreational areas to urban and transport-commercial areas. The list of locations included: Underhill Earth History Park, forest area near Underhill, Pidhiria (residential area), Leybova Hora restaurant, transport area near Leybova Hora, parking area at the entrance to Leybova Hora, Shevchenko Square in Nadvirna, Hetmana Mazepy Street in Nadvirna, central market in Nadvirna, area near the fountain in the centre of Nadvirna, OKKO petrol station (Yaremche), souvenir market (Yaremche), MORVA Premium Spa Resort (Yaremche), the Stanislavsky Hotel (Yaremche), the shopping area near the railway track (Yaremche), Svobody Street (Yaremche), Car Park №1 (Polianytsia), Lake of Youth (Bukovel), the Ferris wheel (Bukovel), the Syayevo restaurant (Bukovel), Car Park №2 (Bukovel), entrance to Bukovel, ski area (Bukovel), remote area of Bukovel. The selection of sites was based on the need to cover a variety of environmental conditions and tourist traffic intensity.

The first stage of the study involved an analytical review of scientific publications on the impact of tourism on the environment, air

pollution by solid particles, sustainable tourism, and environmental monitoring. The information base consisted of peer-reviewed articles for the period 2020-2024. The literature was selected based on criteria of relevance, scientific novelty, and relevance to the research topic. The second stage involved the organisation and conduct of field measurements of atmospheric air in selected tourist locations, conditionally divided into three groups according to the level of anthropogenic load: low (recreational areas), medium (tourist and recreational areas) and high (transport and trade hubs). The measurements were carried out according to a single scheme at specific points at a height of 1.5 metres above ground level.

Three specialised devices were used to collect data. The CEM DT-9881M portable air quality analyser (brand CEM Instruments, country of registration and manufacture China) was used to measure concentrations of PM_{2.5} and PM₁₀ fine particles, formaldehyde, as well as air temperature and relative humidity. The device operates on the basis of a diffusion sampling method and is equipped with a built-in battery and memory. To determine the concentrations of carbon monoxide (CO) and nitrogen dioxide (NO₂), a Walkom W-K-600 gas analyser (manufacturer Walcom Instruments, Italy) was used, known for its high accuracy in mobile monitoring of gaseous pollutants. The volume fraction of oxygen was determined using a CEM GD-3803 oxygen monitor (brand CEM Instruments, country of manufacture China). The use of several devices ensured a multifactorial assessment of the state of atmospheric air.

The third stage involved processing the obtained data using Microsoft Excel software for primary analysis: calculating average values, standard deviations, and coefficients of variation. For in-depth statistical analysis, regression analysis methods (fourth-order polynomial regression) were used to model the dependence of PM₁₀ on PM_{2.5}, taking into account load categories, as well as correlation analysis to establish the relationship between air pollution levels and

relative humidity. In order to identify the nature of the relationship between the concentrations of PM_{2.5} and PM₁₀ particulate matter in the air at tourist locations in the Ivano-Frankivsk region, mathematical modelling methods, in particular regression analysis, were used. Given the empirical distribution of the studied variables and the need to take into account possible nonlinear dependencies, the linear model was found to be insufficiently informative to accurately reflect the relationship between these parameters. For a comparative analysis of the concentrations of fine particles in tourist locations in the Ivano-Frankivsk region, the standards approved by the current national documents in the field of atmospheric air protection were applied. In particular, the focus was on maximum permissible concentrations (MPCs).

In view of this, it was appropriate to use fourth-order polynomial regression, which allows higher degrees of the PM_{2.5} variable to be taken into account to model its impact on PM₁₀ levels. In addition, the model included a categorical variable "Load Category", represented as two binary predictors: for areas with low and medium levels of anthropogenic load. The high load zone is the base (reference) category. That is why fourth-order polynomial regression was used to build the model:

$$PM_{10} = \beta_0 + \beta_1 PM_{2.5} + \beta_2 PM_{2.5}^2 + \beta_3 PM_{2.5}^3 + \beta_4 PM_{2.5}^4 + \beta_5 (\text{Low load}) + \beta_6 (\text{Medium load}), (1)$$

where: β_0 – is a free term (constant) corresponding to the baseline level of PM₁₀, β_1 is a linear coefficient showing how PM₁₀ changes when PM_{2.5} changes, β_2 , β_3 , β_4 are polynomial coefficients reflecting the nonlinear effect of PM_{2.5} on PM₁₀, β_5 – correction of the PM₁₀ level for low load, β_6 – correction of the PM₁₀ level for medium load.

Regression analysis results = Coefficient of determination $R^2 = 0.87$ (the model explains 87% of the variation in PM₁₀). All polynomial coefficients are statistically significant ($p < 0.05$).

Results and Discussion

One of the main environmental problems in tourism is the accumulation of pollutants in the air due to the growth of tourist flows and the development of transport infrastructure in popular recreational areas. Fine particles, in particular PM_{2.5} and PM₁₀, are the main markers of anthropogenic load, as their excessive accumulation in the air negatively affects both the state of natural ecosystems and human health. The problem is compounded by the fact that most tourist regions are traditionally positioned as areas with “clean air”, which does not always correspond to the actual environmental situation in conditions of intensive exploitation of the territories.

Fine aerosol particles have the ability to penetrate deep into the human respiratory system, causing a wide range of negative effects, including respiratory and cardiovascular diseases and an increase in overall mortality risk. At the same time, the accumulation of PM_{2.5} and PM₁₀ in tourist regions leads to a decrease in the ability of vegetation to naturally purify the air, impairs the photosynthetic activity of green spaces and accelerates soil degradation due to particle deposition.

It is particularly important to analyse the distribution of fine particles depending on the level of anthropogenic load: from areas with a predominantly natural environment to areas with developed transport infrastructure. As part of the study, the concentrations of fine particles PM₁₀ and PM_{2.5} in the air of tourist areas in the Ivano-Frankivsk region were determined, with subsequent classification

of observation objects according to the level of anthropogenic load into three categories:

- recreational areas (low load);
- tourist and recreational areas (medium load);
- transport and trade hubs (high load).

Areas with minimal anthropogenic impact include natural parks, forest areas, and areas with low building density and limited traffic. The dominant sources of PM₁₀ and PM_{2.5} fine particles are natural processes, in particular soil dust and biogenic emissions. Concentrations of pollutants in such conditions remain the lowest due to effective natural filtration of the atmosphere by vegetation.

Tourist and recreational areas are characterised by a moderate level of anthropogenic impact. The main factors of pollution include tourist activity, commercial activity, and traffic flows near recreational facilities. The concentration of solid particles in these areas is intermediate between the levels recorded in recreational and transport and commercial areas, as the influence of natural and man-made sources is combined here. City centres, transport hubs, commercial areas, markets, bus stations, car parks and petrol stations are characterised by the highest level of anthropogenic impact. The main sources of pollution are motor vehicle exhaust gases, industrial emissions and aerosols generated by economic activity. The high intensity of traffic flows and commercial processes leads to increased concentrations of PM₁₀ and PM_{2.5}, which negatively affects the quality of atmospheric air and the ecological state of the region (Table 1).

Table 1. Air quality indicators in different locations of Ivano-Frankivsk region (August 2024)

Nº	Location	Load category	PM _{2.5} (µg/m ³)	PM ₁₀ (µg/m ³)	HCHO, ppm	O ₂ , %	CO ₂ , ppm	°C	Relative humidity, %
1	Earth History Park “Underhill”	Low load	11.6	28.0	0.0	20.4	455	23.1	65
2	Forest area near Underhill	Low load	10.8	26.0	0.0	20.4	450	22.9	65
3	Pidhiria, residential district	Low load	11.2	24.0	0.0	20.4	454	23.3	64
4	Leibova Hora Restaurant	Medium load	12.0	25.0	0.01	20.2	435	23.9	61

Table 1. Continued

N ^o	Location	Load category	PM2.5 (µg/m ³)	PM10 (µg/m ³)	HCHO, ppm	O ₂ , %	CO ₂ , ppm	°C	Relative humidity, %
5	Transport zone near Leibova Hora	Medium load	12.5	29.0	0.01	20.2	435	24.1	61
6	Parking area near the entrance to Leibova Hora	Medium load	13.0	31.0	0.01	20.2	435	24.1	61
7	Shevchenko Square, Nadvirna	High load	13.0	32.0	0.0	20.4	444	23.5	67
8	Hetman Mazepa Street, Nadvirna	High load	13.5	33.6	0.0	20.4	439	23.5	70
9	Central Market, Nadvirna	High load	14.0	40.0	0.0	20.4	439	23.5	70
10	Area near the fountain in the centre of Nadvirna	Medium load	13.2	41.0	0.0	20.4	444	23.5	67
11	OKKO Petrol Station, Yaremche	High load	13.0	39.0	0.0	20.3	576	28.7	50.2
12	Souvenir Market, Yaremche	High load	13.0	35.0	0.0	20.4	502	29.1	49.2
13	MORVA Premium Spa Resort, Yaremche	Medium load	14.0	38.0	0.01	20.3	494	29.3	42.6
14	Hotel Stanislavsky, Yaremche	Medium load	13.0	40.0	0.02	20.4	499	29.4	47.7
15	Shopping area near the railway line, Yaremche	High load	13.5	37.0	0.02	20.4	480	29.4	47.5
16	Svobody Street, Yaremche	High load	13.8	38.5	0.02	20.4	440	29.4	47.9
17	Car Park N ^o 1, Polianytsia	High load	19.0	39.2	0.02	20.4	442	24.7	50.7
18	Lake of Youth, Bukovel	Medium load	12.0	30.0	0.01	20.4	412	26.0	46.3
19	Ferris Wheel, Bukovel	Medium load	11.0	25.0	0.01	20.2	420	30.1	49.3
20	Syaevo Restaurant, Bukovel	Medium load	8.0	22.0	0.01	20.2	424	29.8	48.5
21	Car Park No. 2, Bukovel	High load	17.0	23.0	0.01	20.2	456	29.8	48.9
22	Entrance to Bukovel	High load	16.5	29.0	0.01	20.2	429	29.8	48.2
23	Ski area, Bukovel	Medium load	10.5	28.5	0.01	20.2	424	29.8	48.5
24	Remote area, Bukovel	Low load	9.0	24.0	0.01	20.2	438	29.8	48.5

Source: compiled by the authors

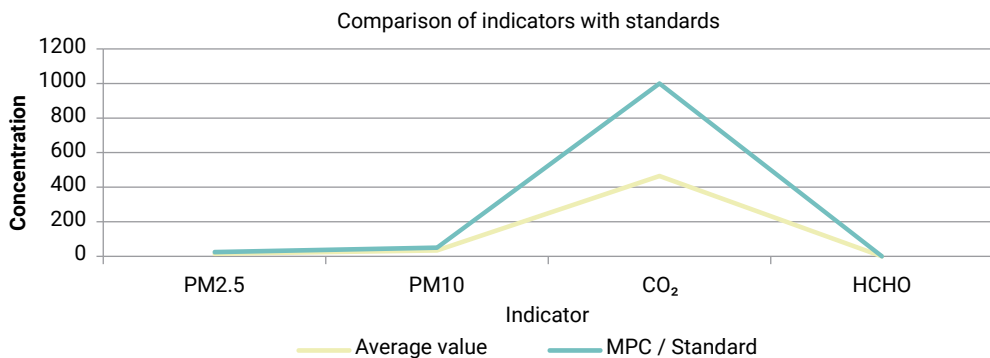
Background concentrations are determined in accordance with Order of the Ministry of Ecology and Natural Resources of Ukraine No. 286 (2001), as amended in 2021. The document has been in force since 1 March 2000 and defines the basic standards for the permissible

content of chemicals in the air, in particular components that are hazardous to human health, such as PM2.5, PM10, formaldehyde, carbon monoxide and nitrogen dioxide, established and recommended by the World Health Organisation (2021). (Table 2, Fig. 1).

Table 2. Comparison with permissible standards

Indicator	Average value (high load)	Standard in Ukraine (MPC)	Conclusion
PM2.5	14.63 $\mu\text{g}/\text{m}^3$	up to 25 $\mu\text{g}/\text{m}^3$ (daily average)	Within the normal range
PM10	34.63 $\mu\text{g}/\text{m}^3$	up to 50 $\mu\text{g}/\text{m}^3$ (daily average)	Within the normal range
CO ₂	464.7 ppm	approximately up to 1,000 ppm (ventilation recommendations)	Within the normal range
HCHO	≤ 0.02 ppm	up to 0.035 ppm	Within the normal range

Source: compiled by the authors based on Order of the Ministry of Ecology and Natural Resources of Ukraine No. 286 (2001)

**Figure 1.** Comparison with permissible standards

Source: compiled by the authors based on Order of the Ministry of Ecology and Natural Resources of Ukraine No. 286 (2001)

Thus, the average PM2.5 value was $14.63 \mu\text{g}/\text{m}^3$, which is significantly lower than the daily maximum permissible concentration of $25 \mu\text{g}/\text{m}^3$. The recorded value indicates that there is no excess of fine dust, which can adversely affect the respiratory system. A similar trend can be observed for PM10, whose concentration was $34.63 \mu\text{g}/\text{m}^3$ with a permissible level of $50 \mu\text{g}/\text{m}^3$. Both indicators remain within sanitary and hygienic standards, which confirms the absence of critical levels of particulate matter pollution. The average carbon dioxide level was 464.7 ppm, which corresponds to the recommended standards for areas of mass human presence. The formaldehyde content was within the range of 0.00–0.02 ppm, not exceeding the maximum permissible value of 0.035 ppm.

A comparative analysis showed that none of the indicators exceeded the established standards.

Accordingly, the atmospheric air in tourist locations in the Ivano-Frankivsk region is characterised by a relatively favourable ecological state, despite the increased recreational and transport load. Fourth-order polynomial regression was chosen as the most suitable tool for modelling the dependence of the concentration of PM10 suspended particles on the level of PM2.5 fine dust, given the empirical nature of the data distribution and the need to take into account non-linear relationships that are not covered by the linear model. The additional inclusion of the categorical variable “Load category” in the form of binary predictors made it possible to take into account the influence of the intensity of anthropogenic pressure on the concentration of PM10, where a high level is the baseline. The dependence is shown in (Fig. 2):

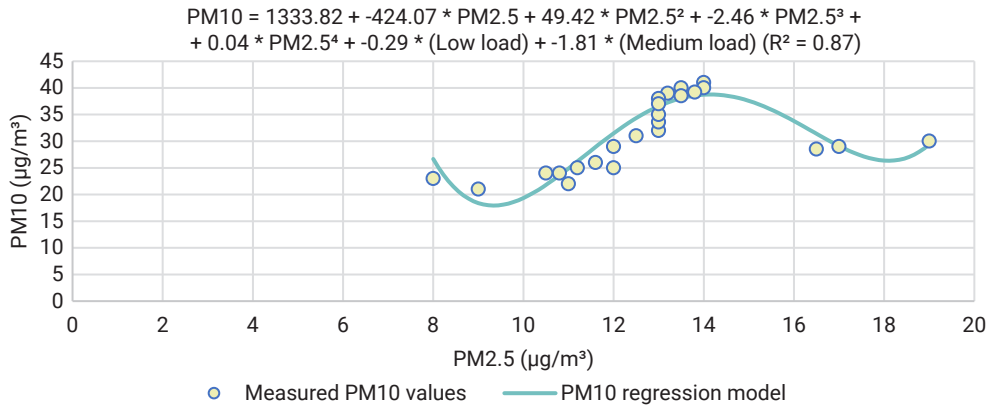


Figure 2. Dependence of PM10 concentration on PM2.5 in tourist locations in Ivano-Frankivsk region

Note: 4th order polynomial approximation, $R^2 = 0.87$

Source: compiled by the authors

Considering that relative humidity influences the behaviour, aggregation and deposition of aerosol particles, analysing its relationship with PM2.5 concentration is justified for a deeper understanding of the mechanisms of air quality formation in tourist areas. An increase in relative humidity can promote the condensation of water vapour on the surface of PM2.5 particles, increasing their size and changing their

deposition properties, which in turn affects the concentration of particles in the air. On the other hand, excessive humidity can cause particles to be washed out of the atmosphere, reducing their content. Therefore, establishing the nature of the interdependence between these parameters is necessary to assess the environmental status of tourist areas and the potential risk to human health (Fig. 3).

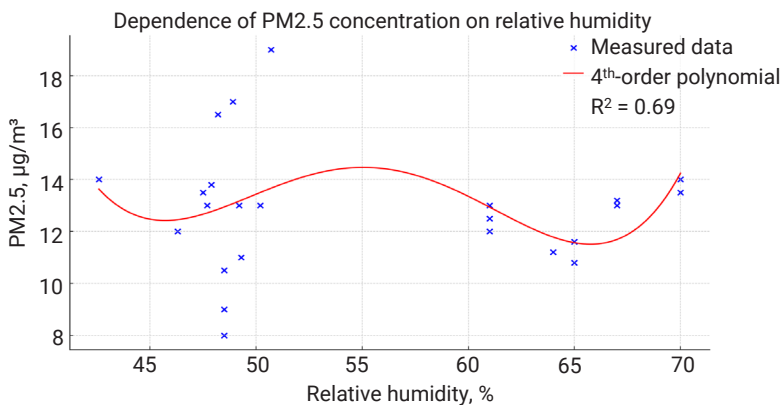


Figure 2. Polynomial regression of PM2.5 concentration on relative humidity in tourist locations of Ivano-Frankivsk region

Source: compiled by the authors

The results of the regression analysis showed that the coefficient of determination

$R^2 = 0.69$, which indicates a fairly strong dependence between the concentration of fine particles

PM2.5 and the level of relative humidity in the atmospheric air of tourist locations in the Ivano-Frankivsk region. The value of the coefficient of determination indicates that approximately 69% of the variation in PM2.5 concentration can be explained by changes in humidity, which indicates the presence of a significant nonlinear relationship between these indicators. Analysis of the constructed polynomial model demonstrates the nonlinear nature of the relationship. In particular, as relative humidity increases, there is a tendency for the concentration of fine particles in the air to decrease, which may be due to the physical and chemical processes of aerosol deposition under the influence of hygroscopic growth of droplets and gravity towards the Earth's surface. However, when a certain threshold level of humidity is reached, a change in the trend is recorded - a secondary increase in PM2.5 concentrations is possible, which is probably explained by the mechanisms of particle aggregation into larger aggregates, a decrease in their precipitation rate, or the formation of secondary particles due to heterogeneous reactions on the surface of water aerosols.

The results obtained emphasise the role of relative humidity as one of the key meteorological factors influencing the spatial and temporal variability of fine dust in tourist regions. Under conditions of medium and high anthropogenic load, typical for recreational and tourist areas, fluctuations in air humidity can significantly modify the quality of the atmospheric environment, intensifying or mitigating the impact of pollution sources. The results of the conducted research allow for the formation of substantiated conclusions regarding the impact of anthropogenic load on the spatio-temporal distribution of fine aerosol concentrations of PM2.5 and PM10 in the atmospheric environment of tourist locations in the Ivano-Frankivsk region. The obtained indicators demonstrate significant variability depending on the level of technogenic pressure, which fully correlates with the conclusions made in the work of S.Yu. Adamenko *et al.* (2024), where the patterns of PM10 particle accumulation in the urban

environment of Ivano-Frankivsk were studied. In particular, in transport and commercial areas, particle concentrations exceed the corresponding values recorded in recreational areas with minimal urbanisation, indicating a direct relationship between the nature of land use and the level of atmospheric dustiness.

The coefficient of determination $R^2 = 0.87$, obtained in the process of approximating the dependence between PM2.5 and PM10 using fourth-order polynomial regression, confirms the existence of a pronounced nonlinear relationship between the two main markers of aerosol pollution, which was also recorded in a study of PM2.5 concentrations in various natural and territorial conditions in the Ivano-Frankivsk region (Adamenko *et al.*, 2024). The inclusion of a categorical variable reflecting the level of anthropogenic load in the model allowed for a deeper understanding of the spatial dynamics of pollution and its dependence on the functional purpose of tourist areas.

Changes in PM2.5 particle content also showed a noticeable dependence on the level of relative air humidity, which is consistent with the conclusions presented in international publications by J.S. Rodríguez-Alcántara *et al.* (2024) and M. Pásková *et al.* (2024), which emphasised the role of atmospheric moisture as a factor influencing particle aggregation, hygroscopic growth and deposition. The analysis found that an increase in humidity to a certain threshold level contributes to a decrease in the concentration of fine particles due to the intensification of deposition processes. At the same time, at high humidity levels, a change in trend is observed, which is probably due to the secondary formation of aerosol aggregates or a slowdown in their deposition, which is characteristic of heterogeneous chemical transformations in a humid environment.

It should be emphasised that the results of the study are relevant in the context of the scientific community's growing attention to the impact of environmental indicators on the quality of tourism services and the attractiveness of destinations. The work of C. Eusébio *et al.* (2022)

indicates that air quality is one of the critical factors in choosing tourist routes, especially in the ecotourism segment. A decrease in environmental comfort due to increased concentrations of PM10 and PM2.5 during periods of intense tourist traffic can negatively affect the perception of the region, which is strategically important for areas with high recreational potential.

The detected PM2.5 and PM10 concentrations in tourist locations demonstrate a close relationship between air quality and environmental comfort, which, according to a number of studies, directly affects the intensity of tourist flows. In particular, a spatial econometric analysis in China demonstrated a negative relationship between air pollution levels and the number of foreign visitors (Deng *et al.*, 2017), while regression models showed a significant reduction in inbound tourism in conditions of increased concentrations of harmful pollutants (Dong *et al.*, 2019). Air quality in the region of origin also determines the demand for outbound tourism, with disposable income acting as an integrating factor (Wang *et al.*, 2018).

In parallel with changes in anthropogenic load, special attention should be paid to meteorological factors, in particular relative humidity, which modifies the chemical composition of atmospheric aerosols and their deposition processes. This is confirmed by monitoring observations in Tehran, where significant fluctuations in PM1, PM2.5 and PM10 particle levels have been detected under the influence of dust storms and local pollution (Jaafari *et al.*, 2017; Naddafi *et al.*, 2019).

In a global comparative context, a correlation has been established between air pollution levels and the Human Development Index, allowing the HDI to be considered a marker of environmental quality (Santra, 2014). Indoor pollution problems in low-income countries, associated with inadequate ventilation and the dominance of traditional energy sources, are considered a critical public health challenge (Bruce *et al.*, 2000). Air quality in rural areas is often overlooked in environmental policy, but it has a significant impact on vulnerable population groups (Majra, 2011).

In general, the identified patterns of change in PM2.5 and PM10 concentrations indicate the need to establish a systematic monitoring system for the air environment in tourist regions, adapt environmental policy to the intensity of tourist traffic, and implement preventive measures to reduce anthropogenic impact, especially in areas with a high density of transport and commercial facilities. The study confirms that monitoring atmospheric air quality is a key element in ensuring the sustainable development of recreational areas, and integrating monitoring results into spatial planning practices can significantly improve the environmental safety and tourist attractiveness of the region.

Conclusions

Thus, the results of the study show that the atmospheric air in tourist locations in the Ivano-Frankivsk region is in satisfactory ecological condition, despite the presence of differentiated anthropogenic load. It has been established that the average concentrations of fine particles PM2.5 and PM10 in areas with high tourist and transport loads do not exceed the maximum permissible concentrations specified by national and international regulatory documents, but show a steady upward trend compared to natural recreational areas.

Regression modelling revealed a high level of nonlinear dependence between PM2.5 and PM10 concentrations (coefficient of determination $R^2 = 0.87R$), which indicates the importance of fine fractions in the formation of overall atmospheric pollution. It has been established that the increase in PM2.5 levels largely determines the increase in the total mass of PM10 dust, regardless of the type of anthropogenic load, which confirms the universality of the physicochemical processes responsible for the formation of aerosol pollution in tourist regions.

Additional analysis of the influence of meteorological factors showed the significant role of relative humidity in the variation of fine particle levels. Polynomial modelling of the dependence of PM2.5 on air humidity showed a coefficient of

determination $R^2 = 0.69R^2$, indicating a noticeable non-linear relationship between these indicators. An increase in humidity to a certain optimal level contributes to a decrease in PM_{2.5} concentrations due to the effect of particle precipitation, while excessive humidity can cause a secondary increase in pollution due to aerosol aggregation and heterogeneous reactions.

Prospects for further research include conducting multi-season measurements, expanding the set of analysed indicators (in particular, organic and inorganic gases), integrating spatial pollution models based on geoinformation technologies, and studying long-term trends in the

impact of tourism on the state of the atmospheric environment in the context of climate change.

