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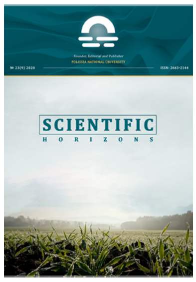

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Isolation and study of a strain of lumpy skin disease virus of cattle

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Abstract. The identification of new virus strains will prevent the development of outbreaks thanks to the development and use of vaccines. The aim of the work was to isolate and sequence the genome of lumpy skin disease virus from an epizootic in the Kostanay region. The genetic material of virions was identified by polymerase chain reaction (PCR); viral antigens and antibodies to them have been determined by enzyme-linked immunosorbent assay (ELISA) or diffusion test methods; to accumulate the material, the virus has been cultivated in lamb testicle monoclonal cell; new generation sequencing has been performed using MiSeq System and FastQ software; strain affiliation has been established by the BLASTN-alignment method. Specific

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amplicons of the virus with a length of 347 bp. were detected in skin samples but not found in blood. Antigens in 1:5-1:320 dilutions were identified in skin material and 2-3 passages of monoclonals; less antigenic activity was found in the blood in a 1:2 dilution. In response to the pathogen, specific immunoglobulins were synthesized in the serum of 67% of the studied animals and were detected in dilutions of 1:100-1:400. The viral material was accumulated in monoclonals and isolated in a sucrose gradient. The whole-genome sequence of the obtained material confirmed the isolation of a new strain of nodular dermatitis virus with a percentage of similarity to the closest homologues of 99.66%. The strain was named *Dermatitis nodularis bovum/2018/Kostanay/KZ*; the sequence has been submitted to GeneBank, and the object has been deposited in the Collection of Microorganisms under accession number M-9-21/D. The obtained information can be used to prevent the spread of foci of cattle infection

Keywords: biomaterial; sequencing; assembly; polymerase chain reaction; virus; deoxyribonucleic acid

INTRODUCTION

Due to the globalisation of economic relations in many countries of the world, which rapidly includes the Republic of Kazakhstan, the epizootic situation in their territories is becoming increasingly vulnerable to the penetration and spread of dangerous and especially dangerous infectious diseases, including diseases of farm animals. Taking into account the wide dissemination of the lumpy skin disease in the direction from the south to the northeast, the migration of this pathogen can be dangerous for Kazakhstan territory. Epizootic outbreaks harm not only the health of animals, but also the economic situation of the region. Therefore, it is important to identify pathogens in time to prevent their spread and implement a vaccination program.

Infectious lumpy skin disease of cattle is an acute particularly dangerous infectious viral disease, accompanied by fever, nodular lesions and necrosis of the skin, swelling of subcutaneous connective tissue and organs, damage to the eyes, mucous membranes of the respiratory and digestive tracts (Issimov *et al.*, 2022). The causative agent is a virus containing deoxyribonucleic acid belonging to the *Poxviridae* family, the genus *Capripoxvirus*. Currently, three varieties are known – *Orphlins*, *Allerton* and *Neethling* – distinguished by the degree of virulence, immunogenicity and cytopathogenic action (Bayantassova *et al.*, 2023). It has been proven that only the virus of the third group causes infectious lumpy skin disease. It has an antigenic relationship with the sheep pox virus. It reproduces in chicken embryos, in primary cell cultures of calves, lambs, rabbits (Whittle *et al.*, 2023; World Animal Health Information System, n.d.). In natural conditions, cattle are most susceptible to this disease. At a temperature of 4°C, the virus remains active for 6 months. It spreads at a speed of 70 to 100 km/day (Namazi & Khodakaram Tafti, 2021).

Lumpy skin disease virus (LSDV) is an African endemic that had spread to Kazakhstan, Serbia, Greece, Bulgaria, Thailand, India and China. As indicated by P.A. Desingu *et al.* (2023), the main risk factor associated with LSDV is its microevolution: the genome constantly undergoes frameshift and nonsense mutations that cause new disease outbreaks in different regions. The wild-type strain has been found only in Africa, as well as only recombinant viruses have been found in Asia. M.B. Orynbayev *et al.* (2021) investigated an epizootic of nodular dermatitis in the region of Atyrau (Makash), Kazakhstan. The first cases of cow disease were found in 2016 (Vygovska *et al.*, 2023). The researchers managed to isolate the virus from animal material, cultivate it in a lamb testicle line monolayer, identify the provoker by polymerase chain reaction (PCR) (receiving a specific amplicon with a length of 192 bp) and sequence the genome of the pathogen.

Amplicons of the virus were detected in all samples of blood, spleen, lymph nodes and lungs of dead animals, as well as in the material of vectors – ticks and ticks. The strain was archived as *Nodulares/Dermatitis/Atyrau-2016*. Based on this strain, O. Chervyakova *et al.* (2022) constructed an attenuated vaccine by knocking out four genes (encoding interleukin-like protein, interferon-gamma receptor, thymidine kinase, and LSDV142). The modified virus was genetically stable for ten passages in monoclonals; in high doses, the one had not caused the disease, but stimulated the synthesis of virus-specific immunoglobulins in vaccinated animals.

As indicated by F. Vandenbussche *et al.* (2022), several vaccinia-like recombinant LSDVs were identified in Kazakhstan and the neighbouring region of China in 2017-2019. Previously, mass vaccination of animals against nodular dermatitis (Lumpivax) was carried out in Kazakhstan (Kropyvka *et al.*, 2024). LSDV was transmitted between animals. Vaccination caused the appearance of new strains, which are conventionally divided into four groups and differ in a number of genetic recombination's (126-146 exchanges). Therefore, it is important to search and identify such recombinants.

The tasks of the work were isolation and identification of the nodular dermatitis virus strain from the focus of infection in the Kostanay region.

MATERIALS AND METHODS

The work was performed on the basis of on the basis of the RSE Research Institute of Biological Safety Problems of the Scientific Committee of the Ministry of Education and Science of the Republic of Kazakhstan. Biomaterials from sick and dead animals from the outbreak of epizootics were collected in September 2018 in the Kostanay region. Treatment of animals took place in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986). To detect the lumpy skin disease virus in cattle, 4 samples of affected skin parts and 4 samples of animal blood were tested in real time by PCR with a pair of specific viral primers LSDV-2-f and LSDV-2-r and *Taq* polymerase. Amplification was carried out in 35 cycles (94°C 3 min-((94°C 20 sec-50°C 20 sec-72°C 40 sec)×35)-72°C 7 min-4°C storage) (Zeedan *et al*., 2019).

To determine the activity and specificity of the antigen, the method of enzyme-linked immunosorbent assay (ELISA) was used. It was taken 8 samples of animal skin and blood as well as 10 samples of virus passages. For this purpose, polystyrene flat-bottomed dies were used, which were previously sensitised with a specific immunoglobulin of the lumpy skin disease virus of cattle. Control antigens (antigen C, antigen H) were used to perform enzyme-linked immunosorbent assay. The test biomaterials were diluted in a start ratio of 1:5, and culture samples in a start ratio of 1:2. Then, control antigens C, H and test samples of 0.1 cm^3 were introduced into the wells. The plate with antigens was incubated (17 h 4°C), washed, contacted with conjugate (1 h