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Conflict of Interest

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Reference

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Дослідження забруднення повітря дрібнодисперсними частками у туристичних локаціях Івано-Франківської області

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Анотація. Активізація туристичної діяльності в Івано-Франківській області актуалізує потребу у системному моніторингу атмосферного повітря, зокрема у частині вмісту дрібнодисперсних аерозольних часток, для оцінки рівня екологічної безпеки в популярних туристичних осередках. Метою дослідження була комплексна оцінка забруднення атмосферного повітря частками PM_{2.5} та PM₁₀ у локаціях із різним ступенем антропогенного навантаження, а також ідентифікація факторів, що детермінують просторову варіативність їх концентрацій. У межах емпіричного етапу було здійснено серію інструментальних польових вимірювань із застосуванням портативного моніторингового обладнання. Локації класифіковано на три групи: рекреаційні території (низьке навантаження), туристично-рекреаційні зони (середнє навантаження) та транспортно-торговельні вузли (високе навантаження). Для статистичної обробки даних використано методи багатовимірного аналізу, зокрема поліноміальну регресію четвертого порядку та регресійне моделювання залежності між рівнями PM₁₀ і PM_{2.5} з урахуванням типології навантаження. Встановлено, що середні концентрації PM_{2.5} та PM₁₀ у зонах високого навантаження становили відповідно 14,63 мкг/м³ та 34,63 мкг/м³, що не перевищує гранично допустимі значення, визначені національними та міжнародними нормативами. У природних рекреаційних зонах зафіксовано найнижчі показники, що зумовлено природною фільтраційною здатністю зелених насаджень і відсутністю транспортного трафіку. У зонах із середнім навантаженням виявлено проміжні рівні забруднення, які вказують на вплив як стаціонарних, так і мобільних джерел викидів. Проведений регресійний аналіз засвідчив наявність нелінійного зв'язку між PM_{2.5} і PM₁₀ ($R^2 = 0,87$), що свідчить про домінування дрібнодисперсної фракції у загальній масі аерозольного пилу. Додатково встановлено, що підвищення відносної вологості повітря корелює зі зниженням концентрацій дрібнодисперсних часток, що має значення для прогнозування рівня забруднення повітря у залежності від метеоумов

Ключові слова: екологічний моніторинг; туристичне навантаження; атмосферні аерозолі; просторовий аналіз; PM_{2.5}; PM₁₀; регресійне моделювання



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Abscisic acid and biopreparations as markers of organic soybean adaptation to hydrothermal stress

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Abstract. Given the growing demand for organic soybeans and the need to mitigate the adverse effects of climate change, the study of biopreparations of various origins and their influence on plant adaptive mechanisms under stress conditions has become increasingly relevant. This study aimed to assess changes in abscisic acid (ABA) content under the influence of biopreparations in a multi-year field experiment and to analyse the relationship between ABA and the biochemical, physiological, and agronomic indicators of soybean adaptation to hydrothermal stress under organic farming conditions. The study involved the use of biological preparations based on mycorrhizal fungi, rhizosphere and nitrogen-fixing bacteria, as well as phytohormones. A range of field, laboratory (physiological and biochemical), and statistical methods was employed. The findings revealed the integration of molecular, biochemical, physiological, and agronomic stress resistance markers as a result of the enhanced adaptive potential of soybean following the application of biopreparations. Their effectiveness was evident through a comprehensive influence on key adaptive mechanisms, including: a 9.2%-36.3% reduction in ABA concentration, indicating alleviation of stress pressure; a 10.0%-27.6% increase in relative water content, contributing to the maintenance of optimal cellular water balance; a 19.2%-65.4% improvement in stomatal conductance, attributed to the optimisation of stomatal function; a 15.6%-41.8% reduction in lipid peroxidation levels (malondialdehyde), indicating effective membrane protection against oxidative stress; a 9.1%-46.6% increase in proline content, suggesting the activation of natural defence mechanisms and the maintenance of osmotic balance. Correlation analysis confirmed that the biopreparations facilitated a complex modulation of the plant stress response, optimising the balance between defence reactions and productivity, which led to a 12.3%-45.5% increase in yield even under adverse weather conditions. It was established that under water stress, the biological preparations modulated ABA biosynthesis and its regulatory role, contributing to coordinated changes in osmoprotective mechanisms, antioxidant defence, and the plant water regime. The most effective approach proved to be the triple combination of biopreparations, which ensured an optimal balance between phytohormonal regulation, physiological processes, and soybean productivity under hydrothermal stress. The results obtained may be recommended for the cultivation of organic soybeans to improve yield under climate-related challenges

Keywords: organic farming; relative water content; stomatal conductance; proline; malondialdehyde; yield

Introduction

An integrative study of stress resistance in agricultural crops – encompassing molecular-biochemical processes, physiology, and productivity – is crucial for developing effective adaptation strategies to climate-related risks, particularly drought and temperature extremes. This is especially relevant for soybean (*Glycine max* L. Merr.), which holds strategic importance for Ukraine's economy and export potential (Bobos et al., 2025). Investigating the relationships between the application of biopreparations, phytohormonal regulation (particularly abscisic

acid (ABA)), and the physiological mechanisms of plant adaptation to hydrothermal stress offers a pathway toward the development of environmentally safe technologies and enhances the competitiveness of organic agricultural production under climate change conditions.

According to K. Bogati & M. Walczak (2022), global climate change is transforming soil environments and adversely affecting the functioning of the soil biota, including microorganisms and plants, which significantly complicates crop cultivation. Under such conditions, potential

yield losses may reach up to 50%, as reported by S. Poudel *et al.* (2023). Soybean is one of the most important protein-oil crops in global agriculture, with yield losses under severe water deficiency potentially reaching 100%.

According to the findings of H.M. Zabolotnyi *et al.* (2020), soybean holds a leading position on both the global and Ukrainian agricultural markets, serving not only as an export crop but also as a strategically important raw material for the food and feed industries. According to the European Commission (EU Imports of Organic Agri-food Products, 2025), Ukraine ranks second among suppliers of organic soybeans to the EU, making this crop critically important for national food and economic security. However, as demonstrated by A. Abhilasha & S.R. Choudhury (2021), increasing hydrothermal stress necessitates a deeper understanding of the mechanisms underlying plant adaptation to extreme growing conditions. A key role in regulating adaptive responses is played by phytohormones, particularly ABA, which coordinates complex signalling pathways aimed at maintaining water balance in plants. The study by S. Das *et al.* (2025) confirmed that ABA synthesis is activated in the roots under water deficit conditions, after which the hormone is transported to the aerial organs, triggering a cascade of adaptive responses. These include the regulation of stomatal conductance, accumulation of osmoprotectants, reinforcement of the cuticular wax layer, and enhancement of the antioxidant defence system.

Considerable attention has been devoted to investigating the relationship between rhizosphere microbiota and the functioning of ABA-dependent signalling pathways. As shown in the study by B. Wang *et al.* (2025), microorganisms contained in biopreparations are capable of influencing endogenous ABA levels, modulating its signalling pathways, and activating the physiological and biochemical mechanisms of stress tolerance. In the study by M. del C. Orozco-Mosqueda *et al.* (2023), the potential of biopreparations for targeted regulation of the plant

phytohormonal status was emphasised. However, the relationship between ABA levels, the activity of physiological stress markers, and soybean productivity under contrasting hydrothermal conditions remains insufficiently explored.

Research by I. Yaghoubian *et al.* (2022) demonstrated that the application of plant growth-promoting microorganisms (PGPM) helps to mitigate the adverse effects of drought on soybean yield. As confirmed by the findings of J. Naamala *et al.* (2022), plant growth-promoting rhizobacteria (PGPR) can activate phytohormone synthesis – particularly ABA – increase the activity of antioxidant enzymes, and regulate the expression of stress-responsive genes. According to G.A. dos Reis *et al.* (2024), microbial inoculants are playing an increasingly important role in agriculture by reducing reliance on chemical fertilisers, enhancing crop productivity, and restoring soil biota. Thus, recent research highlights the need for further investigation into the interaction between biological preparations and ABA as key markers of organic soybean adaptation to hydrothermal stress in the context of climate change.

This study aimed to evaluate the patterns of ABA content variation in soybean plants under the influence of biopreparations of various origins in the context of a multi-year field experiment, and to analyse the relationships between ABA levels and a set of biochemical, physiological, and agronomic indicators of soybean adaptation to hydrothermal stress within an organic farming system. This objective is directed towards identifying the mechanisms through which biopreparations modulate the hormonal status of soybean to enhance its resilience to adverse climatic conditions.

Materials and Methods

The experimental research was conducted over a three-year period (2022-2024) under the agroecological conditions of the Left-Bank Forest-Steppe of Ukraine, within the territory of Kremenchuk District, Poltava Region. The subject of the study was the early-maturing soybean

variety Khorol, developed by breeders at the Soybean Research Institute Limited Liability Company (LLC). The soil cover of the experimental plots was represented by residual-solonchic chernozem on loess deposits. According to the agrochemical analysis of the arable layer (0-20 cm), carried out using a multiparameter photometer Palintest SK500 (Palintest Ltd., United Kingdom), the following characteristics were recorded: slightly acidic soil solution (pHKCl – 6.3); high humus content (5.2%); moderate availability of nitrogen compounds (total nitrogen – 58.6 mg/kg) and phosphorus (available phosphorus – 78.3 mg/kg); and a high level of exchangeable potassium (138.4 mg/kg). Field experiments were laid out using a randomised block design with three replications. The total area of the experimental site was 0.3 ha, with an accounting (measured) plot area of 0.1 ha. The cultivation technology was consistent with the typical practices for the relevant agroclimatic zone, adapted to meet the requirements of organic farming. In the crop rotation structure, the preceding crop for soybean was spring barley.

As soybean is a thermophilic crop, the sowing date in each year of the study was determined based on soil temperature reaching 10°C-12°C at a depth of 5 cm (from 20 April to 5 May). The spatial arrangement of the plants was defined by a row spacing of 38 cm and a seeding rate of 700,000 viable seeds per hectare, which ensured the formation of an optimal plant density for varieties of this maturity group under conditions of variable water availability. An analysis of the temperature regime during the growing season (May-August) showed relative stability in 2022 and 2023, with average monthly temperatures ranging from 14.5°C-21.3°C and 15.5°C-22.0°C, respectively. In contrast, 2024 was characterised by elevated temperatures, particularly in July and August, when average monthly values reached 24.1°C and 22.4°C, respectively – exceeding those of previous years. The research complied with ethical standards as outlined in the Convention on Biological Diversity (1992).

Regarding the moisture regime, the following dynamics were observed: in 2022, rainfall was evenly distributed, with monthly totals ranging from 38 to 57 mm. The year 2023 was marked by a slightly higher amount of precipitation, ranging from 47 to 67 mm per month. In stark contrast, the situation in 2024 was characterised by unprecedentedly low rainfall during critical stages of the growing season: 9 mm in May, 3 mm in July, and 3 mm in August. These values fall within the classification of extreme drought. The hydrothermal coefficient for the 2024 growing season was significantly below optimal levels, resulting in stressful conditions for soybean plants. Particularly detrimental was the combination of critically low atmospheric precipitation and elevated air temperatures during the formation of generative organs and seed filling – stages that are among the most sensitive in the ontogenesis of this crop. The prolonged lack of adequate moisture supply led to pronounced hydrothermal stress, which had a negative impact on soybean yield.

To obtain representative data on the effectiveness of biopreparation application in soybean cultivation under organic farming systems, preparations with different mechanisms of action on plant physiological processes and various forms of symbiotic interactions were selected. Particular attention was given to the potential for synergistic effects between components:

1. In the preparations Mycofriend-wp® and Mycofriend-sc® (BTU-Centre, Ukraine), synergy is achieved through the mutually enhancing action of mycorrhizal fungi (*Glomus* sp., *Trichoderma harzianum*) and rhizosphere bacteria (*Pseudomonas fluorescens*, *Streptomyces* sp., *Bacillus subtilis*, *B. megaterium* var. *phosphaticum*, *B. mucilginosus*, *Enterobacter* sp.). For instance, *Pseudomonas fluorescens* secretes metabolites that stimulate the development of *Glomus* sp. hyphae, while the mycorrhizal fungi improve phosphorus availability for *Bacillus* species.

2. Strains of *Bradyrhizobium (japonicum* strain USDA442 (532 C) and *diazoefficiens*

strains SEIMA 5079 and SEIMA 5080), included in the Profix® preparation (Certis Belchim, Belgium), demonstrate functional complementarity due to differences in root colonisation dynamics and varying temperature optima for nitrogenase activity. This ensures stable nitrogen fixation across a wide range of agroecological conditions.

3. In the Violar® preparation (IC Bioinvest-Agro LLC, Ukraine), synergy is realised through

a balanced composition of phytohormones from different classes (auxin, cytokinin, and gibberellin types), as well as free amino acids, lipids, sterols, and abscisic acid. This combination enables the integrated regulation of growth and adaptive processes within the plant organism.

The experimental design included eight treatment variants involving seed treatment and foliar application of bioproducts during the BBCH 61 growth stage (Table 1).

Table 1. Experimental design involving biopreparation application

No.	Variants	Doses	Treatment
1	Control (water)	–	seed treatment 30 minutes before sowing
2	Profix (Prof)	1.25 kg/500 kg	seed inoculation 30 minutes before sowing
3	Violar (Vio)	0.5 L/t + 100 mL/ha	seed treatment 1 hour before sowing + foliar spraying
4	Mycofriend (Myc)	1.5 L/t +	seed treatment 1 hour before sowing
5	Profix + Violar (Prof + Vio)	1.25 kg/500 kg + 100 mL/ha	seed inoculation 30 minutes before sowing + foliar spraying
6	Mycofriend + Profix (Myc + Prof)	1.5 kg/t + 1.25 kg/500 kg	seed treatment for 30 minutes + inoculation 30 minutes before sowing
7	Mycofriend + Violar (Myc + Vio)	1.5 kg/t + 100 mL/ha	seed treatment + foliar spraying
8	Mycofriend + Profix + Violar (Myc + Prof + Vio)	1.5 kg/t + 1.25 kg/500 kg + 100 mL/ha	seed treatment for 30 minutes + inoculation 30 minutes before sowing + foliar spraying

Source: developed by the authors

The relative water content (RWC) in soybean leaves was measured weekly over a seven-week period, starting from the second half of May. For the analysis, one leaf was collected from ten representative plants in each experimental plot. Samples were immediately weighed to determine their fresh weight, after which they were immersed in distilled water for 24 hours at room temperature to achieve full

turgor. The following day, the leaves were carefully blotted to remove excess moisture and reweighed to determine their turgid weight. The samples were then dried to a constant weight at 70°C to determine their dry weight. The RWC was calculated as a percentage using the following formula:

$$RWC = \frac{(FW - DW)}{(TW - DW)} \cdot 100, \quad (1)$$

where FW (g) is the fresh weight of the leaf immediately after sampling, DW (g) is the dry weight of the leaf after drying at 70°C , and TW (g) is the turgid weight of the leaf. The analytical determination of ABA content was carried out using fluorescence spectroscopy with a PerkinElmer LS-45 fluorescence spectrometer (PerkinElmer, Inc., USA). The slit widths for both emission and excitation were set at 5 nm. The spectral range covered 200-700 nm. The measurement error in determining peak positions was ± 2 nm, with a spectral band half-width of 5 nm. Fluorescence intensity measurements of ABA solutions were performed using thin quartz cuvettes (spatula-type) with a path length of 1.00 cm. The procedure for preparing ABA leaf extracts for fluorimetric measurements followed the method described by Y.-N. Li *et al.* (2009).

Stomatal conductance (SC) is a direct indicator of stomatal opening and closing in plants (Jiang *et al.*, 2006) and is typically measured using a porometer. In this study, SC in soybean leaves ($\text{mol}/\text{m}^2 \cdot \text{s}$) was measured using an SC-1 porometer (Decagon Devices, Inc., USA), with 30-second intervals and three replications for each treatment variant. The concentrations of proline and malondialdehyde (MDA) in soybean leaf tissues were determined at the BBCH 61 growth stage, following modified methodological protocols developed by M.K. Fatema *et al.* (2023). Soybean productivity was assessed by fully harvesting plants manually from the accounting plots after cutting the above-ground phytomass. Seed yield from each experimental plot was adjusted to a standard moisture content of 15% and expressed in tonnes per hectare. To summarise the results and identify key factors influencing soybean adaptive responses under varying hydrothermal conditions, principal component analysis (PCA) was conducted.

For each experimental treatment, the arithmetic mean (\bar{X}) and standard error of the mean (SE) were calculated. The statistical

significance of differences between treatment groups was assessed using one-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test at a significance level of $P < 0.05$. Data visualisation and statistical processing were performed using RStudio. Relationships between experimental parameters were evaluated through correlation analysis, with Pearson correlation coefficients calculated at a significance level of $P < 0.05$. Statistical analysis of the experimental data was conducted using Statistica 12.0 software.

Results and Discussion

Determining the level of ABA is of fundamental importance, as this phytohormone acts as a key regulator of stress-induced signalling cascades that trigger complex plant adaptation mechanisms under water deficit conditions. An integrated analysis of physiological markers (RWC, SC), in combination with biochemical indicators (proline, MDA), provides a comprehensive understanding of the functional state of the plant at various levels of organisation. It also enables the identification of correlation links between molecular-biochemical processes and the final agronomic performance of soybean cultivation under hydrothermal stress conditions (Table 2).

Based on the experimental data obtained, a detailed analysis was conducted to evaluate the effects of climatic factors and different biological seed treatments on the ABA content in plants of the early-maturing soybean variety Khorol. Weather conditions were found to correlate strongly with changes in ABA levels. In 2024, regardless of treatment type, a significant increase in ABA content was observed compared to 2022-2023: in the control variant, it reached 8.5 nmol/g, compared to 6.8 and 6.5 nmol/g, respectively. This increase of 25.0%-30.8% confirms the activation of plant defence mechanisms in response to water deficit, as ABA is a key regulator of drought response in plants.

Table 1. Effect of biopreparation treatments on molecular and physiological parameters and soybean yield under hydrothermal stress conditions (20222024)

Treatment	ABA, nmol/g DW		Proline, µg/g		MDA, µg/g		RWC, %		SC, mol/m ² ·s		Yield, t/ha			
	2022	2023	2024	2022	2023	2024	2022	2023	2024	2022	2023	2024		
Control	6.8 ± 0.10 ^a	6.5 ± 0.10 ^a	8.5 ± 0.13 ^a	5.1 ± 0.07 ^e	5.4 ± 0.08 ^c	6.9 ± 0.10 ^e	9.4 ± 0.14 ^a	73.8 ± 1.09 ^e	75.1 ± 1.10 ^e	69.7 ± 1.02 ^d	0.31 ± 0.005 ^g	2.24 ± 0.033 ^f	2.46 ± 0.035 ^f	1.86 ± 0.029 ^g
Profix	6.1 ± 0.09 ^b	5.9 ± 0.09 ^{ab}	7.8 ± 0.11 ^b	6.2 ± 0.09 ^c	6.4 ± 0.09 ^{cd}	8.6 ± 0.13 ^c	7.2 ± 0.11 ^c	80.5 ± 1.18 ^{cd}	82.5 ± 1.21 ^{cd}	77.5 ± 1.14 ^c	0.37 ± 0.005 ^{ef}	2.50 ± 0.037 ^e	2.82 ± 0.041 ^e	2.11 ± 0.029 ^f
Violar	5.4 ± 0.08 ^{cd}	5.2 ± 0.08 ^{cd}	6.9 ± 0.10 ^c	6.8 ± 0.10 ^b	7.5 ± 0.11 ^{ab}	9.5 ± 0.14 ^b	5.9 ± 0.09 ^d	11.3 ± 0.17 ^e	87.2 ± 1.28 ^{bcd}	84.0 ± 1.24 ^b	0.36 ± 0.005 ^{cd}	2.71 ± 0.099 ^d	2.93 ± 0.044 ^{de}	2.32 ± 0.035 ^e
Mycofriend	5.6 ± 0.08 ^c	5.4 ± 0.08 ^c	7.1 ± 0.10 ^c	5.7 ± 0.08 ^d	5.9 ± 0.09 ^{de}	7.4 ± 0.11 ^e	8.2 ± 0.12 ^{ab}	14.8 ± 0.22 ^b	81.2 ± 1.20 ^{de}	77.8 ± 1.14 ^c	0.35 ± 0.005 ^d	2.73 ± 0.039 ^{cd}	3.01 ± 0.045 ^{de}	2.41 ± 0.036 ^{de}
Prof + Vio	5.1 ± 0.07 ^d	4.8 ± 0.07 ^e	6.7 ± 0.10 ^c	7.4 ± 0.11 ^a	7.8 ± 0.11 ^a	10.4 ± 0.15 ^a	6.1 ± 0.09 ^d	10.9 ± 0.16 ^e	88.7 ± 1.30 ^{abc}	85.7 ± 1.26 ^b	0.43 ± 0.006 ^c	2.91 ± 0.044 ^{bc}	3.10 ± 0.045 ^{cd}	2.53 ± 0.037 ^{cd}
Myc + Prof	5.3 ± 0.08 ^{cd}	5.0 ± 0.07 ^{de}	6.2 ± 0.09 ^d	6.1 ± 0.09 ^{cd}	6.3 ± 0.09 ^{cd}	8.0 ± 0.12 ^d	7.3 ± 0.11 ^c	12.6 ± 0.19 ^d	87.8 ± 1.29 ^{abcd}	84.6 ± 1.25 ^b	0.41 ± 0.006 ^{bc}	2.93 ± 0.043 ^{cd}	3.24 ± 0.048 ^{bc}	2.65 ± 0.039 ^{bc}
Myc + Vio	4.6 ± 0.07 ^e	4.4 ± 0.06 ^f	6.0 ± 0.09 ^{de}	6.3 ± 0.09 ^c	6.4 ± 0.09 ^b	8.3 ± 0.12 ^{cd}	6.9 ± 0.10 ^c	6.4 ± 0.09 ^c	89.5 ± 1.32 ^{ab}	86.7 ± 1.27 ^{ab}	0.46 ± 0.007 ^b	3.02 ± 0.045 ^{ab}	3.36 ± 0.050 ^{ab}	2.76 ± 0.041 ^b
Myc + Prof + Vio	4.2 ± 0.06 ^f	4.1 ± 0.06 ^f	5.6 ± 0.08 ^e	6.5 ± 0.10 ^{bc}	6.6 ± 0.10 ^{cd}	8.7 ± 0.13 ^c	6.3 ± 0.09 ^d	9.4 ± 0.14 ^f	94.7 ± 1.39 ^a	91.9 ± 1.35 ^a	0.41 ± 0.006 ^a	3.15 ± 0.046 ^a	3.57 ± 0.053 ^a	2.96 ± 0.045 ^a

Note: data are presented as mean ± SE (n = 10). Values followed by different letters (a, b, c, d, e, f, g) for each variable indicate statistically significant differences at the p < 0.05 level according to Tukey's HSD post hoc test

Source: compiled by the authors

The findings showed that the use of biopreparations with different modes of action contributed to a reduction in ABA levels compared to the control in all years of the study, indicating their beneficial role in enhancing plant stress resistance. The greatest reduction in ABA content relative to the control was observed under the combined treatment Myc + Prof + Vio, which led to a 36.9%-38.2% decrease in 2022-2023 and 34.1% in the drought-stricken year 2024. The Myc + Vio combination also demonstrated high effectiveness, reducing ABA levels by 32.3% in favourable years and by 29.4% in 2024. Among single treatments, Violar proved the most effective, resulting in a 20.0%-20.6% reduction in ABA content in favourable years and an 18.8% reduction in 2024.

Analysis of the results from 2022-2024 confirmed a significant influence of climatic factors on the biochemical indicators of soybean adaptive responses, with the most pronounced changes observed in 2024, a year marked by extreme weather conditions (Table 2). In particular, in response to the 2024 drought, a substantial increase in proline content was recorded across all treatment variants compared to 2022-2023. In the control, the proline level reached 6.9 $\mu\text{g/g}$, which was 26.7%-34.7% higher than in previous years. This indicates the activation of natural defence mechanisms, as proline is a key osmoprotectant that accumulates in plant cells under water deficit conditions, helping to maintain osmotic balance.

The highest proline accumulation was recorded under the combined Prof + Vio treatment: in 2022-2023, proline levels ranged from 7.4 to 7.8 $\mu\text{g/g}$ (an increase of 43.9%-45.1% compared to the control), and in 2024 reached 10.4 $\mu\text{g/g}$ (51.4% higher). This effect can be attributed to the synergistic action of nitrogen-fixing bacteria and the phytohormonal complex in regulating metabolic processes. The integrated application of all three biopreparations (Myc + Prof + Vio) also led to a significant increase in proline content – up to 6.5-6.6 $\mu\text{g/g}$ in 2022-2023 and 8.7 $\mu\text{g/g}$ in

2024 (21.0%-27.6% above the control), confirming the effectiveness of a combined approach in stimulating plant adaptive mechanisms.

The changes in MDA content proved particularly significant. In 2024, the control treatment recorded an MDA level of 17.9 $\mu\text{g/g}$, nearly double that of 2022-2023 (9.2-9.4 $\mu\text{g/g}$). This sharp increase indicates an intensification of lipid peroxidation processes in plant cell membranes under drought conditions, which negatively affects their structural integrity and function. The most effective treatment in reducing MDA content was the combined application of three biopreparations (Myc + Prof + Vio), which resulted in a 33.2%-37.2% decrease in 2022-2023 and a 47.7% reduction in 2024. These findings suggest a strong antioxidant effect and the development of a comprehensive cellular defence system against oxidative stress. The Myc + Vio combination also demonstrated high efficacy, reducing MDA content by 26.4%-29.8% in 2022-2023 and by 37.8% in 2024, confirming the important role of mycorrhizal fungi and phytohormones in maintaining membrane integrity under abiotic stress.

Relative water content (RWC) and stomatal conductance (SC) are key physiological indicators that reflect a plant's water status and its capacity to withstand drought. The relationship between these parameters highlights various aspects of water exchange, the adaptive potential of plants under adverse environmental conditions, and overall physiological activity. Analysing the dynamics of these indicators concerning climatic conditions and the influence of biopreparations provides a valuable foundation for developing effective agronomic strategies for soybean cultivation in the context of climate change and increased aridity.

In the control treatments, RWC was recorded at 73.8% in 2022, 75.1% in the wetter year of 2023, and dropped to a critical 69.7% during the drought conditions of 2024. This 7.2% decrease in 2024 compared to 2023 indicates a significant disruption of the plant's water balance under severe water deficit. It is well established

that a decline in RWC to 70%-80% in plant cells suppresses key metabolic processes, including photosynthesis and respiration, signalling severe physiological stress and potentially irreversible damage to the structure of cell membranes (Filipović, 2020). The SC in the control plots decreased by 38.7% in 2024 compared to the wetter 2023 season, reflecting the activation of plant defence responses aimed at reducing water loss through stomatal closure.

The application of biopreparations significantly improved the water status of plants in all study years, although the most pronounced effect was observed under drought conditions. In the favourable year of 2023, Profix application increased RWC to 82.5% (+ 9.9% compared to control), Violar to 87.2% (+ 16.1%), and Mycofrend to 81.2% (+ 8.1%). The combination of biopreparations produced an even more pronounced effect: Prof + Vio increased RWC to 88.7% (+ 18.1%), Myc + Prof to 87.8% (+ 16.9%), and Myc + Vio to 89.5% (+ 19.2%). The most effective treatment was the comprehensive application of Myc + Prof + Vio, which raised RWC by 26.1% compared to the control, indicating a strong synergistic effect of biological agents with different mechanisms of action.

Under the acute drought conditions of 2024, the impact of biopreparations on plant water status was even more evident. The combined application of Myc + Prof + Vio increased RWC by 31.9% relative to the control, demonstrating the biopreparations' effectiveness in enhancing cellular water retention. Notably, even under extreme drought, plants treated with the full combination reached an RWC of 91.9%, significantly exceeding the control value under favourable 2023 conditions (75.1%). This confirms the high efficacy of biopreparations in improving stress tolerance and mitigating the adverse effects of water deficit.

The application of biopreparations also significantly influenced SC, ensuring optimal levels in line with environmental conditions. In 2023, Profix seed treatment increased SC by 19.4% compared to the control, Violar by 25.8%, and

Mycofrend by 12.9%. The most pronounced effects were observed with combined treatments: Prof + Vio enhanced SC by 38.7%, Myc + Prof by 32.3%, and Myc + Vio by 48.4%. The highest stomatal conductance was recorded with the full combination Myc + Prof + Vio, reaching $0.51 \text{ mol/m}^2 \cdot \text{s}$ (+ 64.5% compared to control), which ensured a high level of photosynthetic activity.

Under the drought conditions of 2024, the action of the biopreparations was not aimed at maximising stomatal opening but rather at its optimal regulation. The combined treatment with Myc + Prof + Vio increased SC to $0.39 \text{ mol/m}^2 \cdot \text{s}$, which was 2.1 times higher than the control. This ensured a sufficient level of photosynthesis while simultaneously limiting water loss. The unfavourable climatic conditions of 2024 led to a marked reduction in soybean yield across all experimental treatments (Table 2). In the control variant, the yield was 1.9 t/ha, which was 17.0%-24.4% lower compared to 2022-2023. A decrease in RWC below 70% confirms that water deficit is a critical factor disrupting water exchange, photosynthesis, respiration, and assimilate transport, all of which directly affect yield formation.

The highest yield was achieved through the combined application of the three biological preparations, which increased yield by 40.6%-45.1% in 2022-2023 and by 59.1% in 2024 relative to the control. These results are supported by statistical analysis and confirm the high effectiveness of an integrated approach to enhancing drought tolerance and soybean productivity. High yield levels were also ensured by the Myc + Vio combination (+ 34.8%-36.6% in 2022-2023 and + 48.4% in 2024), highlighting the key role of mycorrhizal symbiosis and hormonal regulation in shaping soybean performance.

To systematise the results and facilitate interpretation of the effects of biological preparations on plant adaptive traits, a heat map was constructed. This approach enables a clear assessment of the effectiveness of individual and combined biotreatment variants under varying hydrothermal conditions (Fig. 1).

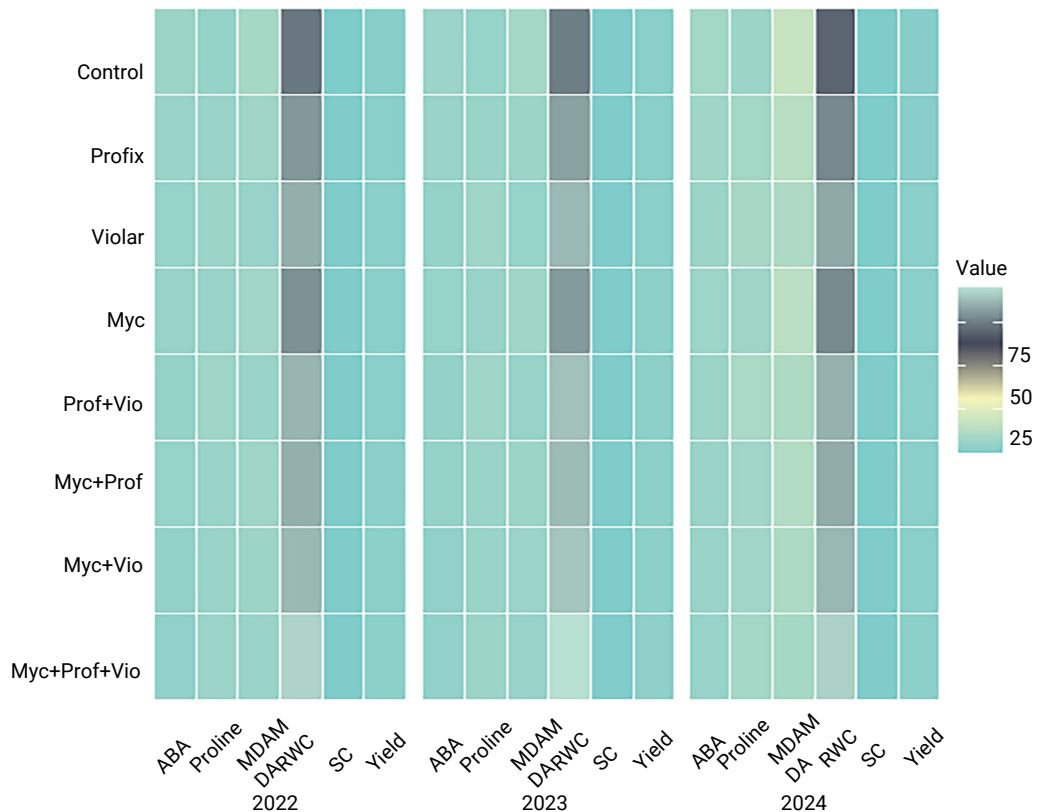


Figure 1. Heat map of comprehensive experimental indicators of soybean under different biotreatment variants in contrasting hydrothermal conditions (2022-2024)

Source: compiled by the authors

The heat map results reveal a clear dependence of ABA levels on hydrothermal conditions and the influence of biopreparations. In 2022, all variants exhibited moderate concentrations of this stress-related phytohormone. In contrast, the extreme weather conditions of 2024 led to a peak accumulation of ABA in the control treatment, indicating the activation of stress-induced mechanisms in response to water deficit. The lowest ABA values were observed in the treatments with combined application of Myc + Prof + Vio and Myc + Vio, even under drought conditions in 2024, confirming their effectiveness in reducing stress levels.

The accumulation dynamics of proline correlated with the intensity of abiotic stress. The highest concentrations of this osmoprotectant were recorded in 2024 across all variants; however, the

combined biopreparations resulted in the smallest increase, demonstrating a stabilising effect on metabolic processes. Throughout all study years, the Myc + Prof + Vio treatment ensured the most consistent proline concentrations. The MDA content, as a marker of the intensity of lipid peroxidation processes, increased sharply in 2024 in the control variant, indicating the activation of oxidative stress. The most effective reduction in MDA levels was achieved through the use of biopreparations containing mycorrhizal components, particularly Mycofriend, confirming their antioxidant properties.

The RWC index exhibited an inverse trend in relation to the intensity of hydrothermal stress. The lowest values were recorded in 2024 under the control conditions, reflecting a significant disruption of water exchange. Biopreparations

effectively supported water balance, particularly due to mycorrhizal symbioses, which enhanced the water-absorbing capacity of the root system. The highest RWC values were recorded in the combined treatment variants, notably Myc + Prof + Vio, even under the extreme water deficit conditions of 2024. The dynamics of SC reflected the complex nature of plant adaptive responses to water stress. In 2024, a critical reduction in SC was observed in the control, associated with stomatal closure aimed at reducing water loss. Biopreparations contributed to the optimisation of stomatal functioning, maintaining a balance between adequate photosynthetic activity and water conservation. The most stable SC values were demonstrated by the combined biopreparation treatments.

Soybean yield showed a direct correlation with the effectiveness of adaptive mechanisms. The highest productivity levels were achieved in the combined treatments incorporating mycorrhizal fungi, nitrogen-fixing bacteria, and phytohormonal formulations. Under the conditions of severe water deficit in 2024, biopreparations significantly mitigated the negative effects of stress, whereas a marked decline in yield was observed in the control treatment. The use of individual biopreparations (Profix, Violar, Mycofriend) resulted in moderate improvements in adaptive parameters; however, their effectiveness was insufficient under extreme conditions. Combinations of two biopreparations (Prof + Vio, Myc + Prof, Myc + Vio) exhibited a synergistic effect, particularly in stabilising water balance and reducing oxidative stress indicators. The highest overall efficacy across all experimental parameters was achieved with the integrated application of Myc + Prof + Vio, which led to a reduction in stress markers (ABA, MDA), maximal preservation of RWC, SC, balanced proline accumulation, and consistently high yields.

The combined action of the biopreparations was driven by several interacting factors: improved nitrogen nutrition provided by *Bradyrhizobium japonicum* and *B. diazoefficiens* (Profix); the formation of mycorrhizal symbioses involving

Glomus sp. and *Trichoderma harzianum* (Mycofriend); hormonal balance regulation via naturally derived phytohormones (Violar); and the antagonistic activity of *Bacillus subtilis* and *Pseudomonas fluorescens* against pathogenic microorganisms.

To validate the observed patterns, a correlation analysis was conducted between the physiological and biochemical indicators of soybean adaptation to hydrothermal stress. Most notably, a strong negative correlation was observed between ABA content and yield ($r = -0.909$), confirming the central role of this phytohormone in reducing soybean productivity under stress conditions. A strong positive correlation was also identified between ABA and MDA levels ($r = 0.882$), indicating a direct relationship between the activation of ABA synthesis and the onset of oxidative stress in plant tissues. ABA content showed a strong negative correlation with SC ($r = -0.885$), confirming the physiological role of ABA as a key regulator of stomatal closure in response to water deficit. A negative correlation was also observed between ABA and relative water content (RWC) ($r = -0.723$), indicating a deterioration of the plant's water status as the concentration of this stress hormone increased. A weak positive correlation between ABA and proline content ($r = 0.306$) suggests their parallel yet partially independent involvement in stress-adaptive mechanisms.

A significant negative correlation was found between MDA content and SC ($r = -0.799$), reflecting the disruption of gas exchange processes under oxidative stress. Furthermore, the correlation between MDA and RWC ($r = -0.574$) confirms the adverse impact of enhanced lipid peroxidation on the water balance of plants. A strong negative correlation between MDA and yield ($r = -0.754$) highlights the direct inhibitory effect of oxidative stress on soybean productivity. Conversely, a strong positive correlation between RWC and SC ($r = 0.832$) reflects the close relationship between the plant's water regime and stomatal function. A similarly high positive correlation between RWC and yield ($r = 0.847$) underscores the critical role of maintaining optimal water status in

achieving high productivity. The strongest positive correlation was recorded between SC and yield ($r = 0.955$), emphasising the decisive importance of stable stomatal function in ensuring maximum soybean productivity.

The results obtained clearly confirmed that the application of biopreparations contributes to the optimisation of the water regime, the reduction of oxidative stress intensity, and the maintenance

of crop productivity under hydrothermal stress, through a complex influence on key physiological and biochemical mechanisms of plant adaptation. Figure 2 presents the findings of a three-year study (2022-2024) on the effects of biopreparations with different functional properties on the molecular and physiological-biochemical indicators, as well as the yield, of the soybean cultivar Khorol under organic farming conditions.

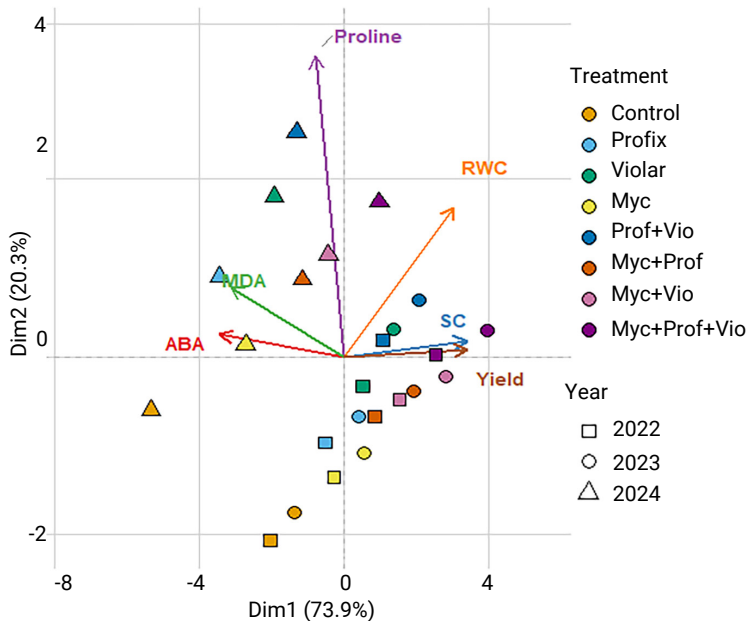


Figure 2. Principal component analysis – relationship between the action of biopreparations and the set of experimental indicators (2022-2024)

Source: compiled by the authors

The total explained variance accounted for 91.8% (Dim1 – 63.3%, Dim2 – 28.5%), indicating the high informativeness of the data obtained and the validity of the applied approach. The biplot analysis clearly illustrates the differentiation of experimental data by year, reflecting the decisive influence of the hydrothermal regime on the development of adaptive responses in plants. For instance, the year 2022 was characterised by moderate moisture availability and optimal temperature conditions, as evidenced by the clustering of relevant points predominantly in the lower section of the biplot. The 2023 season, marked by evenly

distributed rainfall throughout the growing period, resulted in the highest recorded yields, which is confirmed by the grouping of “Yield-2023” data points in the right-hand section of the biplot.

Of particular interest are the results from 2024, when extreme drought conditions were observed, especially during the critical stages of reproductive organ development (July August). The corresponding data points are concentrated in the upper section of the biplot and exhibit a strong correlation with indicators of stress-induced metabolites and physiological/biochemical adaptive mechanisms. In particular, a positive

correlation was established between drought conditions and the accumulation of proline, a typical osmoprotectant whose synthesis intensifies in response to water deficit. The elevated levels of MDA in plants grown in 2024 reflect the activation of lipid peroxidation processes in membrane structures triggered by oxidative stress.

The most significant outcome of the PCA was the clear differentiation in plant responses to various biopreparation treatments depending on the level of hydrothermal supply. Complex treatment schemes, especially Myc + Prof + Vio, demonstrated the highest efficacy under drought conditions in 2024, reflected in increased RWC and SC values. This finding is of fundamental importance for understanding the mechanisms underlying the development of drought tolerance in soybean agroecosystems under the influence of biological preparations. The synergistic action of arbuscular mycorrhizal fungi, nitrogen-fixing bacteria, and phytohormonal regulators facilitates the formation of a multi-level adaptive response to water deficit. It was shown that single treatments produced intermediate effectiveness, whereas control variants consistently exhibited the poorest performance, particularly under stress conditions. This confirms the rationale for using complex biopreparation schemes to ensure multi-component protection of the plant organism against abiotic stress factors.

The analysis of the contrasting hydrothermal conditions in 2023 and 2024 revealed that, under optimal moisture availability, the effectiveness of biopreparations was primarily expressed through increased yield and the optimisation of the water regime. In contrast, under drought conditions, their action was directed towards the activation of defence responses through the accumulation of the osmolyte proline and the reduction of oxidative stress levels. Of particular note is the consistent effectiveness of the Myc + Prof + Vio treatment across years with contrasting weather conditions, which is of critical importance for developing adaptive technologies for organic soybean cultivation in the context of climate change.

The findings are consistent with current scientific evidence regarding the influence of biopreparations of various origins on the hormonal status of plants, particularly regarding abscisic acid levels. ABA is one of the key signalling molecules that regulates numerous aspects of plant growth, development and physiological condition, playing a particularly important role in shaping adaptive responses to abiotic stresses, as noted in the study by Y. Osakabe *et al.* (2014). Hormone-mediated signalling pathways, particularly those involving ABA and auxin, are considered by V. Verma *et al.* (2016) to be essential for maintaining the balance between growth processes and the activation of defence responses under stress conditions.

The physiological role of ABA lies in its ability to initiate structural and functional changes in plant cells aimed at preserving the organism's viability under adverse conditions. The strong negative correlation identified in this study between ABA content and stomatal conductance ($r = -0.885$) confirms the regulatory function of this phytohormone in controlling stomatal activity. According to S. Munemasa *et al.* (2015), an increase in ABA concentration in response to hydrothermal stress induces the closure of stomata – microscopic pores on the leaf surface formed by guard cells, which regulate gas exchange and water vapour loss. This mechanism serves as an effective adaptive response, allowing plants to reduce transpirational losses and maintain water balance during drought. The observed relationship between ABA concentration and stomatal activity confirms the crucial role of this phytohormone in regulating the water regime of soybean under hydrothermal stress. The established correlation indicates the high activity of ABA-dependent signalling cascades in managing water exchange processes in soybean plants exposed to hydrothermal stress.

It is important to emphasise the role of rhizospheric microbiota – PGPR and arbuscular mycorrhizal fungi – in modulating the phytohormonal status of plants, particularly the level of ABA. PGPR strains present in the biopreparations

Mycofriend and Profix, as well as in the metabolite-based preparation Violar, are known to influence the hormonal balance of plants both directly and indirectly, enhancing productivity and stress resilience. Research by R. Porcel *et al.* (2014) confirms that these effects involve regulation of ABA synthesis and modulation of ethylene levels.

According to D. Barnawal *et al.* (2017), certain rhizobacterial strains are capable of metabolising ABA, thereby reducing its concentration in plant tissues, which positively influences growth processes. Similar properties are characteristic of mycorrhizal fungi. For example, H. Esch *et al.* (1994) identified the presence of ABA in the spores and hyphae of *Glomus* species, confirming their ability to synthesise or accumulate this hormone. Research by A. Martinez-Medina *et al.* (2013) showed that root colonisation by *Trichoderma harzianum* enhances the expression of ABA-responsive genes, thereby increasing the adaptive capacity of plants. These findings are supported by later studies (e.g. Rawal *et al.*, 2022), which demonstrated the ability of *T. harzianum* to improve plant tolerance to abiotic stresses, including drought, through the regulation of phytohormonal signalling pathways.

The role of rhizobacteria in regulating ABA levels is further supported by the findings of M. Saakre *et al.* (2017), who described the ability of *Pseudomonas fluorescens* to enhance plant drought tolerance through modulation of ABA levels. The positive impact of PGPR on soybean growth has also been confirmed in recent studies by S. Gouli *et al.* (2024). Certain *Streptomyces* strains, according to F.M. Rashad *et al.* (2015), demonstrate the capacity to synthesise ABA *in vitro*, reinforcing their potential role in the hormonal regulation of growth processes. The interaction of *Bacillus megaterium* var. *phosphaticum* with plant hormonal balance highlights the complex nature of phytohormonal signalling in microbe-plant associations, as discussed by H. Etesami *et al.* (2023). Particularly noteworthy are the findings of R. Hidri *et al.* (2024), which showed that dual inoculation with *Bacillus subtilis* and

arbuscular mycorrhizal fungi effectively protects plants from salt stress by modulating ABA levels and activating antioxidant mechanisms.

The findings of the present study underscore the complexity and variability of plant phytohormonal responses depending on the combination of environmental conditions and type of biological treatment. As shown by E.A. Kazerooni *et al.* (2021), PGPR treatment can reduce ABA levels in plants exposed to stress factors by 45.2%-86.5%, which is consistent with the present results. Specifically, under hydrothermal stress, an increase in ABA content by 31.5%-32.2% was recorded in soybean plants inoculated with mycorrhizal fungi (Myc) or rhizobacteria (Profix), compared to plants grown under favourable conditions. Comparison of the treated variants with the stress control revealed a tendency towards a reduction in ABA levels by 8.2%-16.5%, depending on the treatment scheme. This indicates the effectiveness of biopreparations in reducing the need for activation of ABA-dependent defence mechanisms by improving the physiological state of the plants.

The most effective result was observed with the combined treatment of Myc + Prof, which led to a 27.1% reduction in ABA levels, indicating optimised water status and mineral nutrition. The decrease in ABA under stress conditions, recorded in this study (up to 34.1% following Myc + Prof + Vio treatment), is consistent with the findings of R.A. Repke *et al.* (2022), who noted that biostimulants are capable of lowering ABA concentrations under stress, thereby helping to maintain stomatal function and photosynthetic potential.

These results are also in line with previous studies on the impact of seed priming on the physiological and biochemical parameters of emmer wheat. In particular, UV-C seed irradiation combined with treatment using the 1r Seed Treatment solution led to an increase in photosynthetic pigments and a corresponding rise in yield by 21.4%-31.0% (Korotkova *et al.*, 2023). Similar outcomes were obtained in soybean studies, where the application of the phytohormonal preparation Violar in combination with a

bacterial inoculant enhanced chlorophyll content by 5.5%-17.2% and leaf surface area by 13.6%-21.2% (Chaika & Korotkova, 2025). Thus, the results of the study confirm the effectiveness of biopreparations in enhancing the molecular and physiological-biochemical resilience of plants to hydrothermal stress. This is attributed to their ability to modulate hormonal status, optimise water balance, and support crop productivity.

Conclusions

The study confirmed the key role of abscisic acid (ABA) in shaping the adaptive responses of soybean plants to hydrothermal stress through the regulation of structural and functional changes, particularly stomatal activity and water status. Under water-deficit conditions, ABA concentrations increased by 25.0%-30.8%, indicating the activation of protective mechanisms. The application of biopreparations led to a reduction in ABA levels by an average of 9.2%-36.3%, confirming their positive effect on the drought tolerance of soybean plants. In response to the drought conditions of 2024, a significant increase in proline content was observed across all experimental treatments, indicating the activation of natural osmoregulatory mechanisms. The greatest accumulation of proline was recorded following the combined application of Prof + Vio, with an average increase of 47.1% compared to the control.

The drought conditions also caused a marked rise in the concentration of malondialdehyde (MDA), indicating intensified lipid peroxidation and a disruption of the structural integrity of cell membranes. The combined application of biopreparations, particularly Myc + Prof + Vio, resulted in a 15.6%-41.8% reduction in MDA content and a 9.1%-46.6% increase in proline levels compared to the control, suggesting the formation of an effective antioxidant defence system. The application of biopreparations significantly improved the water status of soybean plants throughout all years of the study – on average by 10.0%-27.6% compared to the control – confirming the development of effective cellular waterretention

mechanisms and enhanced stress tolerance. The optimisation of stomatal activity under the influence of biopreparations was achieved not only through increased conductance (by 19.2%-65.4% relative to the control), but also via the formation of regulatory mechanisms that ensured the adaptive functioning of the stomatal function in accordance with hydrothermal conditions.

Correlation analysis confirmed the key mechanisms influencing productivity: a strong negative correlation was observed between yield and ABA content ($r = -0.909$) as well as MDA content ($r = -0.754$), while a strong positive correlation was found with stomatal conductance ($r = 0.955$) and relative water content ($r = 0.847$). A 17%-24% decrease in yield in the control under drought conditions confirmed the critical role of water deficit in limiting soybean productivity. At the same time, the use of biopreparations – especially the Myc + Prof + Vio complex – contributed to a 12.3%-45.5% increase in yield due to improved water status and reduced concentrations of ABA and MDA, highlighting the effectiveness of an integrated approach to enhancing crop drought tolerance.

The findings clearly demonstrate that the combined use of biopreparations is the most effective strategy for increasing the adaptive potential of organic soybeans to hydrothermal stress. The three-component combination of mycorrhizal fungi, nitrogen-fixing bacteria, and phytohormones provides systemic support for plant metabolic processes and ensures stable productivity even under extreme weather conditions. These results support the development of adaptive technologies for organic soybean cultivation in response to current climate change trends.

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Conflict of Interest

None.

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Абсцизова кислота та біопрепарати як маркери адаптації органічної сої до гідротермального стресу

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Анотація. У зв'язку зі зростанням попиту на органічну сою та необхідністю зменшення негативного впливу кліматичних змін актуальним є дослідження впливу біопрепаратів різної природи на адаптивні механізми рослин до стресових умов. Метою роботи було оцінити зміну вмісту абсцизової кислоти (АВА) під дією біопрепаратів у багаторічному польовому експерименті та проаналізувати взаємозв'язки АВА з біохімічними, фізіологічними й агрономічними показниками адаптації сої до гідротермічного стресу в умовах органічного землеробства. Дослідження проводили з використанням біопрепаратів на основі мікоризних грибів, ризосферних й азотфіксуючих бактерій і фітогормонів. Застосовано комплекс польових, лабораторних (фізіологічних, біохімічних) і статистичних методів. Встановлено інтеграцію молекулярних, біохімічних, фізіологічних і агрономічних маркерів стресостійкості завдяки підвищенню адаптивного потенціалу сої при застосуванні біопрепаратів. Їх ефективність проявлялася через комплексний вплив на ключові адаптивні механізми: зниження концентрації стресового гормону АВА на 9,2-36,3 %, що свідчило про послаблення стресового навантаження; підвищення відносного вмісту води на 10,0-27,6 %, що забезпечувало оптимальний водний статус клітин; покращення продихової провідності на 19,2-65,4 % завдяки оптимізації роботи продихів; зниження рівня перекисного окислення ліпідів (малонового діальдегіду – на 15,6-41,8 %), що вказувало на ефективний захист мембран від оксидативного стресу; підвищення вмісту проліну на 9,1-46,6 %, що свідчило про активацію природних захисних механізмів і підтримку осмотичного балансу. Кореляційний аналіз підтвердив,

що біопрепарати забезпечують комплексну модуляцію стресової відповіді рослин, оптимізуючи баланс між захисними реакціями та продуктивністю, що призводило до підвищення врожайності на 12,3-45,5 % навіть за несприятливих погодних умов. Встановлено, що під час водного стресу біопрепарати модулюють біосинтез АВА та її регуляторну функцію, сприяючи координуваним змінам осмопротекторних механізмів, антиоксидантного захисту та водного режиму рослин. Найбільш ефективною виявилася потрібна комбінація біопрепаратів, яка забезпечувала оптимальний баланс між фітогормональною регуляцією, фізіологічними процесами та продуктивністю сої в умовах гідротермічного стресу. Отримані результати можуть бути рекомендовані для вирощування органічної сої з метою підвищення її врожайності в умовах кліматичних викликів

Ключові слова: органічне землеробство; відносний вміст води; продихова провідність; пролін; малоновий діальдегід; урожайність



Use of innovative technologies to extend the shelf life of dairy products

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Abstract. The aim of the study was to provide a comprehensive assessment of the impact of innovative technologies on the processing and treatment of dairy products in order to extend their shelf life. A comparative analysis of non-thermal methods revealed that high-pressure processing (600 MPa, 15 minutes) and pulsed electric fields (35 kV/cm, 1,000 Hz) were the most effective in inactivating microorganisms, reducing their overall count by 4.2 and 3.8 log CFU/cm³, respectively. Non-thermal techniques showed less impact on the nutritional composition of milk compared to traditional pasteurisation, preserving up to 91.8% of vitamin C and 97.2% of whey proteins. The best organoleptic properties were observed in samples treated with high-pressure processing and pulsed electric fields, scoring 4.9 out of 5 on a 5-point scale. It was also found that ultrasound treatment accelerated

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yoghurt fermentation by 28%-32%, high pressure shortened cheese ripening time by 30%-35%, and ultrafiltration increased the yield of acid-set cheese by 15%-18%. Among antimicrobial packaging materials, the highest activity was demonstrated by films containing silver nanoparticles (1.5%). A synergistic effect was observed when combining non-thermal processing methods with innovative packaging materials, enabling the extension of milk's shelf life to 35 days at a temperature of $4 \pm 1^\circ\text{C}$. Intelligent packaging systems with pH-sensitive indicators were developed for monitoring product freshness. To investigate the synergistic effect of combined treatments, a factorial experimental design was employed with the calculation of synergy coefficients. An analysis of nanoparticle migration from packaging materials showed that their concentration remained within permissible limits when recommended storage conditions were observed. Mathematical models were developed to predict milk shelf life based on processing and packaging parameters. An economic evaluation of the proposed technological solutions demonstrated their competitiveness in comparison with traditional methods, particularly in the production of premium-segment products

Keywords: non-thermal processing; high pressure; pulsed electric field; antimicrobial packaging; nanoparticles; intelligent packaging systems

Introduction

Dairy products constitute an important component of the human diet due to their high nutritional value and the bioavailability of their nutrients. However, these products are characterised by a limited shelf life owing to their microbiological vulnerability and susceptibility to oxidative processes. This issue highlights the need for the development and implementation of innovative processing technologies capable of extending the shelf life of dairy products without adversely affecting their organoleptic and nutritional properties. Traditional preservation methods, such as thermal pasteurisation and sterilisation, do not always provide an optimal balance between product safety and the retention of quality attributes. In particular, high-temperature processing often results in protein denaturation, vitamin loss, and a deterioration in taste.

Significant contributions to research on dairy quality preservation technologies have been made by V. Chiozzi *et al.* (2022), who conducted a comparative analysis of thermal and non-thermal food processing technologies. The authors noted that techniques such as ultrasound, ultraviolet (UV) irradiation, ozonation, and high hydrostatic pressure exhibit considerable potential for

microorganism inactivation in milk while exerting minimal impact on its sensory and nutritional characteristics. The researchers paid particular attention to the safety of applying these technologies in the dairy industry and their compliance with contemporary food safety standards. K.K. Dash *et al.* (2022) and E. Bancalari *et al.* (2020) investigated the effects of various thermal processing regimes on the quality and microbiological safety of milk and dairy products. The authors systematised data on the impact of both traditional and modified thermal treatments on the nutrient composition of milk and proposed alternative approaches aimed at minimising the adverse effects of heat treatment. These studies underscore the need to develop innovative technologies that ensure effective microorganism inactivation while preserving the biological value of dairy products.

In the field of intelligent packaging systems, significant contributions were made by A.M. Alizadeh *et al.* (2022) and P.P. Akhila *et al.* (2022), who analysed trends and opportunities for applying intelligent packaging to dairy products. The researchers categorised various types of active and intelligent packaging materials, including antimicrobial agents, oxygen scavengers,

moisture absorbers, as well as freshness sensors and temperature indicators. They also assessed the potential of these materials to extend the shelf life of different dairy product categories and their influence on consumer perception. S. Pandey *et al.* (2022) explored the application of bio-inspired active packaging materials to enhance the shelf life and safety of food products. The authors focused particularly on materials with antimicrobial properties based on natural compounds such as chitosan, essential oils, bacteriocins, and enzymes. They found that such materials are highly effective against a broad spectrum of microorganisms responsible for dairy spoilage, offering the added benefit of environmental safety in comparison with synthetic antimicrobial agents.

The barrier properties of packaging materials for dairy products were thoroughly examined by K. Sneha & A. Kumar (2022), who investigated the potential of nanoemulsions for enhancing packaging performance. The authors developed and characterised various types of nanoemulsions based on natural compounds with antimicrobial and antioxidant properties. Their findings showed that incorporating such nanoemulsions into packaging materials can significantly extend the shelf life of dairy products by enabling the controlled release of active components and creating an effective barrier against oxygen and moisture. The functional properties of dairy products under innovative processing technologies were studied by H. Sharma *et al.* (2023), who explored the impact of lactic acid bacteria and their metabolites on the techno-functional properties and health benefits of fermented dairy products. The authors found that using specific strains of lactic acid bacteria in combination with innovative processing techniques not only prolongs product shelf life but also enriches the products with bioactive compounds such as bioactive peptides, exopolysaccharides, and vitamins. Contemporary developments in automation and digitalisation in the food industry, including the dairy sector, were explored by S. Sharma *et al.* (2021). They analysed the potential of artificial intelligence and big

data analytics for optimising production processes, quality control, and predicting the shelf life of dairy products. The authors developed conceptual models of intelligent quality monitoring systems that integrate data from various sources – including in-pack sensors, storage conditions, and transport environments – to enable more accurate determination of actual shelf life under specific conditions. The environmental aspects of innovative technologies in the dairy industry were examined by W. Ahmed *et al.* (2022). The researchers conducted a comprehensive life cycle analysis of various packaging materials and processing technologies in terms of their environmental impact. They proposed strategies to reduce the ecological footprint of the dairy industry, including the use of biodegradable packaging materials, energy-efficient processing technologies, and the adoption of circular economy principles.

This study aimed to systematise and compare modern innovative processing and treatment technologies for dairy products with the objective of extending shelf life, while taking into account their effects on organoleptic, physicochemical, and microbiological quality indicators. To achieve this aim, it was essential to analyse the main factors limiting the shelf life of dairy products and to classify non-thermal and combined processing methods for both raw milk and finished dairy goods. It was also necessary to examine the effectiveness of various innovative technologies – including advanced packaging solutions – depending on the category of dairy product.

Materials and Methods

The study was conducted during 2023-2024 at the laboratory complex of the Department of Food Technology and Microbiology, Odesa National University of Technology. For the experimental part of the research, whole cow's milk was selected with a fat content of 3.2%, protein content of 3.0%, and total solids of 12.5%, in accordance with the requirements of DSTU ISO 707:2002 (2002). Special attention was paid to preventing potential contamination of samples during milk

collection. The microbiological indicators of the raw material met the regulatory standards: the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAM) did not exceed 1×10^5 CFU/cm³, and coliform bacteria and pathogenic microorganisms were not detected. For the comparative analysis of non-thermal processing methods, the following equipment was used: a UVC lamp unit (254 nm) with a radiation intensity of 30 mW/cm² for UV irradiation; a high-pressure system operating in the range of 300–600 MPa; a pulsed electric field generator with a field strength of 20–35 kV/cm and a frequency of 100–1,000 Hz; an ultrasonic generator operating at a frequency of 20 kHz and a power output of up to 1,000 W. To investigate antimicrobial packaging materials, samples of polyethylene film modified with silver, zinc oxide, and chitosan nanocomposites were used at concentrations of 0.5%, 1.0%, and 1.5%.

The impact of non-thermal processing methods on the technological properties of milk was studied under the following conditions: UV irradiation in a thin layer (4 mm) with exposure times of 10, 20, 30, and 40 seconds; high-pressure treatment at 300, 400, 500, and 600 MPa for 5, 10, and 15 minutes at a temperature of $15 \pm 2^\circ\text{C}$; pulsed electric field treatment with a field strength of 20–35 kV/cm, pulse duration of 1–4 μs , and frequency of 200–1,000 Hz in a continuous-flow system with a throughput of 1 L/min; ultrasound treatment at intensities of 30, 50, and 70 W/cm² for 5, 10, and 15 minutes at a temperature of $25 \pm 3^\circ\text{C}$. To assess structural changes in milk proteins caused by non-thermal methods, differential scanning calorimetry was employed. Microscopic analysis of milk structure was carried out using an optical microscope with a digital camera at magnifications of $\times 400$ and $\times 1,000$. The evaluation of the effectiveness of non-thermal methods in milk processing included the following: investigating the effect of ultrasound treatment (50 W/cm², 5 minutes) on yoghurt fermentation; applying high-pressure processing (500 MPa, 10 minutes) to accelerate cheese

ripening; using ultrafiltration in the production of acid-set cheese; employing pulsed electric field treatment of milk prior to drying. To assess the effectiveness of packaging materials, samples of milk treated using different methods were stored at a temperature of $4 \pm 1^\circ\text{C}$, with sampling carried out on days 1, 7, 14, 21, 28, and 35 of storage. The samples were analysed for microbiological parameters (total microbial count, psychrotrophs, and spore-forming bacteria), physicochemical indicators (pH, titratable acidity, viscosity, degree of fat oxidation, and vitamin content), and organoleptic characteristics using a 5-point scale. To investigate the synergistic effect of combining non-thermal processing methods with innovative packaging materials, factorial experimental design was applied, and synergy coefficients were calculated using the following formula 1:

$$S = \log\left(\frac{N_x}{N_a} \times N_b\right), \quad (1)$$

where N_x is the number of microorganisms after combined treatment; N_a and N_b are the number of microorganisms after individual treatments.

Energy efficiency of the studied technologies was evaluated by measuring electricity consumption (kWh/L) for each processing method. An economic assessment of the technological solutions was conducted, taking into account capital and operating costs, potential shelf life extension, and expansion of market reach. For statistical analysis of the results, analysis of variance was used. The significance of differences was assessed using Student's t-test at a confidence level of $p < 0.05$. All experiments were performed in five replicates.

Results

The study of non-thermal milk processing methods revealed significant differences in their effects on microbiological parameters and product shelf life. To enable an objective comparison of the effectiveness of different technologies, a comprehensive analysis was conducted, encompassing not only microbiological indicators but also technological aspects of applying these methods

in the processing of raw milk and the production of various dairy products. It was found that the highest level of microorganism inactivation in raw milk was achieved using high-pressure processing (600 MPa, 15 minutes) and pulsed electric field treatment (35 kV/cm, 1,000 Hz), which

extended the shelf life of milk to 21-28 days when stored at $4 \pm 1^\circ\text{C}$. A key consideration for the practical application of these technologies is their impact on subsequent processing stages and the quality characteristics of the final dairy products (Table 1).

Table 1. Effects of non-thermal processing methods on the technological properties of milk

Method of processing	Processing mode	Effect on protein fraction	Effect on technological properties	Energy consumption (kWh/L)
Control (untreated)	–	Native structure	Standard properties	–
Thermal pasteurisation	72°C, 15 s	Partial denaturation of whey proteins (18%-20%)	Improved cheese yield, reduction in vitamin content	0.12 ± 0.01
Thermal pasteurisation	90°C, 20 s	Significant denaturation of whey proteins (28%-30%)	Substantial improvement in water-holding capacity	0.18 ± 0.02
Ultraviolet irradiation	40 s	Minimal changes (2%-3%)	Preservation of native properties	0.04 ± 0.01
High-pressure processing	600 MPa, 15 min	Reversible denaturation (15%-18%)	Improved rennet coagulation, increased cheese yield	0.08 ± 0.01
Pulsed electric field	35 kV/cm, 1,000 Hz	Minimal changes (3%-5%)	Enhanced enzymatic processes	0.05 ± 0.01
Ultrasound treatment	70 W/cm ² , 15 min	Partial fragmentation (8%-10%)	Improved homogenisation, reduced fermentation time	0.09 ± 0.02

Note: values presented are means ± standard deviation (n = 5)

Source: compiled by the authors

As shown in Table 1, non-thermal processing methods offer significant advantages in preserving the native structure of milk proteins compared to thermal pasteurisation. Of particular interest are high-pressure processing and pulsed electric field treatment, which not only effectively inactivate microorganisms but also enhance the technological properties of milk for further processing. For instance, high-pressure processing (600 MPa, 15 minutes) leads to reversible denaturation of whey proteins, improving rennet coagulation and increasing cheese yield by 4%-6% compared to conventional thermal pasteurisation. Pulsed electric field treatment (35 kV/cm, 1,000 Hz) has minimal impact on the milk's protein fraction (changes of only 3%-5%), allowing the functional properties of the milk to be preserved almost at the level of raw milk,

while still ensuring a high degree of microbiological safety. Moreover, this technology has the lowest energy consumption among the methods studied (0.05 kWh/L), making it economically attractive for industrial implementation.

An analysis of the impact of different processing methods on the physicochemical parameters of milk showed that non-thermal techniques better preserve thermolabile components. The highest retention of vitamin C and whey proteins was observed following pulsed electric field treatment (91.8% and 96.5%, respectively) and ultraviolet irradiation (87.5% and 97.2%, respectively). These findings are particularly relevant for the production of functional dairy products with enhanced biological value. The study of structural changes in milk proteins using differential scanning calorimetry showed that high-pressure

processing (600 MPa, 15 minutes) induces conformational changes similar to those observed during mild thermal treatment (65°C, 30 minutes). However, unlike thermal processing, these changes are largely reversible, which explains the preservation of the proteins' functional properties. Pulsed electric field and ultraviolet irradiation caused only minimal structural alterations, with negligible effects on protein functionality.

Microscopic analysis of milk structure after different treatments revealed that ultrasound processing led to partial disintegration of fat globules and casein micelles, resulting in improved product homogeneity. This effect is particularly beneficial in the production of fermented dairy beverages and desserts, as it provides a more uniform texture and reduces the risk of whey separation. In addition to the impact on raw

materials, an important focus of the study was the effectiveness of combined technologies in extending the shelf life of finished dairy products. The combined use of non-thermal milk processing methods and innovative antimicrobial packaging materials demonstrated a synergistic effect. Milk treated with high-pressure processing and packaged in film containing silver nanoparticles (1.5%) maintained microbiological stability for up to 35 days, nearly twice as long as when each technology was applied individually. The study highlighted the broad potential of non-thermal technologies not only for raw material treatment but also at various stages of dairy product manufacturing. Experiments were conducted using non-thermal methods in the production of yoghurts, cheeses, and other dairy products, with the results presented in Table 2.

Table 2. Impact of innovative technologies on dairy product quality

Product	Technology	Technological effect	Impact on product quality	Economic effect
Yoghurt	Ultrasound treatment (50 W/cm ² , 5 min)	Fermentation time reduced by 28%-32%	Increased viscosity by 15%-20%, syneresis reduced by 25%-30%	Productivity increased by 23%
Yoghurt	High-pressure processing (400 MPa, 5 min)	Starter culture activity increased by 20%-25%	Improved texture, probiotic content increased by 1.2 log CFU/g	Shelf life extended by 14-18 days
Acid-set cheese	Ultrafiltration	Protein concentration increased 3.5-4 times	Product yield increased by 15%-18%, improved water-holding capacity	Production costs reduced by 12%-15%
Hard cheese	High-pressure processing (500 MPa, 10 min)	Ripening accelerated by 30%-35%	Intensified flavour compound formation, more uniform texture	Production cycle shortened by 20%-25%
Powdered milk	Pulsed electric field pre-drying	Dispersibility increased by 20%-25%	Improved solubility by 18%-20%, preservation of native protein properties	Energy consumption reduced by 10%-12%
Ice cream	Ultrasound treatment of mix (40 W/cm ² , 3 min)	Improved homogenisation, ice crystal size reduced by 40%-45%	Enhanced texture, heat stability increased by 15%-18%	Freezing time reduced by 22%-25%

Source: compiled by the authors

One of the most promising directions identified was the use of ultrasound treatment in yoghurt production. Treating the standardised milk mixture with ultrasound (50 W/cm², 5 minutes) before the addition of starter cultures reduced the fermentation time by 28%-32% compared to

traditional processing. This effect is attributed to ultrasonic cavitation, which causes partial disintegration of casein micelles and increases the availability of nutrients for the starter cultures. In addition, the final product exhibited increased viscosity (by 15%-20%) and reduced syneresis (by

25%-30%), contributing positively to its sensory properties and storage stability.

Studies on the effect of ultrasound treatment on yoghurt quality during storage showed that samples produced using this method maintained their sensory characteristics significantly longer than the control samples. After 21 days of storage at $4 \pm 1^\circ\text{C}$, yoghurts produced with ultrasound-treated milk showed 35%-40% lower syneresis and retained higher viscosity. Microbiological analysis revealed that the number of viable probiotic cultures in these samples was 0.8-1.0 log CFU/g higher compared to the control, confirming the positive effect of ultrasound treatment on probiotic viability. High-pressure processing has demonstrated considerable potential in the production of hard cheeses. Treatment during the ripening stage (500 MPa, 10 minutes) allowed the ripening process to be accelerated by 30%-35%, due to the intensification of proteolytic activity under high pressure. Flavour and aroma profile analysis showed that cheeses treated with high pressure had a richer taste and aroma compared to control samples at the same ripening stage. The economic benefit of this technology lies in the shortened production cycle and reduced maturation costs. A detailed study of the effects of high pressure on cheese proteolysis was carried out using chromatographic analysis of peptide profiles. It was found that high-pressure treatment (500 MPa, 10 minutes) led to the formation of low-molecular-weight peptides (under 5 kDa) in significantly greater quantities than in control samples after 60 days of ripening. These peptides play a key role in the development of flavour and aroma, and may also exhibit potential biological activity. Moreover, microstructural analysis of cheese after high-pressure treatment showed a more uniform distribution of moisture and fat within the cheese matrix, contributing to an improved texture in the final product.

Membrane technologies, particularly ultrafiltration, were investigated in the production of acid-set cheese. The use of ultrafiltration made it possible to increase the protein concentration

by 3.5 to 4 times without adversely affecting its functional properties. This led to a 15%-18% increase in product yield and improved water-holding capacity. An economic efficiency analysis showed that incorporating ultrafiltration into the production process reduced manufacturing costs by 12%-15%, owing to increased product yield and lower energy consumption. The ultrafiltration technique was also studied in combination with high-pressure processing for the production of functional cultured dairy products. It was found that pre-treatment of milk by ultrafiltration, followed by high-pressure treatment of the concentrate (400 MPa, 5 min) before the addition of starter cultures, resulted in a product with a higher protein content (8%-10%) and improved rheological properties. This approach is particularly promising for the manufacture of specialised dairy products for sports nutrition and geriatric diets, where a high protein content is a key factor. Pulsed electric field technology demonstrated high effectiveness in the production of powdered milk. Treatment of milk before drying (25 kV/cm, 500 Hz) enhanced dispersibility by 20%-25% and preserved the native properties of proteins. The resulting product exhibited improved solubility (by 18%-20%) and retained the functional properties of the proteins – an especially important characteristic in the production of infant formula and functional food products.

The study of the impact of pulsed electric field treatment on the milk drying process revealed that this technology can reduce energy consumption by 10%-12% by enhancing heat transfer and shortening drying time. Furthermore, microstructural analysis of the resulting milk powder showed that samples treated with pulsed electric field prior to drying exhibited a more porous particle structure, which accounted for their improved solubility. The functional properties of proteins, such as foaming capacity and emulsifying ability, were retained at 92%-95% of their original values, whereas these indicators dropped to 75%-80% in the control samples. Ultrasound-assisted re-emulsification

technology was explored in the production of butter and spreads. Treating cream with ultrasound (60 W/cm², 3 minutes) prior to churning resulted in a product with smaller water droplets (average diameter 2-3 µm compared to 5-7 µm in the control) and their more uniform distribution within the fat phase. This led to improved texture, enhanced thermal stability, and reduced tendency towards phase separation. Additionally, the product showed improved spreadability at low temperatures, broadening its potential applications. The microencapsulation technology using drum drying was investigated for the development of encapsulated probiotic cultures resistant to adverse environmental conditions. It was found that using sodium alginate and maltodextrin as coating materials significantly enhanced the survival of probiotic cultures under low pH conditions (pH 2.0, simulating gastric juice) by 2.5-3.0 log units compared to non-encapsulated cultures. This opens up new opportunities for the creation of functional dairy products with guaranteed delivery of probiotics to the consumer's intestines. As part of the study, intelligent packaging systems with pH-sensitive indicators based on anthocyanins were developed and tested for monitoring the freshness of dairy products. It was established that the indicator colour changed from blue to pink when the milk reached a critical acidity level (pH ≤ 6.0), allowing for visual assessment of product freshness without opening the package.

Experimental testing of the stability of the pH-sensitive indicators showed that they retained their activity throughout the entire shelf life of the dairy products (up to 45 days), provided that the recommended storage conditions were followed. An important feature of the developed indicators is their ability to respond specifically to changes caused by microbiological spoilage, without reacting to normal physico-chemical processes occurring in milk during storage. To enhance the effectiveness of quality control for dairy products, a concept for an integrated monitoring system was developed, combining intelligent packaging materials with automated

control systems. The system incorporates miniature sensors for pH, temperature, and gas composition, embedded into the packaging material; Radio Frequency Identification (RFID) tags for product identification and tracking; a central data collection and analysis platform using machine learning algorithms; and a mobile application for consumers, enabling them to obtain real-time information about product status by scanning a QR code. A unique feature of the system is its ability to predict the remaining shelf life of the product based on the dynamics of monitored parameters. The machine learning algorithms, trained on a large dataset of experimental results, can accurately (up to 92%-95%) forecast the point at which critical quality thresholds are reached. This enables optimised logistics and reduced product losses due to expired shelf life.

Experimental testing of the system at a dairy processing facility demonstrated that its implementation led to a 35%-40% reduction in product returns due to non-compliance with quality standards and improved supply chain efficiency through better inventory management. Additionally, the system provides full traceability from producer to consumer, enhancing brand trust and aligning with current trends in the food industry. The economic assessment of the proposed technological solutions demonstrated their competitiveness compared to traditional methods. Specifically, the implementation of a combined technology (high-pressure milk processing and antimicrobial packaging) enables a 20%-25% reduction in energy consumption relative to conventional thermal pasteurisation, and extends the product's shelf life by three to four times, significantly enhancing distribution opportunities. A detailed analysis of the energy efficiency of various milk processing technologies revealed that pulsed electric field treatment has the lowest energy consumption among the methods studied, at just 0.05 kWh/L. This is due to the process operating at room temperature, with energy expenditure limited to generating the electric pulses. Although high-pressure processing involves

substantial capital investment in equipment, it also shows strong energy efficiency (0.08 kWh/L) compared to traditional high-temperature pasteurisation (0.18 kWh/L). In the long term, these technologies offer the potential to substantially reduce operational costs and the environmental footprint of production.

The economic feasibility of implementing the developed technologies at a medium-capacity dairy facility (processing 50 tonnes of milk per day) showed that the capital investment in non-thermal processing equipment can be recouped within 2.5 to 3 years due to reduced operating costs, higher product yields, and expanded market reach. For enterprises producing premium segment products, the payback period may be reduced to 1.5 to 2 years, owing to the potential for premium pricing of products with enhanced sensory qualities and extended shelf life. The analysis of current trends in the food industry has identified several promising directions for the development of innovative technologies in the dairy sector. Among these, 3D printing of dairy products stands out as particularly noteworthy, enabling the creation of complex structures from dairy-based materials with customised properties. An experimental 3D-printing unit for dairy applications was tested using cheese mass enriched with probiotics. The results demonstrated that this technology allows for the formation of products with intricate internal structures and controlled release of active compounds (Dinkçi *et al.*, 2019; Alirezalu *et al.*, 2020). Testing of various formulations for 3D printing revealed that protein concentrate-based mixtures supplemented with hydrocolloids (such as pectin, gelatine and agar) serve best as structure-forming agents. These compositions ensure stable shape retention of printed structures during storage while maintaining acceptable sensory qualities. Enrichment with probiotic cultures enables the production of functional foods with targeted health-promoting properties.

Another promising direction is fermentation using immobilised cultures, which involves

fixing starter cultures onto specialised carriers to enhance their stability and productivity. Experimental research has shown that the use of immobilised cultures can reduce fermentation time by 35%-40% and increase the yield of bioactive compounds. Various materials were tested as carriers for immobilisation, including calcium alginate, carrageenan, chitosan, and synthetic polymers. The best results in terms of culture viability and technological feasibility were achieved using calcium alginate. Microscopic analysis showed that the starter culture cells were evenly distributed within the carrier matrix and maintained their metabolic activity over an extended period (up to 30 fermentation cycles with repeated use). Lyophilised methods for producing dry fermented products represent another promising area of research. This technology combines fermentation with subsequent freeze-drying, allowing the preservation of probiotic viability and the bioactive properties of fermented products. It was found that freeze-dried cultured dairy products retained up to 80%-85% of viable probiotic cultures after 12 months of storage at room temperature. Optimisation of the freeze-drying process revealed that pre-freezing the product at -40°C, followed by drying at a pressure of 0.1 mbar and plate temperature of 30°C, provided the best retention of probiotics and bioactive compounds. The addition of protective agents such as trehalose and inulin prior to drying further increased probiotic survival by 15%-20%. The resulting lyophilised products were characterised by high porosity, rapid rehydration when water was added, and the preservation of sensory properties comparable to those of fresh fermented products.

A comprehensive study of the rheological properties of reconstituted lyophilised fermented dairy products revealed that their consistency depends on the composition of the protective media used prior to drying. The addition of prebiotics, such as inulin and fructooligosaccharides, not only enhances the survival rate of probiotic cultures but also improves the texture of the reconstituted product, giving it a creamy consistency

similar to that of fresh yoghurt. Furthermore, these components increase the functional value of the final product by creating a synbiotic complex. Research into the microstructuring of dairy proteins using a combined approach – including controlled fermentation, high-pressure processing, and ultrasound treatment – has enabled the development of new types of protein ingredients with unique functional properties. It was found that such ingredients exhibit significantly higher water-holding capacity (by 30%-35% compared to traditional dairy protein concentrates), improved emulsifying properties, and the ability to form thermostable gels at low protein concentrations (3%-4%). The microstructured protein ingredients were successfully applied in the production of low-calorie dairy desserts, where they allowed for a 50%-60% reduction in fat content without compromising the sensory qualities of the product. Instrumental profilometry analysis of the texture of these desserts demonstrated that their rheological properties were comparable to traditional high-fat analogues, and in some instances, even surpassed them in terms of homogeneity and creaminess. Automated control systems incorporating artificial intelligence demonstrate considerable potential for optimising technological processes in the dairy industry. An experimental system for predicting the shelf life of dairy products, based on neural networks, achieved an accuracy rate of 92%-95%, enabling optimisation of logistics and a reduction in product waste.

To train the neural network, a large dataset was collected and analysed. This included changes in physicochemical, microbiological, and organoleptic parameters of various dairy products during storage under different conditions. A multilayer perceptron neural network with three hidden layers was trained on 80% of the available data, with validation carried out on the remaining 20%. The results showed that the system could accurately predict the residual shelf life of products based on an analysis of initial parameters and storage conditions (Fadiji *et al.*, 2023). In addition to shelf-life prediction, the developed

artificial intelligence system was adapted to optimise the processing conditions in dairy product manufacturing. Machine learning algorithms analysed correlations between raw material processing parameters (temperature, pressure, duration, etc.) and the quality characteristics of the final product. This allowed for the identification of optimal regimes to achieve the desired product properties with minimal energy and time expenditure. The practical implementation of an automated control system at a dairy facility significantly improved product quality consistency, reduced the proportion of defective batches by 40%-45%, and optimised the use of raw materials and energy resources. An additional advantage of the system lies in its capacity for continuous self-improvement through the accumulation of new data and refinement of predictive models.

An assessment of the energy efficiency of various technological combinations revealed that the most promising for industrial application is the integration of pulsed electric field treatment during the initial processing stage with high-pressure processing applied to the packaged end product. This combination enables a 30%-35% reduction in energy consumption compared to traditional technologies, while maintaining an equivalent or superior level of product safety. An evaluation of the environmental impact of innovative technologies, carried out using Life Cycle Assessment (LCA) methodology, indicated that non-thermal processing methods can reduce the carbon footprint of dairy production by 20%-25%, primarily by lowering energy consumption and decreasing food waste due to extended shelf life. Moreover, the use of biodegradable packaging materials modified with natural antimicrobial agents contributes to a further reduction in environmental burden (Morales-de la Peña *et al.*, 2019). A comprehensive analysis of the quality of dairy products produced using innovative technologies included not only standard physicochemical and microbiological parameters but also an assessment of the bioavailability of key nutrients. It was found that non-thermal

processing methods, such as high-pressure processing and pulsed electric fields, ensure greater bioavailability of calcium and essential amino acids compared to traditional thermal pasteurisation. This effect is attributed to a lower degree of protein denaturation and the preservation of the native structure of calcium-binding components in milk.

A study of the impact of innovative technologies on the allergenicity of milk proteins showed that high-pressure treatment (600 MPa, 15 minutes) can reduce the allergenic potential of β lactoglobulin – one of the main milk allergens – by 25%-30%. This is explained by pressure-induced conformational changes in the protein that result in the masking of certain epitopes responsible for allergic reactions. These findings open up opportunities for developing hypoallergenic dairy products using high-pressure processing instead of traditional thermal treatment. To evaluate the broader prospects for the implementation of innovative technologies in the dairy industry, a SWOT analysis was conducted. The key findings were as follows: strengths include improved product quality, extended shelf life, and increased energy efficiency; weaknesses involve high initial investment costs and the need for specialised personnel; opportunities relate to expanding the product range and entering new markets; threats stem from potential changes in regulatory requirements and consumer hesitancy towards new technologies. Marketing research conducted as part of this study showed that consumers are willing to pay 15%-20% more for dairy products produced using innovative “green” technologies that preserve the natural properties of milk. Willingness to pay a premium was particularly high among consumer segments focused on healthy lifestyles and environmental responsibility. Thus, the findings of this study demonstrate that innovative technologies for processing and manufacturing dairy products have considerable potential to improve production efficiency, enhance product quality, and extend shelf life. Combined approaches – integrating various methods of raw milk processing, product manufacturing, and

packaging, along with automated quality control systems – are especially promising.

Discussion

The study confirmed the high effectiveness of non-thermal milk processing methods and innovative packaging materials in extending the shelf life of dairy products. These findings should be viewed in the context of current scientific research in this field. The demonstrated high efficiency of high-pressure processing in microbial inactivation and minimal impact on organoleptic properties aligns with the results obtained by L. PicartPalmade *et al.* (2019). The authors noted that treatment at 500-600 MPa can achieve levels of microbial inactivation comparable to thermal pasteurisation, while ensuring significantly better preservation of thermolabile components in milk. They also conducted detailed studies on the impact of high pressure on specific microbial cultures and found that Gram-negative bacteria exhibit greater sensitivity to this method compared to Gram-positive strains. Studies have shown that high-pressure processing is particularly effective in accelerating cheese ripening. This aligns with the findings of A.Ch. Stratakos *et al.* (2019), who investigated the impact of high-pressure processing on the safety and quality of raw milk. Their research confirmed that the technology not only inactivates pathogenic microorganisms but also has a beneficial effect on enzymatic processes during cheese production, contributing to faster ripening and the development of a more intense flavour and aroma profile.

The results regarding the effectiveness of ultraviolet irradiation treatment in extending the shelf life of milk demonstrate significant microbial inactivation when applied in a thin layer. However, the issue of uneven exposure due to the shadowing effect in opaque liquids such as milk remains a limiting factor for large-scale applications. M.M. Delorme *et al.* (2020) proposed innovative technical solutions to address this challenge, including the use of turbulent flow and specially designed reactors with a high surface-to-volume

ratio, which enable more uniform irradiation. They also established that UV treatment has a selective effect on different groups of microorganisms in milk, showing the highest efficacy against coliform bacteria and psychrotrophic microorganisms – both of which are major contributors to the spoilage of dairy products during storage. The theoretical aspects of applying ultraviolet irradiation for milk treatment are comprehensively discussed in the research of T. Koutchma (2019), who proposed mathematical models for calculating the effective radiation dose based on the optical properties of the product, equipment geometry, and the kinetics of microbial inactivation. The author also systematised data on the impact of UV radiation on the nutrient composition of milk and established that, under optimised processing conditions, up to 90%-95% of thermolabile vitamins are preserved – findings that are consistent with those obtained in the present study. In this research, pulsed electric field technology demonstrated strong potential not only for microbial inactivation but also for improving the technological properties of milk. Of particular interest is the influence of this method on fermentation processes and texture formation in fermented dairy products. These results complement the study by M.M. Goodarzi *et al.* (2020), who found that pulsed electric fields can stimulate the activity of starter cultures and accelerate fermentation by 15%-20% due to increased permeability of microbial cell membranes.

The data obtained regarding the effectiveness of ultrasound treatment are supported by the findings of R. Mahendran *et al.* (2019), who investigated the use of pulsed light to extend product shelf life. Although the authors focused on a different technology, their conclusions on the mechanisms of microbial inactivation and the optimisation of processing parameters closely align with the results of the present study. In particular, they found that combining pulsed light with other non-thermal methods (including ultrasound) produces a synergistic effect, allowing for a higher degree of microbial

inactivation at less intensive processing levels, thus minimising the impact on the sensory and nutritional qualities of dairy products. The study of the effectiveness of automated quality control systems based on machine learning aligns with the findings of Z.H. Zhang *et al.* (2019), who conducted a comprehensive analysis of nonthermal technologies and their current and future applications in the food industry. The authors emphasised that the advancement of digital technologies and sensor systems is a key factor in the successful large-scale implementation of non-thermal processing methods. They proposed an integrated approach to process automation that includes continuous monitoring of critical processing parameters and product quality prediction using mathematical models – an approach closely aligned with the shelf-life prediction system for dairy products developed in the present study. The findings regarding the use of membrane technologies, particularly ultrafiltration, to increase protein concentration and improve the functional properties of dairy products complement the research by A. Soni *et al.* (2021). These authors analysed the application of emerging processing technologies aimed at enhancing the safety and bioactivity of milk. They demonstrated that membrane technologies not only enable the concentration of valuable milk components but also facilitate the removal of toxic substances and allergens, opening new opportunities for developing specialised products for specific consumer groups, including individuals with dairy protein allergies.

The study's findings on the impact of different processing methods on the allergenicity of milk proteins are supported by the research of H. Hernández *et al.* (2022), who explored the potential for developing innovative and healthier dairy products through the addition of microalgae. The authors found that certain components of microalgae, particularly polyphenols and peptides, are capable of binding to allergenic epitopes of milk proteins, thereby masking them and reducing the overall allergenicity

of the product. This insight opens up promising prospects for combining non-thermal processing methods with the addition of bioactive compounds to develop hypoallergenic dairy products. The effectiveness of combining various technologies, as demonstrated in this study, is supported by the research of N. Teixidó *et al.* (2022), who investigated the use of biological agents as alternatives to chemical preservatives. The authors conducted a comprehensive study of various bio-preservatives, including bacteriocins, the lactoperoxidase system, and natural extracts, and determined their effectiveness against the primary spoilage organisms in dairy products. The synergistic effect observed in this study when combining non-thermal processing methods with antimicrobial packaging materials complements the findings of M. Gogliettino *et al.* (2020). They established that the combination of high-pressure processing with active packaging materials containing antimicrobial peptides can extend the shelf life of meat and dairy products by two to three times compared with the use of each technology individually.

The findings on the development of intelligent packaging systems with freshness sensors are supported by the research of M.R. Yan *et al.* (2022), who analysed innovative packaging solutions in terms of quality, safety and consumer perception. The authors systematised various types of smart packaging, including temperature, time and freshness indicators, as well as QR codes and RFID tags for tracking product movement. They established that such systems not only provide improved quality control but also enhance consumer trust – an important factor in the adoption of new technologies within the food industry. The economic viability of introducing innovative technologies, as demonstrated in the present study, aligns with the conclusions of R. Vanaraj *et al.* (2024), who conducted a comprehensive assessment of the cost-effectiveness of different shelf-life extension technologies. The authors developed an integrated evaluation model that takes into account both

direct costs associated with equipment and its operation, and indirect benefits such as reduced product returns and the expansion of distribution networks.

Research into the development of functional dairy products using non-thermal processing methods complements the study of A. Sridhar *et al.* (2020), who examined the application of preservation technologies and nanotechnology for extending the shelf life of various food products. Particular attention was given to the relationship between processing methods and the bioavailability of functional ingredients, which is critical in the development of products with enhanced biological value. They found that non-thermal methods, such as high-pressure processing and pulsed electric fields, better preserve bioactive components and their functionality compared to conventional thermal technologies, which is fully consistent with the results obtained in this study. The environmental aspect of innovative packaging solutions explored in this study complements the findings of S. Pandey *et al.* (2022), who conducted a comprehensive analysis of current trends in active packaging. The authors assessed the life cycle of various packaging materials, including conventional polymers, biodegradable materials and nanocomposites, in terms of their environmental impact and potential for recycling or biodegradation.

Unlike previous studies, this research proposes a comprehensive approach to extending the shelf life of dairy products, encompassing the entire technological chain – from initial raw milk processing to packaging and storage of finished products. Such an approach maximises the efficiency of each technological solution through the synergistic interaction of different components within the system. The findings of the study lay the groundwork for the further development of innovative technologies in the dairy industry.

Conclusions

As a result of the study, a comparative analysis of modern innovative technologies for the

processing and treatment of dairy products was conducted. It was found that non-thermal methods – such as high-pressure processing, pulsed electric fields, ultrasound, and UV irradiation – ensure a higher retention of nutrients and superior organoleptic properties compared to conventional thermal processing. The most effective methods were high-pressure processing (600 MPa, 15 minutes) and pulsed electric fields (35 kV/cm, 1,000 Hz), which reduced the total microbial count by 4.2 and 3.8 log CFU/cm³, respectively, while preserving up to 91.8% of vitamin C and 96.5% of whey proteins. Combining these methods with antimicrobial packaging materials (silver nanoparticles, 1.5%) extended the shelf life of milk to 35 days. The study demonstrated the significant potential of non-thermal methods in dairy processing: ultrasonic treatment accelerated yoghurt fermentation by 28%-32%; high-pressure processing reduced cheese maturation time by 30%-35%; and ultrafiltration increased the yield of acid-set cheese by 15%-18%. Smart packaging systems with pH-sensitive indicators, combined with machine learning technologies, enabled shelf-life prediction with an accuracy of up to 92%-95%.

The energy efficiency of non-thermal technologies (0.04-0.08 kWh/L compared to 0.18 kWh/L for conventional pasteurisation)

contributes to a 20%-25% reduction in the carbon footprint of production. Economic analysis confirmed the commercial viability of implementing these technologies, with a payback period of 1.5 to 3 years, making them particularly attractive for producers of premium dairy products. Promising directions for future development include 3D printing of dairy products, fermentation using immobilised cultures, lyophilic technologies for the production of functional foods, and micro-structuring of dairy proteins to create low-calorie products with enhanced sensory properties. An integrated approach that combines various innovative technologies for processing, treatment and packaging enables the production of safe, functional, and energy-efficient dairy products that meet the demands of the modern market, consumer preferences, and the principles of sustainable food industry development with minimal environmental impact.

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Conflict of Interest

None.

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Використання інноваційних технологій для збільшення терміну придатності молочних продуктів

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Анотація. Метою дослідження було надати комплексну оцінку впливу інноваційних технологій на процеси обробки та переробки молочних продуктів з метою подовження строку їх придатності. Порівняльний аналіз нетермічних методів показав, що найвищу ефективність інактивації мікроорганізмів забезпечують технологія високого тиску (600 МПа, 15 хв) та імпульсне електричне поле (35 кВ/см, 1000 Гц), які знижують загальну кількість мікроорганізмів на 4,2 та 3,8 \log КУО/см³ відповідно. Нетермічні методи демонстрували менший вплив на нутрієнтний склад молока порівняно з традиційною пастеризацією, забезпечуючи збереження до 91,8 % вітаміну С та до 97,2 % сироваткових білків. Найкращі органолептичні характеристики спостерігалися у зразках, оброблених технологією високого тиску та імпульсним електричним полем (4,9 бала за 5-бальною шкалою). Встановлено, що ультразвукова обробка прискорює ферментацію йогуртів на 28-32 %, високий тиск прискорює дозрівання сирів на 30-35 %, а ультрафільтрація підвищує вихід кисломолочного сиру на 15-18 %. Серед антимікробних пакувальних матеріалів найвищу активність продемонстрували плівки з наночастинками срібла (1,5 %). Виявлено синергетичний ефект при комбінованому застосуванні нетермічних методів обробки та інноваційних пакувальних матеріалів, що дозволяє збільшити термін придатності молока до 35 діб при температурі $4 \pm 1^\circ\text{C}$. Розроблено інтелектуальні пакувальні системи з рН-чутливими індикаторами для моніторингу свіжості продукту. Для дослідження синергетичного ефекту комбінованої обробки застосовувалося факторне планування експерименту з розрахунком коефіцієнтів синергії. Аналіз міграції наночастинок з пакувальних матеріалів показав, що їх концентрація не перевищувала допустимих рівнів при дотриманні рекомендованих умов зберігання. Розроблено математичні моделі для прогнозування

терміну придатності молока залежно від параметрів обробки та пакування. Проведено економічну оцінку запропонованих технологічних рішень, яка показала їх конкурентоспроможність порівняно з традиційними методами при виробництві продукції преміального сегменту

Ключові слова: нетермічна обробка; високий тиск; імпульсне електричне поле; антимікробне пакування; наночастинки; інтелектуальні пакувальні системи



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Justification of the suspension type of a single-axle fruit transport trailer

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Abstract. The relevance of the study is due to the importance of preserving fruit products, the quality of which is affected by mechanical damage to fruits during transportation, associated with specific difficulties due to their substantial sensitivity to mechanical influences, so reducing the dynamic impact on fruits requires improving the design parameters of special vehicles and recommendations for their use. The purpose of the study was to conduct a comparative examination of the level of vertical

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accelerations of a trailer with balanced and unbalanced suspensions and prove the feasibility of using the latter for fruit transportation. The influence of design parameters and operational factors of single-axle trailers on vertical accelerations and their distribution in the energy spectrum of vehicle vibrations during fruit transportation was assessed. Theoretical and experimental studies were conducted, and a comparative analysis of vertical accelerations of two types of suspensions was performed. In the course of theoretical research, a differential equation for estimating the level of vertical accelerations was solved, considering the following factors: suspension type; balancer parameters; road profile irregularities; radial deformation and tire stiffness; speed of the vehicle. An experimental evaluation of vertical accelerations of a single-axle trailer with balanced and unbalanced suspensions was conducted when transporting fruits in a garden on a dirt Inter-block road to confirm the results of theoretical studies. The results showed that the RMS values of vertical accelerations in the operating speed range that occur in trailers with unbalanced suspension are higher than in trailers with balanced suspension. The dominance of harmonics of vertical vibrations in the range of 1.75-2 Hz was established, which accounted for more than 55% of the power of the acceleration spectrum, which is crucial for assessing fruit damage. It was experimentally proven that at an average transportation speed (about 20 km/h), the level of vertical accelerations of a trailer with both suspensions did not exceed the permissible level. The practical value of the work lies in the possibility of applying the results obtained in the design and improvement of single-axle trailers for fruit transportation, which will help reduce product losses and increase the efficiency of horticultural farms

Keywords: soil irregularities; vibrations; mechanical damage to fruits; differential equations; vertical accelerations; amplitude-frequency characteristics

Introduction

The process of harvesting and transporting fruits is one of the most labour-intensive processes in horticulture, which accounts for more than 30% of all costs for growing fruit products, and at the same time, more than 15-20% of products are lost. The causes of losses are known, but not fully understood (Khalid *et al.*, 2024). A substantial part of scientists in their publications focus on logistics research, timely execution and productivity of vehicles (Lozova, 2019), and other studies are aimed at reducing the loss of fruit products in the process of logistics transportation (Stupska *et al.*, 2022). One of the most difficult tasks is to reduce losses due to mechanical damage to fruits, where an important role belongs to vehicles. Reduced marketability and even spoilage of apples are most often the result of incorrect choice of packaging, type, and design of vehicles and transportation conditions.

In many cases, the absence of roads with hard and smooth surfaces and the tendency to increase the performance of vehicles lead to insufficient smoothness and leads to an increase in the level of mechanical damage to products. M. Al-Dairia *et al.* (2021) investigated the mechanisms of mechanical damage to apples during harvesting and transportation, and proposed the design of an experimental platform for testing, accounting for dynamic load parameters. The authors demonstrated that uneven movement of cars on rough terrain is one of the key factors of fruit damage. The study determined the relationship between the intensity of vibrations and the frequency of damage and also confirmed the need to develop adaptive collection and transportation systems that consider mechanical loads. B. Suleimani & E. Ahmadi (2021) analysed the effect of road conditions, suspension, and transport speed on the level of vibrations transmitted to

fruits during transportation. It is established that the parameters of the road surface, in particular, bumps, and the type of suspension of the vehicle are the main factors determining the amplitude and frequency of vibrations. This study confirmed that insufficient smoothness when transporting products on irregular roads substantially increases the risk of mechanical damage to the fruit. Insufficient smoothness of vehicles is associated with a large number of external influences from roads and their nature.

Damage to products is mainly affected by two components: the characteristics of the cargo (physical and mechanical properties of the transported product, the method of its packaging and wrapping), and the characteristics of the vehicle (body type and suspension). Thus, in the study by D. Cigdem & M.S. Aday (2022), indicators that characterise the smoothness of the vehicle were evaluated depending on the speed of movement, active accelerations, but their frequency distribution was not researched. Mechanical damage to the fruit during transportation is caused by three factors: co-impacts, compressive force, and vibration. In the paper of F. Han *et al.* (2023), the authors presented that vibration accounts for half to two-thirds of the total amount of fruit damage during transport, but did not reveal the structure of vibrations, nor did they perform frequency analysis of the distribution of vertical accelerations. Similar examination of the vehicles is given in a publication of X. Liu *et al.* (2022), where it was confirmed that one of the most important factors is difficult road conditions, when vehicles make vibrations with accelerations, the value of which reaches the limit of $3.5g$ (g – acceleration of gravity), not specifying the type and parameters of the suspension.

Transportation of fruits from the garden is conducted by both general-purpose vehicles (cars, tractor trailers) and special ones. I. Hazbavi (2013) found that the disadvantages of general-purpose vehicles include excessive platform height and insufficient smoothness, which affects the increase in mechanical damage and

deterioration of fruit quality. For the most part, special vehicles are single-axle trolleys with or without balanced suspension, with low-pressure tires and a wide profile. Their use allows increasing the yield of standard products, fully mechanising loading and unloading operations, substantially improving labour productivity, reducing material costs for containers, and preserving the quality of fruit products. Trailers with tandem axles are widely used. Their advantage is evident in the case of heavy-duty vehicles. In a study by B. Jarimopas *et al.* (2005), the use of balanced suspension was explained by the high permissible loads on running systems and the fact that it absorbs various shocks well and, as a result, provides for moving at an increased speed on uneven roads. It remains unclear how the use of a balancing suspension affects the level of vertical accelerations and their frequency distribution compared to an unbalanced one.

The study aimed to investigate the influence of the efficiency of using the balanced suspension of a single-axle trailer with fruits on the level of vertical accelerations and the feasibility of using an unbalanced suspension in the process of on-farm transportation. The goal of the paper is to prove the feasibility of using a single-axle trailer with an unbalanced suspension for on-farm transportation of fruits with acceptable oscillation modes.

Materials and Methods

In this paper, theoretical and experimental studies of vertical vibrations of a single-axle trailer with an unbalanced or balanced tandem suspension are conducted. It is customary for a trailer loaded with fruit to move along a dirt road at a constant speed. Given that the trailer is a complex oscillatory system, vertical accelerations were taken as the main indicator of vibrations, which most affect the mechanical damage to fruits during their transportation (Khodaei *et al.*, 2019). For theoretical studies of vertical vibrations of vehicles, a model with one degree of freedom for moving over randomly located road bumps

is adopted, considering non-sprung masses, the impact of cargo, and tire stiffness (Martishko & Volyansky, 2018). An equivalent oscillatory system

of a uniaxial container ship was used (Fig. 1) with unbalanced and balanced suspensions to determine and analyse vertical accelerations.

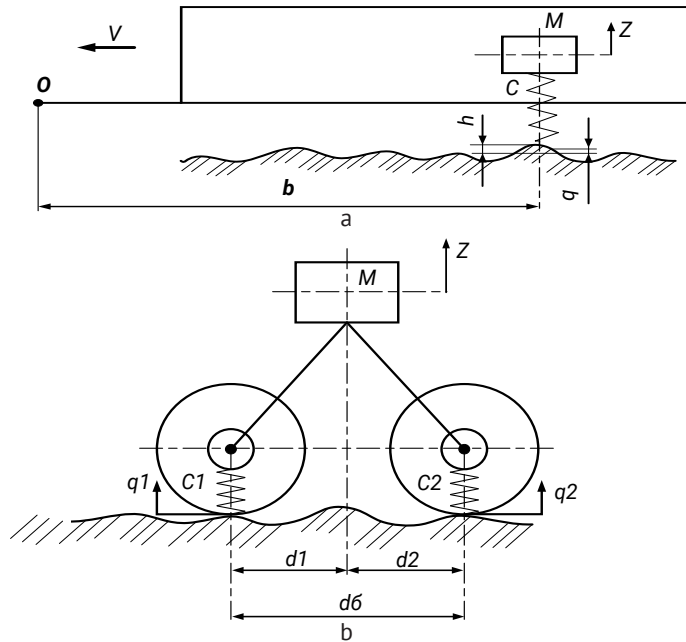


Figure 1. Equivalent single-axle trailer oscillation system

Note: a – unbalanced suspension; b – tandem balanced suspension; M – trailer weight shown; V – speed of movement; Z – vertical movement of the reduced mass; c – total tire stiffness; q_1 and q_2 – value of the height of unevenness under the front and rear wheels; d_1, d_2, d_b – balancer parameters

Source: compiled by the authors

An experimental test of the dependence of vertical accelerations on the speed of a container ship with balancing and unbalanced suspensions was conducted when transporting fruits along a dirt road along the edge of the block in the garden of the Institute of Horticulture of the National Academy of Agrarian Sciences to confirm the results of

theoretical research. Measurement and analysis of vertical vibrations of the fruit trailer was conducted using vibration measuring equipment of the company “Brüel & Kjær”, Denmark (Fig. 2) according to the methodology developed by the Forecasting and Testing of Equipment and Technologies for Agricultural Production named after Leonid Pohorily.

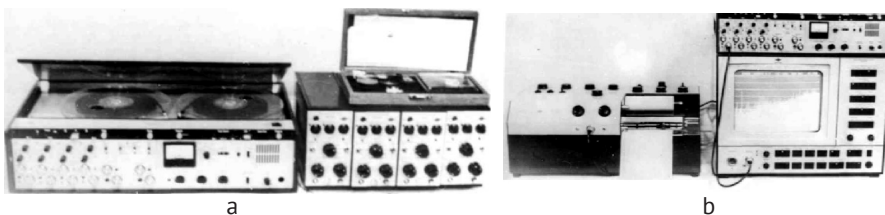


Figure 2. Measuring equipment “Brüel & Kjær”

Note: a – for recording; b – frequency analysis

Source: compiled by the authors

The frequency spectrum and vertical accelerations were analysed at the centre of mass of

the fruit trailer. A block diagram of the experiments is shown in Figure 3.

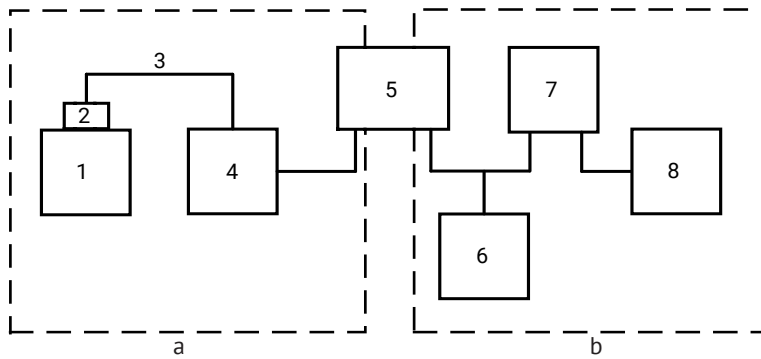


Figure 3. Experiment flowchart: for recording and frequency analysis of container ship vibrations

Note: a – to record results; b – for frequency analysis; 1 – object of the experiment; 2 – accelerometer; 3 – cable; 4 – amplifier; 5 – tape recorder; 6 – RMS measurement device; 7 – frequency analyser; 8 – recorder

Source: compiled by the authors

The root-mean-square value was taken as the indicator for estimating vertical accelerations, from a known dependency (Kalchenko *et al.*, 2017):

$$a = \sqrt{\frac{1}{T_0} \int_0^{T_0} a^2(t) dt}, \quad (1)$$

where $a(t)$ – acceleration from time t ; T_0 – time averaging.

Accelerometers 4370 were used, the sensitivity of which was 10 pC/m.s^2 to measure the oscillation parameters. Oscillation signals from the accelerometer were sent to the frequency amplifier, the lower filter limit of which was 0.25 Hz, and the upper limit –1000 Hz. After amplification, the signals were recorded on a 4-channel tape recorder and then fed to an RMS amplifier. The oscillation level was recorded in the maximum value saving mode. The spectral distribution of accelerations in the frequency spectrum was measured using a frequency analyser in the frequency range 0...100 Hz. The filter bandwidth is 0.25 Hz. The averaged frequency spectra were recorded by a frequency analyser and recorded on frequency-granular paper, where accelerations were denoted in decibels relative to the conditional zero acceleration level $a_0 = 3 \times 10^{-4} \text{ m/s}^2$.

Results and Discussion

Given that road bumps are random in nature, they were considered one-dimensional: in the longitudinal direction, ignoring the probability of movement of the left and right wheels in different profiles. With this assumption, there are no lateral vibrations of the trailer since the change in the micro-profile of the cross-section of the field is usually insignificant, and the track of the unit is not wide. The correlation function for the height of road surface irregularities was taken from the study by M.I. Karbovanets & V.Y. Lazur (2019) according to the formula:

$$K(l) = \Delta e^{-\alpha |l|}, \quad (2)$$

where Δ – variance of inequalities; α – coefficient that characterises the attenuation of the function; l – correlation interval. The field profile is defined by the equation:

$$q = h(1 - e^{-\alpha |l|}), \quad (3)$$

where q – flow value of the inequality height; h – the limit to which the height of the inequality is directed. It is assumed that the ordinates of the

heights of inequalities are distributed according to the normal law, then the largest of them are directed to the value 6σ , where σ – the mean square of the heights of inequalities (Martynenko & Yuryk, 2008). The considered movement of the system (Fig. 1a) around the point O , with the assumption that the displacement of the reduced mass M of the oscillation system occurs in a vertical straight line since the longitudinal base of the trailer B is substantially higher than the height of the bump h . In this case, the motion of the system is described by the differential equation in general form:

$$M\ddot{Z} + c(Z - q) = 0, \quad (4)$$

where Z – vertical movement of the reduced mass; c – total tire stiffness. Inelastic resistance forces in tires were disregarded.

If the possibility of lifting the wheels from the support surface is not accounted for, it can be assumed that there is a condition:

$$f = (Z - q), \quad (5)$$

where f – flow value of the radial strain of the tire.

Then, the equation of motion can be written in terms of the radial deformation of the tire:

$$M\ddot{f} + cf = -M\ddot{q}. \quad (6)$$

It is considered that:

$$\omega = \sqrt{\frac{c}{M}}, \quad (7)$$

where ω – the natural frequency of vibrations of the system, then the equation of motion will acquire a more convenient form for analysis:

$$\ddot{f} + \omega^2 f = -\ddot{q}. \quad (8)$$

After double differentiation of equation (3) over time t , given that $l = vt$, and substituting the result in expression (8), the forced motion equation of the system will have the form:

$$\ddot{f} + \omega^2 f = h\alpha^2 v^2 e^{-\alpha vt}. \quad (9)$$

Equation (9) was solved for the steady-state oscillation process, yielding:

$$f = \frac{h\alpha^2 v^2}{\omega^2 + \alpha^2 v^2} \left(\frac{av}{\omega} \sin \omega t - \cos \omega t \right). \quad (10)$$

Considering condition (8), the vertical acceleration of the system (trailer at the driving point) was found at the known values of its components:

$$\ddot{Z} = \frac{h\alpha^2 v^2 \omega^2}{\omega^2 + \alpha^2 v^2} \left(\cos \omega t - \frac{av}{\omega} \sin \omega t \right). \quad (11)$$

Thus, from expression (11), the vertical accelerations of a trailer depend on the size of road irregularities, the speed of movement, and the natural frequency of vibrations. Further, the features of vertical vibrations of vehicles with balanced suspension were considered (Fig. 1b). The parameters and assumptions were the same as without it, with the difference that the signals from the wheel are different, and therefore their effect on the overall axis of the balancers is not the same. The moment of inertia of the balancers relative to the suspension oscillation axis was not considered. The effect of road bumps on the axle of a car with a balanced suspension can be recorded as follows:

$$q = \frac{d_2}{d_1 + d_2} q_1 + \frac{d_1}{d_1 + d_2} q_2, \quad (12)$$

where d_1, d_2 – balancer parameters; q_1, q_2 – value of the height of unevenness under the front and rear wheels. If the balance beam arms are the same, then:

$$d_1 = d_2 = \frac{1}{2} d_0, \quad (13)$$

The road roughness function that affects the car's axle will look like:

$$q(t) = \frac{1}{2} [q_1(t) + q_2(t)]. \quad (14)$$

The equation of motion of the system, written in terms of radial deformation of the tire, has the same form as for unbalanced suspension (8). Considering the symmetry of the suspension scheme, including the fact that the speed of the trailer

remains constant during overcoming the bumps, the road profile can be set by the expression:

$$q = h \left\{ 1 - \frac{1}{2} [e^{-\alpha|l|} + e^{-\alpha|l'|}] \right\}. \quad (15)$$

After double differentiation of equation (9) over time, and also considering that $l' = v(t - t_0)$, where t^0 – difference in time when the wheels hit a given bump (q), and substituting the obtained value into the equation of motion of the system written through the radial deformation of the tire, the following is obtained:

$$f + \omega^2 f = \frac{h\alpha^2 v^2}{2} [e^{-\alpha vt} + e^{-\alpha v(t-t_0)}]. \quad (16)$$

The solution of such an equation by conventional methods is quite complex. This is easier to do using operational computing. The task is simplified assuming that after hitting the first inequality after $2t_0$, the wheels of the trailer will again run over the same unevenness, after $4t_0$ – over the third, etc. Impact $q(t)$ will be periodic with a period of t_0 starting from the moment $t_0 = 0$ when the front wheels of the trailer, moving on a flat road, touch bumps. The assumption is formal in nature since the following inequality does not affect fluctuations during the first $2t_0$, when the previous bump is overcome. In this case, equation (16) has the form:

$$f + \omega^2 f = A [q(t) + q(t - t_0) + q(t - 2t_0) + \dots], \quad (17)$$

where $q(t)$, $q(t - t_0)$, $q(t - 2t_0)$... – corresponding unit functions; $A = \frac{1}{2} h\alpha^2 v^2$. To solve equation (17), the Laplace-Carson transform and the delay theorem are applied. The function and its derivatives have the form (Massalitina, 2017):

$$\begin{aligned} f &= \varphi(p), \frac{df}{dt} = p\varphi(p) - pf_0, \\ \frac{d^2 f}{dt^2} &= p^2 \varphi(p) - p^2 f_0 - pf_0. \end{aligned} \quad (18)$$

Provided that at the initial moment $t=0$, the left side of equation (16) will be as follows:

$$\ddot{f} + \omega^2 f = \varphi(p)(p^2 + \omega^2). \quad (19)$$

To obtain the right-hand side, it is noted that, according to the delay theorem, the image of a piecewise continuous unit function takes the form:

$$q(t - t_0) = e^{-pt_0}. \quad (20)$$

Therefore, the right side of equation (20) has the form:

$$\begin{aligned} A [q(t) + q(t - t_0) + q(t - 2t_0) + \dots] = \\ = A (1 + e^{-pt_0} + e^{-2pt_0} + \dots). \end{aligned} \quad (21)$$

Thus,

$$\varphi(p)(p^2 + \omega^2) = A (1 + e^{-pt_0} + e^{-2pt_0} + \dots). \quad (22)$$

Therefore:

$$\varphi(p) = \frac{A}{p^2 + \omega^2} (1 + e^{-pt_0} + e^{-2pt_0} + \dots). \quad (23)$$

To return to the original expression and apply the delay theorem, the following result is obtained:

$$\begin{aligned} \frac{A}{p^2 + \omega^2} (1 + e^{-pt_0} + e^{-2pt_0} + \dots) = \frac{A}{\omega^2 + \alpha^2 v^2} \times \\ \times \{1 - \cos \omega t + [1 - \cos \omega(t - t_0)] + \\ + [1 - \cos \omega(t - 2t_0)] + \dots\}. \end{aligned} \quad (24)$$

Flow value of radial tire deformation:

$$\begin{aligned} f = \frac{h\alpha^2 v^2}{2(\omega^2 + \alpha^2 v^2)} \times \\ \times \{1 - \cos \omega t + [1 - \cos \omega(t - t_0)] + \\ + [1 - \cos \omega(t - 2t_0)] + \dots\}. \end{aligned} \quad (25)$$

According to expression (5), the vertical displacement of the reduced mass has the following form:

$$\begin{aligned} Z = h^* \left\{ 1 - \frac{1}{2} [e^{-\alpha vt} + e^{-\alpha v(t-t_0)}] \right\} + \frac{h\alpha^2 v^2}{2(\omega^2 + \alpha^2 v^2)} \times \\ \times \{1 - \cos \omega t + [1 - \cos \omega(t - t_0)] + \\ + [1 - \cos \omega(t - 2t_0)] + \dots\}. \end{aligned} \quad (26)$$

After twice differentiating the displacement by time, the vertical acceleration of the system

at the driving point is obtained. For steady-state fluctuations, it has the form:

$$\ddot{z} = \frac{h\alpha^2 v^2 \omega^2}{2(\omega^2 + \alpha^2 v^2)} [\cos \omega t + \cos(t - t_0)]. \quad (27)$$

Based on the results of theoretical studies, it is established that the vertical vibrations of vehicles with a balanced suspension are slightly less than those with an unbalanced suspension (Fig. 4).

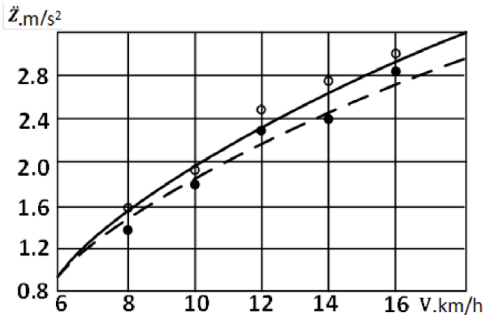


Figure 4. Dependence of vertical accelerations on the speed of the unit if $\omega = 6 \text{ rad/s}$, $\alpha = 0.15 \text{ m}$
Note: \circ – with unbalanced suspension; \bullet – with balanced suspension
Source: compiled by the authors

The difference depends on the length of the balancer, (the larger, the smaller the vertical fluctuations will be). Since the length of the balancer is limited, the vertical vibrations differ little. The results of experimental studies and their analysis confirmed the random nature of changes in vertical accelerations over time (Fig. 5).

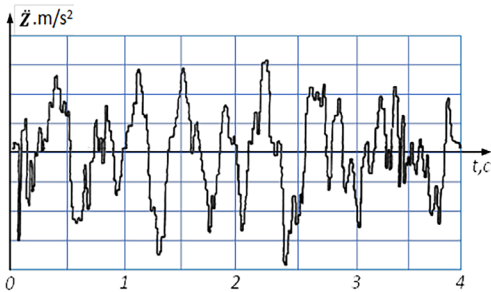


Figure 5. Nature of changes in vertical accelerations over time
Source: compiled by the authors

In the process of moving a trailer with both types of suspension on a dirt road in the Garden on an inter-block road, vertical accelerations depending on changes in the speed of the unit from 6 to 24 km/h and were: balanced suspension in the range of 0.7...2.1 m/s², and unbalanced – 1.1...3.4 m/s². In both suspensions, the maximum RMS acceleration values did not exceed the permissible values of 9.8 m/s² (Fig. 6)

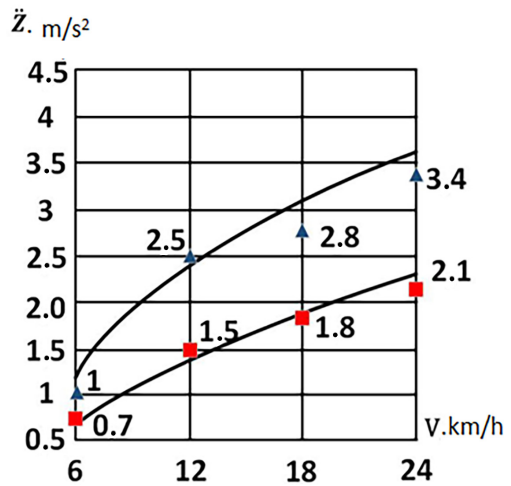


Figure 6. Changing the vertical accelerations of the fruit trailer while driving, depending on the speed of movement suspension type:
Note: \blacktriangle – unbalanced suspension; \blacksquare – balanced suspension
Source: compiled by the authors

As a result of the analysis of the frequency spectrum, the dominance of harmonic components at low frequencies of 1.75 Hz...2 Hz, 5...5.5 Hz, and 12.5 Hz was established. At subsequent harmonics, the increase in acceleration is insubstantial, and starting from a frequency of 20 Hz or more, this phenomenon was not observed. It was established that regardless of the type of suspension and the speed of the trailer, harmonics in the range of 1.45 Hz 5 Hz predominate. (Fig. 7).

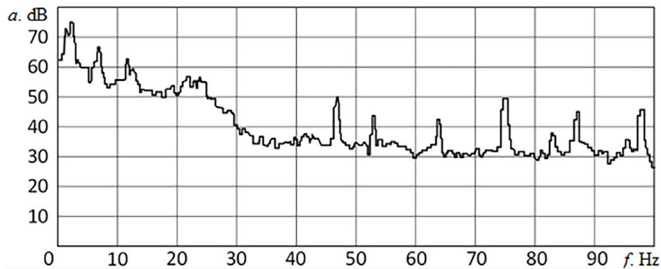


Figure 7. Spectral distribution of vertical accelerations

Source: compiled by the authors

The transition from the logarithmic scale in decibels (DB) to natural acceleration values was estimated by the formula:

$$N_{[dB]} = 20 \lg \frac{a}{a_0}, \quad (28)$$

where N – number of decibels; $a_0 = 3 \times 10^{-4} \frac{m}{s^2}$ – zero level; a – the numerical value of the intermediate acceleration is expressed in m/s^2 .

In the energy spectrum of frequencies, the highest level of vertical accelerations is present at the harmonic of 1.75 Hz...2 Hz, while the rest have lower accelerations. When the unit with a balanced suspension is moving at a speed of $V=24$ km/h, the total level of vertical acceleration is 3.4 m/s^2 , and at a frequency of 2 Hz – 1.9 m/s^2 . In this case, the main harmonic accounts for:

$$\frac{1.9}{3.4} 100\% = 55.8\%. \quad (29)$$

Comparing the spectra of vertical accelerations, the largest of them are at a frequency of 2 Hz and make up 55.8% of the full spectrum. When choosing the technological speed of a single-axle trailer with fruits, it is necessary to focus on the first harmonic, which accounts for approximately half of the power of the spectrum.

Examinations of vibrations of a single-axle trailer confirm the random nature of the change in vertical acceleration (Fig. 5). In this paper, an analytical method for calculating vertical accelerations is developed, considering the reduced mass of the trailer (M), the speed of movement (V), vertical displacement of the reduced mass (Z), total tire

stiffness ($with$), the height of irregularities (q), and the dimensions of the balancing and unbalanced suspension (d). The obtained equations for calculating vertical accelerations allow focusing on the choice of operating speeds, considering the permissible operating accelerations. This approach is fundamentally different from the model of X. Liu *et al.* (2022), in which vibration analysis was based mainly on the assessment of internal damage to fruits by the finite element method under static load conditions. In the proposed study, a system of equations is developed that allows quantifying the influence of dynamic factors already at the level of the trailer design, which allows not only assessing the risks of cargo damage but also reasonably adapting kinematic driving modes.

In real-world conditions of fruit transportation, the vertical acceleration of the trailer depends on the accepted theoretical calculations along with other factors. The paper shows that the distribution density of random factors has a spectrum either in the form of a wide band or in the form of several narrow ones (Fig. 7). Thus, to assess possible mechanical damage to the fruit, it is necessary to simulate the transportation process, considering the frequency of vibrations.

The data obtained as a result of the study confirm that the type of suspension of a single-axle trailer does not substantially affect the level of vertical vibrations, which are the main cause of mechanical damage to fruits during transportation. As demonstrated by E.J. Timm *et al.* (1996), the use of a balancing suspension can slightly reduce the intensity of fruit damage

compared to an unbalanced one, as it better dampens dynamic loads that occur on uneven road surfaces. These results are also supported by the paper of Van Zeebroeck *et al.* (2007), which showed that the most critical factor in apple damage is the pulsed forces transmitted through containers as a result of vibrations and shocks during transportation. In the current study, similar conditions were modelled for different suspension types, and the acceleration level in the frequency range of 1.5-3.5 Hz was found to correspond to the highest level of vertical acceleration, regardless of the suspension type.

In addition, according to the review of M. Al-Dairi *et al.* (2022), mechanical damage received during transportation is one of the main causes of loss of presentation of products, and their number and magnitude increase more with uneven road surface and higher speed than with the type of vehicles. The present study confirms these conclusions: the level of vertical acceleration when using an unbalanced suspension exceeded 0.7 g, while when using a balancing suspension, it did not exceed 0.45 g under similar conditions. B. Suleimani & E. Ahmadi (2015) proved that the quality of the road surface, the speed of transportation, and the type of suspension form the overall oscillatory load on products. Analysis in the current study shows that at speeds up to 24 km/h, both balanced and unbalanced suspensions provide an acceptable level of acceleration. Therefore, the use of structurally simple suspensions, but with sufficient shock-absorbing properties, is an important direction for adapting vehicles to agricultural conditions.

The practical value of this study increases in the context of simplifying the design of trailers for on-farm use. The data show that when driving at speeds of up to 20-24 km/h, even an unbalanced suspension can provide an acceptable level of vertical acceleration that does not exceed the thresholds specified in works S.J. Pretorius & W.J. Steyn (2012). This enables the reduction of the cost of technical means without compromising the quality of fruits during transportation,

which is also confirmed by V.M. Martyshko *et al.* (2012) on the feasibility of simplified suspension designs. The results obtained are logically confirmed in previous studies with a similar subject focus. Researchers A. Harrison & R. Van Houck (2008), in the context of transport logistics of agricultural products, noted that reducing the intensity of vibration loads in systems with adaptive or air suspension not only reduces the risk of fruit damage, but also increases the overall efficiency of logistics chains, especially at the stage of primary transportation from the producer to sorting points. A similar pattern was demonstrated by an experiment of T. Acican *et al.* (2007), which shows that when transporting apples in wooden boxes, the amplitude of vibrations on uneven surfaces causes substantial mechanical damage due to resonant effects, which can be mitigated by changing the stiffness of the supporting elements. B. Soleimani & E. Ahmadi (2015) stated that it is the type of vehicle suspension that is the fundamental factor determining the level of transmitted vibrations: particularly, pneumatic systems demonstrate the ability to adapt to changes in speed and type of road surface, providing a reduction in vibration intensity of up to 27% compared to their mechanical counterparts. The correlation of the above results with those obtained in this study indicates the universality of the effect of reducing vibrations due to the introduction of air suspension, which confirms the feasibility of its use in the conditions of fruit transportation on a mixed roadbed.

Thus, the results of the study confirm the need to consider the dynamic parameters of the suspension when designing trailers for on-farm fruit transportation. Optimisation of suspension types can be a pivotal factor in preserving the quality of agricultural products when transported from the field to primary processing or storage points. In this paper, on the example of fruit transportation by a single-axle trailer with a balanced suspension, it is proved that the spectrum of the greatest vertical accelerations falls on two lanes that require the most focus in the case of a safe

speed of movement. The possibility of using an unbalanced suspension is confirmed.

Conclusions

The suspension must ensure acceptable smoothness of the vehicle at different speeds, on roads with different surfaces. Smoothness of movement is determined by the value of RMS accelerations. It was established that the vertical accelerations of vehicles with a balanced suspension are less than those with an unbalanced suspension. The difference depends on the length of the balancer, the larger it is, the smaller the vertical accelerations will be. Since the length of the balancer is limited, the vertical vibrations differ little. The spectrum of vehicle vibrations is dominated by vertical accelerations at low frequencies of 1.75...12.5 Hz, and the largest of them at frequencies of 1.75...2 Hz. The predominant harmonic components are in the range of 1.45 Hz...5 Hz. If the transport unit moves along the edge of the block at a speed of up to 24 km/h, the vertical accelerations of the container ship with balanced suspension varied in the range of 0.7...2.1 m/s², and with an unbalanced – 1.0...3.4 m/s².

Within the existing speeds at which fruits are transported during on-farm transportation, to

simplify and reduce the cost of the design of a single-axle trailer, it is permissible to use an unbalanced suspension. It is advisable to use a balanced suspension only in cases where, based on the permissible loads on the axle, it is necessary to install more than two wheels. If two wheels can withstand the load, then, for reasons of simplifying the design of the vehicle, it is suggested to use an unbalanced suspension.

Further research should be aimed at selecting the speed of fruit transportation, both accounting for the characteristics of road bumps and suspension shock absorption properties, and considering the natural frequency of vehicle vibrations. It is also necessary to assess the natural frequency of vehicles with fruits to avoid the phenomenon of resonance, which occurs when the natural frequency coincides with the road caused by its roughness.

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Conflict of Interest

None.

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Обґрунтування типу підвіски одновісного причепа для транспортування плодів

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Анотація. Актуальність дослідження обумовлена важливістю збереження плодової продукції, на якість якої впливають механічні пошкодження плодів під час транспортування, пов'язані із специфічними труднощами через їх значну чутливість до механічних впливів, тому зменшення динамічної дії на плоди вимагає удосконалення конструктивних параметрів спеціальних транспортних засобів та рекомендацій щодо їх використання. Метою роботи було провести порівняльні дослідження рівня вертикальних прискорень причепа з балансиною та безбалансиною підвісками та довести доцільність використання останньої для перевезення плодів. Досліджено вплив конструктивних параметрів та експлуатаційних факторів одновісних причепів на вертикальні прискорення та їх розподіл в енергетичному спектрі коливань транспортного засобу при перевезенні фруктів. Проведено теоретичні та експериментальні

дослідження і виконано порівняльний аналіз вертикальних прискорень двох типів підвісок. В ході теоретичних досліджень розв'язано диференціальне рівняння для оцінки рівня вертикальних прискорень з урахуванням наступних факторів: типу підвіски; параметрів балансира; нерівностей профілю дороги; радіальної деформації та жорсткості шин; швидкості транспортного засобу. Для підтвердження результатів теоретичних досліджень була проведена експериментальна оцінка вертикальних прискорень одновісного причепа з балансованою та безбалансною підвісками при перевезенні плодів у саду по ґрунтовій міжквартальній дорозі. Результати дослідження показали, що середньоквадратичні значення вертикальних прискорень у діапазоні робочих швидкостей які виникали у причепів з безбалансною підвіскою вищі, ніж у причепів з балансною підвіскою. Встановлено домінування гармонік вертикальних коливань в діапазоні 1,75-2 Гц, на які припадає понад 55 % потужності спектра прискорень, що є визначальним для оцінки пошкодження плодів. Експериментально доведено, що при середній швидкості транспортування (близько 20 км/год), рівень вертикальних прискорень причепа з обома підвісками не перевищував допустимий рівень. Практичне значення роботи полягає в можливості застосування отриманих результатів при проектуванні та удосконаленні одновісних причепів для перевезення плодів, що сприятиме зменшенню втрат продукції та підвищенню ефективності роботи садівничих господарств

Ключові слова: нерівності ґрунту; коливання; механічні пошкодження плодів; диференціальні рівняння; вертикальні прискорення; амплітудно-частотні характеристики



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Effectiveness of biotechnological tools in improving the health of pigs and environmentalisation of production

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Abstract. In the context of the global growth of pathogen resistance to antibiotics and environmental challenges in animal husbandry, there is a need to implement effective biotechnological solutions. The purpose of the study was to establish the effectiveness of using biotechnological tools in pig breeding, considering their impact on animal health, productivity, and environmental sustainability of production. The experiment was conducted in the period from August to December 2024 at two pig farms in the Kirovohrad and Vinnytsia oblasts with a total sample of 240 pigs divided into control and three experimental groups. The study examined the effects of probiotics, vaccines, and genetic selection on live weight gain, morbidity, haematological and immunological parameters, frequency of

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antibiotic use, environmental parameters, and economic profitability. As a result of the use of probiotics, the average daily weight gain increased by 80 g, the morbidity decreased by half, and immunoglobulin levels increased by 30%. Vaccination provided an increase in the safety of livestock by 4.3%, a reduction in the need for antibiotic therapy by 40%, and a reduction in residual antibiotics in products. Genetic selection yielded the highest results – an increase of up to 810 g/day, the lowest rates of morbidity, ammonia emissions, and antibiotic residues. Economic analysis confirmed the effectiveness of all three approaches: the ROI was 287.5% for probiotics, 50% for vaccination, and 123% for breeding. The practical effectiveness of the results is confirmed by examples of similar approaches in Denmark, Poland, and Brazil, where a reduction in the use of antibiotics was achieved without compromising productivity. The study also showed a positive effect on soil microbial activity, reduced acid load, and eutrophication risks. The results can be used in the practice of pig farms to increase animal productivity, reduce dependence on antibiotics, and reduce the environmental burden on agroecosystems.

Keywords: probiotics; vaccination; genetics; animal husbandry; veterinary medicine

Introduction

Intensive pig production remains one of the key industries for ensuring food security, but it is accompanied by a number of systemic challenges, in particular, the growing environmental burden, the spread of pathogen resistance to antibiotics, risks to product quality, and the need to improve energy efficiency. Under these conditions, biotechnological tools that can reduce the veterinary burden on animals, optimise physiological processes, and reduce the negative consequences of intensive animal husbandry for the environment become particularly relevant. These tools include probiotics that help to normalise the intestinal microflora, vaccines to control infectious morbidity, and selection based on signs of pathogen resistance. When used in an integrated manner, these technologies can provide performance improvements without over-reliance on antibiotic therapy. Simultaneously, this helps to reduce the frequency of gastrointestinal diseases and reduce the environmental burden on agroecosystems.

In the world literature, the problems of biotechnologies in animal husbandry are given increasing attention, in particular, in the context of the transition to sustainable agricultural practices and reducing dependence on antimicrobials. Based on the findings of S. Zira *et al.* (2022), the integration of approaches based on the concept

of One Health allows for synchronous improvement of animal health, human health, and ecological balance. The researchers analysed several scenarios for the future development of pig farming, emphasising the role of genetics, feeding, environmental management, and antibiotic restriction as interrelated elements of biosafety. M. Hasan *et al.* (2024) gives an example of China as a country with a high concentration of livestock complexes, where large-scale application of biotechnology has proved critical to reducing the veterinary burden. The researchers proved that achieving a balance between economic profitability and environmental safety is possible only if the four components – biosafety, genetic selection, nutraceutical support, and systemic immunological monitoring – were combined.

The study by Y.M. Goley *et al.* (2022) analysed global trends in the development of biotechnologies in animal husbandry and pointed out the growing relevance of their implementation in the conditions of Ukraine. In particular, the need to create national platforms for technology transfer to the pig industry was emphasised, considering the specifics of local production systems and limited resources. In turn, according to estimates of A.A. Gaidar (2020), insufficient integration of environmental marketing and biotechnological

approach into the practical activities of agricultural enterprises was recorded in Ukraine. The researcher pointed out the existence of a technological gap: despite access to innovative products, enterprises often do not have mechanisms for effective implementation due to the lack of localised applied research and proper consulting support.

The issue of genetic improvement of animals through marker breeding and genome editing occupies an important place in contemporary pig breeding biotechnology. C.F. Tu *et al.* (2022) considered the potential of genomic interventions as a means of improving the immune status of animals. Special attention was paid to CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats – CRISPR-associated protein 9) technology, which was considered as a promising tool for targeted modification of genes associated with infection resistance in industrial reproduction. In turn, the study by K.M. Whitworth *et al.* (2022) focused on practical advances in pig genome editing using advanced biotechnological platforms. The researchers argued that modification of specific genes, in particular, CD163 (Cluster of Differentiation 163), associated with sensitivity to the Pocrine Reproductive and Respiratory Syndrome Virus (PRRSV), can significantly reduce dependence on veterinary interventions, increase the safety of livestock and optimise production indicators. These data confirm the effectiveness of genetic engineering as a component of long-term biosafety in pig farming.

The study by P.W. Knap (2022) substantiated the importance of selective breeding for resistance to multivariate infectious diseases based on the identification of key genetic markers, in particular, the MUC4 (Mucin) and SLA-1 (Swine Leukocyte Antigen) genes. The researchers emphasised that it is the genetic potential of an animal that determines its adaptive response in conditions of limited use of antibiotics. According to the conclusions of D.N. Das *et al.* (2022), the maximum effect is achieved only when genetic factors are combined with immuno-active components of feeding, such as phytogetic supplements and probiotics. The

researchers proved that this interaction helps to improve the overall physiological status of animals and reduce the risk of metabolic disorders.

The environmental consequences of intensive pig farming were investigated in detail by S. Zhong *et al.* (2022). In particular, the researchers found that one of the main sources of nitrogen loading remains ammonia emissions from manure and antimicrobial residues that enter soil systems and disrupt microbial balance. The use of biotechnological solutions, according to the researchers, can reduce nitrogen emissions, stabilise soil pH, and limit eutrophication of aquatic ecosystems, which is especially important for regions with a high density of livestock facilities. Similar ideas were supported by A. Bodyul (2024), who emphasised the importance of microbiological monitoring to improve the accuracy of biotechnological intervention.

Despite the existence of positive examples, most studies focused only on certain aspects: physiological changes, performance, or immune response. Economic efficiency, impact on soil microbiota, reduction of ammonia load, and payback period for biotechnological tools were also not considered in the unified system. In the context of Ukraine, the effects of combined probiotic use, vaccination, and breeding in real farm conditions remained unexplored, including their impact on antibiotic dependence, environmental parameters, and financial performance.

In this regard, the purpose of the study was to comprehensively assess the impact of three biotechnological approaches on the physiological, productive, veterinary, environmental, and economic indicators of animals to establish their practical feasibility for adaptation in sustainable pig farming systems. Research objectives: to evaluate the effectiveness of probiotics, vaccines and selection in improving the health and productivity of pigs; to determine the impact of these tools on reducing the use of antibiotics and environmental burden; to analyse examples of successful implementation of biotechnologies in industrial practice.

Materials and Methods

The study was conducted during August-December 2024 at two industrial pig farms: Agro-Svit LLC (Kirovohrad Oblast) and Promin Agro PE (Vinnytsia Oblast). The choice of these farms was determined by their compliance with the criteria of scale, technological equipment, and stable breeding work, which ensured the representativeness of the study in the conditions of intensive pig breeding. Both complexes had a well-established veterinary service, advanced systems for monitoring feeding and microclimate, and also maintained documentation on treatment, vaccination, and genetic accounting, which provided complete and reliable data within the experiment. The total number of sows at the enterprises was more than 1,000 units, and the annual turnover was about 15,000 units. The study used crossbreeds of Large White × Landrace breeds (sows) and terminal boars of the Duroc breed, bred as part of internal reproduction.

The experiment included 240 clinically healthy fattened pigs aged 70 ± 2 days with an average body weight of 30.4 ± 0.9 kg. 120 animals were selected from each farm to ensure a uniform representation of the technological conditions of both pig farms. The health status of animals was determined based on a daily clinical examination by the veterinary service of the farm and an additional laboratory blood test (general clinical analysis: white blood cells, haemoglobin, haematocrit, red blood cells) using an automatic haematology analyser BC-2800Vet (Mindray, China). Only those individuals who did not have appetite disorders, enteropathies, external malformations, or residual manifestations of previous diseases were allowed to participate in the study. The criteria for exclusion from the experiment were stress reactions, damage to the respiratory or digestive organs, and detected deviations in the haematological status at the time of inclusion.

The animals were randomly divided into four equal groups of 60 units: control (without biotechnological intervention), probiotic, vaccination, and breeding. The choice of these biotechnological

tools – probiotics, vaccination, and breeding – was conditioned by their widespread use in the practice of advanced pig breeding, the presence of convincing experimental data on the positive impact on the immune status of animals and productivity, and their potential in reducing the need for antibiotic therapy and environmental burden on agroecosystems. A comprehensive assessment of the three approaches allowed comparing their effectiveness by physiological, veterinary, environmental, and economic criteria in industrial production.

All animals were kept in standard conditions in compliance with the microclimatic parameters recommended for fattening pigs. The room temperature was maintained within 18–20°C, relative humidity – at the level of 60–70%. The air velocity did not exceed 0.2 m/s, the ammonia concentration did not exceed 10 ppm, and the carbon dioxide content was 0.2%. Ventilation was carried out mechanically using automatic microclimate control systems. The lighting corresponded to the circadian rhythm, with daylight duration of at least 16 hours. These parameters provided optimal conditions for maintaining health, minimizing stress factors, and accurately comparing the effectiveness of biotechnological interventions.

Feeding of animals was carried out with complete mixed feeds (exchange energy – 12.8 MJ/kg; crude protein – 17.5%) based on barley, corn, soybean meal, and premixes. The control group used a basic diet without biological additives. In the probiotic group, a complex containing *Lactobacillus acidophilus* DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen) 20079, *Bacillus subtilis* DSM 5750 and *Enterococcus faecium* DSM 10663/NCIMB (National Collection of Industrial, Marine and Food Bacteria) 10415, from the commercial form Biotil-Feed (Vitagen Biotech, Poland) at a dose of 1×10^9 CFU/g (colony-forming units per gramme), 0.5 g/kg of feed.

In the vaccination group, animals received three vaccinations: against PRRSV (Porcine Reproductive and Respiratory Syndrome Virus) (Ingelvac PRRS MLV, Boehringer Ingelheim, Germany), *Mycoplasma hyopneumoniae* (Hyogen, Ceva

Santé Animale, France), and *Lawsonia intracellularis* (Enterisol Ileitis, Elanco, USA). In the vaccination group, the animals received three vaccinations according to the preventive protocol of the farm: the primary vaccination was carried out on the 28th day of life, revaccination – on the 56th day, and the third vaccination – on the 70th day, which coincided with the moment when the animals were included in the experiment. Thus, pigs that had already passed the first two stages of immunisation were selected for the study, and the final vaccination was carried out as part of the experiment. In the breeding group, animals were selected from genotypes characterised by increased resistance to diseases of the respiratory and digestive systems (determined by the results of genomic testing of herd lines). This testing was conducted at both farms during August – December 2024 and covered the previous two generations. Thus, the selection of animals for participation in the study was based on already selected traits fixed in the breeding work of previous cycles. Along with an increased growth index, this formed a group with significantly higher indicators of hereditary stability.

Animal body weight control was performed individually on S-ElectroScale electronic platforms (SlaughterTech, Czech Republic) on the 30th, 60th, and 90th days of the experiment. This period was chosen considering the length of the standard fattening cycle and the need to assess both the short- and medium-term effects of biotechnological intervention. The frequency of measurements every 30 days helped to record the dynamics of live weight gain and timely identify deviations in growth indicators, which increased the accuracy of assessing the impact of each of the instruments. In addition, these intervals correlated with the physiological growth phases of fattening pigs, which ensured comparability of data with generally accepted criteria for production efficiency. For the growth analysis, the average daily growth, fattening duration, and feed conversion (calculated using individual FeedControl Pro feeders) were considered. Clinical monitoring

of animals was carried out daily by the veterinary service. All haematological, immunological, and environmental parameters were determined in the laboratory using certified equipment and approved analytical methods. Haematological tests included a general clinical blood test (determination of the number of red blood cells, haemoglobin, white blood cells, haematocrit) using an automatic BC-2800Vet analyser (Mindray, China). Immunological status was assessed by the concentration of Class G immunoglobulins (IgG) determined by enzyme-linked immunosorbent assay using pig IgG ELISA Kit test systems (MyBiosource, USA). To assess environmental parameters, ammonia emissions were determined (Dräger X-am 5000 gas analysers, Germany), the concentration of antibiotic residues in meat by HPLC (high-performance liquid chromatography) (Agilent 1260 Infinity, USA), and manure residues by solid-phase extraction followed by spectrophotometric analysis (Shimadzu UV-1800, Japan). Soil samples after manure application were evaluated for microbiological activity in accordance with DSTU 7863:2015 (2015). The analysis covered the total number of bacteria, the number of actinobacteria, saprotrophic fungi, and ammonifying bacteria. Indicators of enzymatic activity were determined separately: urease, phosphatase, and dehydrogenase. The pH value of the soil was measured by potentiometric method using the tool manufactured by Hanna Instruments (Hungary). Economic efficiency was calculated based on indicators of additional profit, which included: savings on antibiotic therapy (fixed by the average cost list of veterinarians), an increase in sales weight, and a decrease in the cost of feed per unit of growth. The return on investment (ROI) was calculated using the equation (1):

$$ROI = \frac{P-C}{C} \times 100\%, \quad (1)$$

where P – total economic benefit per animal unit; C – cost of implementing biotechnology.

The Student's t-test (normal distribution), Mann-Whitney U-criterion (for rank data), ANOVA

analysis of variance, and Fischer's exact test were used to process statistical data. The level of statistical significance was set at $p \leq 0.05$, all values were given with a 95% confidence interval. The analysis was performed in Statistica 13.0 (StatSoft, USA) and MS Excel 365. To verify the results obtained and interpret the applied significance of the study, a comparative analysis of examples of the introduction of biotechnological tools in pig farming in other countries was carried out. The sample included farms in Denmark, Poland, and Brazil – countries that are among the leaders in the implementation of biosafety, vaccination, and probiotic technologies on commercial pig farms. The main purpose of including these cases was to compare the effectiveness of such interventions in conditions close to industrial scale, and to confirm the relevance of Ukrainian results in a broader international context (Jacobson, *et al.*, 2009; Stefanik *et al.*, 2021). The experimental part of the study was conducted in accordance with the provisions of the Law of Ukraine No. 3447-IV (2006), and in compliance with the requirements of the European Directive 2010/63/EU (European Parliament & Council of the European Union, 2010) on the protection of animals used for scientific purposes.

Results and Discussion

The study evaluated the effectiveness of three biotechnological approaches – probiotic drugs, vaccines, and genetic breeding – in terms of their impact on the health, productivity, haematological, and immunological status of pigs. Special attention was paid to the analysis of the action of probiotic agents that contained active cultures of *Lactobacillus spp.*, *Bacillus subtilis*, and *Enterococcus faecium*. These microorganisms perform a number of important functions in the gastrointestinal tract of pigs, in particular, they contribute to the growth of lactic acid volume, lower pH in the colon, inhibit pathogenic microflora, improve protein digestibility, and reduce endogenous stress. Their use optimised the intestinal microbiocenosis, increased the efficiency of

digestion, and reduced the risk of gastrointestinal disorders. Against the background of probiotic intervention, there was a significant improvement in live weight gain – from 670 to 750 g/day, which was 11.9% more compared to the control group, indicating a positive dynamics of growth processes. Feed conversion improved from 2.9 to 2.6 kg/kg of growth, i.e., by 10.3%, which allowed reducing the consumption of feed resources without reducing the level of productivity. The overall incidence rate decreased from 34.1% in the control group to 15.4% in the probiotic group, i.e., 2.2 times. Analysis of haematological parameters in animals treated with probiotics showed an increase in the haemoglobin concentration from 112 to 118 g/l (+ 5.4%) and normalisation of the number of red blood cells, which indicated an overall improvement in haematopoiesis. However, the level of Class G immunoglobulins increased from 12.3 to 16.1 mg/ml, that is, by 30.9%, which confirmed the activation of a non-specific immune response. Immunological stimulation had a positive preventive effect, which was shown to reduce the need for antibiotic therapy from 87 to 39 cases per 100 animals. This effect was considered as a promising alternative to drug intervention. In the vaccination group, animals received vaccinations against PRRSV, *Mycoplasma hyopneumoniae*, and *Lawsonia intracellularis* according to the triple immunisation protocol. The use of vaccines provided a significant increase in the safety of livestock – up to 97%, compared to 92.5% in the control group. The frequency of antibiotic therapy decreased to 52 cases per 100 animals, which indicates an effective reduction in the risk of secondary infections. The group also showed a decrease in antibiotic residues in meat (up to 5.6% of samples exceeding the MPR (maximum permissible level)) and pus, which confirmed a decrease in the pharmacological load.

Haematological parameters (haemoglobin, white blood cell levels) remained stable, while the level of IgG immunoglobulins increased to an average of 15.7 mg/ml. This indicates the development

of an effective specific immune response, which reduces the need for preventive use of antibiotics during critical periods of growth. In the breeding group, animals were selected based on indicators of hereditary resistance to infections of the respiratory and digestive systems, confirmed by the results of genomic testing (lines with CD163, MUC4, SLA-1 markers). This helped to form a herd with an increased immune index and stable increments. The average daily weight gain in the group reached 810 g/day, and the incidence rate was the lowest among all groups – 12.7%. The content of

IgG immunoglobulins was the highest – 17.3 mg/ml, which indicates increased non-specific immune activity. The reduction of antibiotic residues in meat (up to 2.1%) and manure (13.7 mg/kg), and the lowest level of ammonia emissions (85.3 g/day/animal) confirmed the environmental advantage of the genetic approach. Breeding also provided the highest ROI among long-term solutions – 123%, which confirmed its strategic feasibility for sustainable pig farming. A comparative assessment of the effectiveness of biotechnological measures is given in Table 1.

Table 1. Comparative effectiveness of biotechnological measures in pig breeding

Indicator	Control group	Probiotics (<i>Lactobacillus spp.</i> , <i>B. subtilis</i> , <i>E. faecium</i>)	Vaccination	Breeding
Live weight gain, g/day	670	750	735	810
Feed conversion, kg/kg of weight gain	2.9	2.6	2.7	2.5
Morbidity, %	34.1	15.4	21.2	12.7
Haemoglobin level, g/l	112	118	117	120
Serum immunoglobulins (IgG), mg/ml	12.3	16.1	15.7	17.3
Livestock preservation, %	92.5	94.2	97	98

Source: developed by the authors

Probiotic drugs, in particular, those that contained *Lactobacillus spp.*, *Bacillus subtilis*, and *Enterococcus faecium* were found to be highly effective in improving digestive parameters, reducing enteropathies, and normalising the immune background. Their effect was physiological in nature and was manifested through the stabilisation of the intestinal microbiocenosis, improvement of feed digestibility, and reduction of stress reactions in young animals, especially in the weaning stress period. This provided not only weight gain, but also an improvement in the overall adaptive state of the animals, which is important in the context of reducing antibiotic dependence. Probiotics are characterised by ease of integration into existing technological schemes of pig farms, economic feasibility, and environmental safety, which makes them a universal tool for maintaining animal health and productivity in the short term.

As a result of the introduction of biotechnological tools in the system of intensive pig production, a significant reduction in dependence on conventional antibiotic therapy was recorded. The use of probiotics, vaccination, and genetic selection ensured the development of a more stable immune status in animals, which, in turn, led to a decrease in the number of therapeutic interventions using antibiotics. Initially, the decrease in the use of antimicrobials was explained by a decrease in the frequency of recurrent infections, usually accompanied by the use of a wide range of antibiotics. Biotechnological approaches activated the body's natural resistance, stabilised the gastrointestinal microbiota, and simultaneously helped to maintain the effectiveness of a specific immune response. Animals treated with probiotics had a reduced need for treatment for infections caused by *Escherichia coli*, *Salmonella spp.*, *Clostridium spp.*, and other opportunistic microorganisms.

In the case of vaccination, there was a significant reduction in the need for antibiotic prevention during seasonal outbreaks of viral diseases.

Due to the consolidation of resistant genotypes to the most common swine diseases, the need for emergency chemotherapy has decreased even if the overall microbial load in the environment remains. Thus, hereditary resistance has become one of the main factors of biosafety at the herd level. The study by D.E. Reeves (2006) proved the feasibility of combining biotechnological tools with measures of epidemiological monitoring, genetic selection, and continuous monitoring of animal health. As part of the experiment, this integration was implemented through a combination of selective breeding, vaccine prevention, and microbiological intervention, which reduced the frequency of diseases, reduced the need for therapeutic interventions, and improved the stability of the physiological status of livestock. This confirmed the effectiveness of the integrated approach to biosafety indicated in the previous study, considering the current challenges of animal husbandry in intensive production. A decrease in the volume of antibiotic therapy was accompanied by a decrease in residual substances in the products. There was an improvement in meat quality and a decrease in the frequency of detection of antibiotic residues in animal tissues, especially from the group of tetracyclines and sulphonamides. This ensured compliance with the requirements of international trade regarding the safety of animal products.

Simultaneously with the decrease in product residues, there was an improvement in the environmental characteristics of production. A significant reduction in the content of antibiotic residues in animal excrement has reduced the risk of these substances entering the environment – in soils, surface, and ground water. According to the results given by N. Kemper (2008), veterinary antibiotics can persist in soil and aquatic ecosystems for a long time, which creates a cumulative environmental burden and contributes to the emergence and spread of resistant strains of

microorganisms in the environment. The study confirmed these risks, recording a decrease in the concentration of residual antibiotics in manure and soil in animals for which biotechnological approaches were used, in particular, probiotics and breeding. This was accompanied by a decrease in the content of tetracyclines and sulphonamides, an increase in microbial diversity in soils, a decrease in the activity of ARGs (antibiotic resistance genes) genes, a decrease in the content of tetracyclines and sulphonamides in manure by 41-56% (from 2.4 mg/kg to 1-1.4 mg/kg, respectively), which was accompanied by an improvement in microbial diversity in soils. Due to the stabilisation of the intestinal microbiocenosis and the improvement of digestive processes, more efficient assimilation of feed was achieved, which, in turn, led to a decrease in the volume of manure rich in undigested nitrogen and phosphorus compounds. Reducing ammonia emissions was of particular importance. Under conditions of stable digestion and improved biochemical processing of proteins, the development of volatile nitrogenous compounds decreased. The reduction of ammonia emissions was up to 27% in the probiotic group (96.2 g/day → 70.1 g/day) and up to 32% in the breeding group (96.2 → 65.3 g/day), which directly affected the improvement of the microclimate in livestock premises and the reduction of atmospheric air load. This helped to reduce the load on the microclimate in pigsties and improve the working conditions of personnel. The negative load on atmospheric air in the area where farms are located was also reduced, which is an important condition for compliance with environmental legislation.

The use of manure from animals that have reduced their antibiotic intake due to biotechnological tools has had a positive impact on the biodiversity of soil microorganisms. It was found that after the introduction of such manure, an increase in populations of actinobacteria, saprotrophic fungi, and ammonifying bacteria was observed, which were previously suppressed by the presence of residual antimicrobial drugs. In particular, the activity of phosphatase, dehydrogenase, and

urease enzymes increased, which indicated a restoration of the functional activity of the microbiota. A decrease in the content of residual tetracyclines and sulphonamides in the soil contributed to a decrease in the frequency of detection of antibiotic resistance genes (*tetM*, *sul1*, *blaTEM*) in the microbial pool – from 68% in the control variant to 32% in the probiotic manure variant and 24% in the breeding origin variant, which reduced the risk of their transfer to the agricultural system and food chain. As part of the analysis of the biological activity of soils fertilised with manure from animals with probiotic intervention, an increase in the activity of phosphatase, urease, and

dehydrogenase was recorded, which was consistent with the restoration of microbial balance and a decrease in the genetic load on the soil. As a result of chromogenic and colourimetric analyses, it was determined that the activity of soil enzymes – phosphatase, urease, and dehydrogenase – significantly increased when using manure from animals that were injected with probiotics or selected. All three enzymes are sensitive indicators of the microbiological state of the soil. An increase in their activity indicates the restoration of the functional microbial pool and a decrease in the ecotoxicological load. The summary results are shown in Table 2.

Table 2. Activity of soil enzymes in different approaches to pig feeding

Enzyme	Control group	Probiotic	Breeding
Phosphatase, mcg $P_2O_5/g \cdot h$	3.1±0.2	4.8±0.3	5.2±0.3
Dehydrogenase, mcg TPF/g · 24 h	1.9±0.1	2.8±0.2	3±0.2
Urease, mg $NH_4^+/g \cdot h$	1.6±0.1	2.4±0.2	2.7±0.2

Note: all values are presented as the mean ± standard deviation

Source: developed by the authors

The results show that biotechnological interventions contribute to the activation of soil microbiological processes, especially in terms of organic mineralisation, nitrogen and phosphorus transformation, which are key to maintaining soil fertility and sustainable agricultural production. The reduction of ammonia emissions from manure had a positive effect on the chemical properties of the soil, in particular, on its acidity. In regions with high concentrations of livestock, excess ammonia emissions are subsequently converted into ammonium aerosols, which are involved in the development of acidic sediments that lower the pH of the soil and disrupt the buffer capacity (Zhao *et al.*, 2014). As a result of a decrease in the emission of volatile nitrogenous compounds, pH stabilisation in the surface horizon of the soil and a decrease in the manifestations of acid stress in agricultural crops were recorded. C. Ti *et al.* (2022) proved that reducing ammonia emissions from livestock enterprises not only improves the indoor

microclimate, but also significantly reduces the risk of acid rain, which inhibits the growth of cultivated plants and causes soil degradation. In particular, the indicators of seed germination, root system length, and photosynthetic activity of plants improved, which indicated the restoration of physiological and biochemical balance in agrophytocenoses (European Environment Agency, 2021; Koroban *et al.*, 2023). Thus, the reduction of both residual antibiotics in manure and secondary ammonia emissions provided a comprehensive restoration of the ecological functionality of soils, which is a critical factor for the sustainable development of integrated agroecosystems. Reducing the content of antibiotic residues in animal excrement had not only sanitary and hygienic significance, but also directly affected the biological activity of soils.

Horizontal transfer of antibiotic resistance genes (ARGs) through mobile genetic elements in an environment with an increased content of

residual drugs is a separate danger. Studies conducted in Germany, Denmark, and the Netherlands have shown that long-term application of untreated manure contributes to the accumulation of ARGs in soil DNA (deoxyribonucleic acid) and even in aquifers, which creates risks for water supply and agricultural products (European Environment Agency, 2021). Biotechnologies that reduce the need for antibiotics on farms directly reduce this risk by preventing the circulation of resistant bacteria between animals, humans, and the environment. Another environmentally important consequence of the biotechnological transformation was the reduction of ammonia emissions. Under conditions of improved biochemical processing of proteins, a decrease in the development of volatile nitrogenous compounds was observed, which not only improved the conditions of keeping animals, but also limited emissions into the atmosphere. Ammonia, being a strong nitrogen reducer, is easily washed out into water systems in the free state, stimulating eutrophication of water bodies, mass development of blue-green algae, oxygen deficiency, and degradation of aquatic biocenoses. Every year, due to excessive nitrogen load in coastal areas, fish productivity decreases, and the ecosystem of water bodies is disrupted (European Environment Agency, 2021). Thus, reducing ammonia

emissions in groups with probiotics and breeding also helped to reduce the risk of hypertrophy of soils and water bodies, especially in regions with a high level of agricultural load.

Table 3 shows that breeding and probiotics were most effective in reducing not only the frequency of antibiotic use, but also secondary exposure to their residues. This indicates the possibility of including biotechnological practices in comprehensive programmes of ecosystem sustainability of agricultural production, focused on the circular economy, soil quality preservation, and prevention of environmental pollution. The obtained data indicated the possibility of forming a circular production model based on breeding, microbiological and preventive technologies, which fully corresponds to the approaches proposed in the above-mentioned study. In the context of greening animal husbandry, reducing the level of antibiotic residues in animal excrement, and reducing ammonia emissions to the environment, was of particular importance. As a result of improved digestion of feed protein components caused by stabilisation of the intestinal microbiota, the development of volatile nitrogenous compounds decreased. Reducing ammonia emissions in probiotic and breeding groups helped to reduce the burden on the microclimate of livestock premises and the environment.

Table 3. Indicators of the impact of biotechnological tools on antibiotic therapy and environmental impact

Indicator	Control group	Probiotic	Vaccination	Breeding
Frequency of antibiotic therapy, cases/100 animals	87	39	52	28
Antibiotic residues in meat, % of samples exceeding MPR (maximum permissible level)	12.5	4.2	5.6	2.1
Antibiotic residues in pus, mg/kg	38.6	18.1	21.3	13.7
Ammonia emissions from manure, g/day/animal	135.2	92.7	101.4	85.3
Nitrogen uptake from manure in the soil, %	47	61	58	66

Source: developed by the authors based on own research

The findings clearly suggest that all three biotechnological approaches had a positive impact on both reducing antibiotic use and environmental

parameters. The lowest levels of antibiotic residues and ammonia emissions were observed in the group of animals where breeding was used,

which confirmed its potential for long-term reduction of biogenic loads. Probiotics have significantly improved feed processing and excrement quality, increasing the degree of nitrogen uptake by plants when using manure as fertiliser. The practical implementation of biotechnological innovations in production conditions has shown their high efficiency in ensuring stable growth, improving animal health, and reducing the environmental burden. In Denmark, where the pig industry has a high share in the structure of agriculture, a study was conducted on a medium-scale farm on the effectiveness of vaccination against *Lawsonia intracellularis*. The use of the vaccine has reduced the use of oxytetracycline by 79%, which is especially important in the context of the EU policy on limiting antibiotics in livestock products. In addition, vaccination contributed to an increase in the average daily growth by 46 g, and the slaughter weight of pigs increased by an average of 1.25 kg, which had a positive impact on the economy of production (Jacobson *et al.*, 2009). These results confirm that targeted vaccination is an effective alternative to preventive antibiotic therapy.

A number of pig farms in Poland have implemented breeding programmes aimed at forming animal lines with increased genetic resistance to common infections. According to E. Stefanik *et al.* (2021), these programmes used marker genotyping and selection based on performance and immune resistance indicators. As a result, a 30% reduction in the use of antibiotics was recorded, while the level of productivity remained at a consistently high level. This approach not only reduced the pharmacological load, but also formed an infection-resistant herd with improved reproductive characteristics. Brazil, which is one of the largest pork producers in the world, has implemented a model of integrated biosafety production using probiotics, vaccination, sanitary control, and microbiota monitoring. According to B. Oliveira *et al.* (2024), farms that switched to this model were able to reduce preventive use of

antibiotics without losing productivity. The complex impact of biotechnological interventions helped to simultaneously reduce the risk of antibiotic resistance, stabilise the physiological parameters of animals, and increase the safety indicators of final products. In all the cases described, the combination of genetic, microbiological, and vaccination approaches demonstrated a synergistic effect, which increased economic efficiency and simultaneously reduced biological and environmental risks. Successful examples show the key role of biotechnology in ensuring the sustainable development of pig farming.

In the course of the study, an economic assessment of the effectiveness of implementing biotechnological approaches in the field of pig breeding was carried out. The analysis covered three main technologies: the use of probiotics, the implementation of vaccination programmes, and the use of genetic breeding methods. The assessment was carried out based on the ratio of the cost of implementing these tools to the obtained economic effect, considering improved productivity, increased livestock safety, and reduced treatment costs. It was found that all three tools provided economically feasible use of resources and contributed to the optimisation of the technological process. The approaches differed both in the nature of the impact and in the time horizons of payback. Probiotic drugs showed the most pronounced effect in the short term, due to a rapid reduction in veterinary costs and improved feed conversion. Vaccination proved to be less cost-effective in the absence of outbreaks of diseases, but it ensured the stability of production indicators and reduced the risk of economically significant losses. Breeding approaches required higher start-up costs, but allowed achieving a long-term increase in production efficiency, in particular, by consolidating signs of resistance to diseases and improving growth. A comparative description of the economic performance of biotechnological instruments is given in Table 4 (equation 1).

Table 4. Economic efficiency of biotechnological tools in pig breeding

Tool	Implementation costs, UAH/animal	Additional profit, UAH/animal	ROI, %
Probiotic	80	310	287.5
Vaccination	130	195	50
Breeding	220	490	123

Source: developed by the author

The analysis confirmed the economic feasibility of introducing biotechnologies in pig farms. All the instruments under study provided positive dynamics of profitability, but the degree of profitability varied significantly depending on the nature of the intervention. The most profitable in the short term was the use of probiotics due to the combination of low costs and a tangible economic effect. Breeding, while requiring higher investment, provided a steady return over several production cycles. Vaccination turned out to be less cost-effective, but it was justified in conditions of increased infectious pressure. Thus, biotechnological approaches ensure not only biological efficiency, but also contribute to environmental stabilisation and growth of economic efficiency of the industry.

As part of the study, it was found that all biotechnological measures had a positive effect compared to the control group, and the mechanisms of their action were specific. Similar results were described in the study by R.P. Singh *et al.* (2018), where the combined use of genetic, microbial, and fermentation methods was considered as an effective strategy for adaptive animal husbandry. As in the results obtained, an increase in animal resistance, a decrease in the use of antibiotics, and an increase in economic efficiency were recorded. The reduced need for antibiotics in animals with entrenched genetic resistance to infections was consistent with the findings of M.C. Yunes *et al.* (2021), where genome editing was evaluated as an alternative to drug prevention. Although no genomic intervention methods were used in the study, the effect of increased immune resistance achieved by selective breeding showed similar results. Data by D. Shanmugamprema *et al.* (2022)

showed a positive effect of probiotics on intestinal morphology and immune response. As part of this study, an increase in IgG levels, a decrease in overall morbidity, and microbiota stabilisation were recorded in animals treated with probiotics, which confirmed the effect of physiological immune support without pharmacological intervention. Effects of the use of phyto-genic substances, described by J. Wang *et al.* (2024), included maintaining microbial balance and reducing the need for pharmacological prophylaxis. In the study, similar results were provided by probiotics, which reduced endogenous stress and contributed to better nutrient absorption. The study by G. Korska *et al.* (2024) showed the effectiveness of *Azolla* as an environmentally appropriate feed additive. Although no alternative protein sources were used in the study, probiotic intervention provided a similar improvement in protein uptake, which helped to reduce the metabolic load.

The decrease in the frequency of ARGs detection in soils after the application of biotechnology was consistent with the results of Y. Zhu *et al.* (2013), where a high density of ARGs was found in farms with frequent use of antibiotics. As part of the study, a decrease in prevalence of *tetM*, *sul1*, *blaTEM* was recorded, which indicates a reduced risk of horizontal transfer of resistance genes. R. Puchałka *et al.* (2023) demonstrated the ability of probiotic feed additives to reduce the amount of ARGs in soils fertilised with animal waste. Similarly, this study recorded a decrease in ARGs, which confirmed the effect of biological detoxification of the agroecosystem. The findings of P.P. Nagy *et al.* (2022), which emphasised the importance of selection for immune resistance, were consistent with the data obtained on

reducing the pharmacological load. The use of genetically based selection reduced the frequency of therapeutic interventions without compromising weight gain. G. Wu & F.W. Bazer (2019) showed that the use of biotechnologies contributes to improved feed conversion and economic returns. The results obtained as part of the study were fully consistent with this finding, as there was an improvement in growth rates, a reduction in veterinary drug costs, and an increase in profitability.

F. Khajali & F. Rafiei (2024) have shown that controlling antinutrients in animal diets is crucial for metabolic stability. A similar effect in the study was achieved by using probiotics, which improved digestibility and had a positive effect on haematological parameters. W.M. Rauw *et al.* (2020) substantiated the need to integrate biotechnologies into the system of feeding, breeding, and management to achieve sustainability of animal husbandry. The overall results confirmed the effectiveness of a combination of probiotic, breeding, and vaccination interventions to reduce antibiotic costs and environmental burden. In the study by W. Huangfu *et al.* (2024), *in vitro* and *in vivo* models confirmed the effectiveness of fermentation technologies in reducing nitrogen losses. In the current study, a similar effect was achieved by stabilising the gut microbiota, which helped to improve protein digestion and reduce the synthesis of volatile compounds. The approach to green bioeconomics described by U. Jørgensen *et al.* (2022) provided for the integration of biotechnological innovations to improve the environmental responsibility of agricultural production. The data obtained as part of the study were consistent with this concept, since a decrease in residual antibiotics, a decrease in ammonia emissions, and an increase in the activity of soil microorganisms were recorded. As a result, the study showed that the introduction of biotechnological solutions in pig breeding provides a multi-vector effect: increases the productivity and immune stability of animals, reduces dependence on antibiotic therapy, improves the environmental characteristics of production and

economic feasibility. The systematic use of probiotics, breeding, and vaccination allows not only to meet the requirements of biosafety and sustainable development, but also to form a complete circular model of agricultural production with an emphasis on preserving the health of animals, soils, and consumers.

Conclusions

The study found that all three biotechnological tools under study – probiotics, vaccines, and breeding – contributed to improving health, productivity, and environmental sustainability in industrial pig farming. The use of probiotic drugs based on *Lactobacillus spp.*, *Bacillus subtilis*, and *Enterococcus faecium* provided an increase in the average daily weight gain of pigs by 80 g (from 670 g/day in the control group to 750 g/day in the experimental group), a decrease in feed conversion from 2.9 to 2.6 kg/kg of weight gain, a decrease in morbidity from 34.1% to 15.4%, and an increase in the level of IgG immunoglobulins in serum from 12.3 to 16.1 mg/ml.

Vaccination against PRRSV, *Mycoplasma hyopneumoniae*, and *Lawsonia intracellularis* increased the safety of livestock from 92.5% to 97% and reduced the frequency of antibiotic therapy from 87 to 52 cases per 100 animals. In the vaccine group, there was also a decrease in antibiotic residues in meat to 5.6% of samples with an excess of MPR, compared with 12.5% in the control group. The highest efficiency was demonstrated by breeding: body weight gain reached 810 g/day, feed conversion decreased to 2.5, the morbidity rate decreased to 12.7%, and the safety of livestock increased to 96.1%. The content of antibiotic residues in manure decreased to 13.7 mg/kg (against 38.6 mg/kg in the control group), and ammonia emissions from manure – to 85.3 g/day/animal.

The cost-effectiveness of the analysed solutions was confirmed by ROI calculations: 287.5% for probiotics, 50% for vaccination, and 123% for breeding. The payback period for probiotics amounted to one fattening cycle, and for breeding – 3-5 years. At the ecological level, it was

found that the use of manure with a reduced content of residual antibiotics contributed to an increase in the activity of soil enzymes (urease, phosphatase), stabilisation of soil pH, a decrease in the content of antibiotic resistance genes, and a reduction in the risk of eutrophication of water bodies. Limitations of the study included regional sampling, a limited follow-up period for breeding effects, and the dependence of probiotic effectiveness on feeding conditions. Further research should be directed to the evaluation of combined biotechnological solutions in the long cycle, and

the study of the relationship between the microbiome of animals and soils in the context of biosafety of agricultural systems.

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Conflict of Interest

None.

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Ефективність біотехнологічних інструментів у покращенні здоров'я поголів'я свиней та екологізації виробництва

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Анотація. У контексті глобального зростання стійкості патогенів до антибіотиків та екологічних викликів у тваринництві постає потреба у впровадженні ефективних біотехнологічних рішень. Метою дослідження було встановити ефективність застосування біотехнологічних інструментів у

свинарстві з огляду на їх вплив на здоров'я, продуктивність тварин і екологічну стійкість виробництва. Експеримент проводився у період з серпня по грудень 2024 року на двох свинокомплексах Кіровоградської та Вінницької областей із загальною вибіркою 240 свиней, поділених на контрольну та три дослідні групи. У дослідженні вивчався вплив пробіотиків, вакцин та генетичної селекції на приріст живої маси, захворюваність, гематологічні й імунологічні показники, частоту використання антибіотиків, екологічні параметри та економічну рентабельність. У результаті застосування пробіотиків середньодобовий приріст збільшився на 80 г, захворюваність знизилася вдвічі, а рівень імуноглобулінів зріс на 30 %. Вакцинація забезпечила підвищення збереженості поголів'я на 4,3 %, скорочення потреби в антибіотикотерапії на 40 % і зменшення залишкових антибіотиків у продукції. Генетичний добір дав найвищі результати – зростання приросту до 810 г на добу, найнижчі показники захворюваності, аміачних викидів та залишків антибіотиків. Економічний аналіз підтвердив ефективність усіх трьох підходів: рівень рентабельності інвестицій становив 287,5 % для пробіотиків, 50 % для вакцинації та 123 % для селекції. Практична ефективність результатів підтверджена прикладами впровадження аналогічних підходів у Данії, Польщі та Бразилії, де було досягнуто зменшення використання антибіотиків без шкоди для продуктивності. Дослідження також засвідчило позитивний вплив на мікробну активність ґрунтів, зменшення кислотного навантаження та ризиків евтрофікації. Результати можуть бути використані у практиці свинарських господарств для підвищення продуктивності тварин, зменшення залежності від антибіотиків і зниження екологічного навантаження на агроєкосистеми

Ключові слова: пробіотики; вакцинація; генетика; тваринництво; ветеринарія

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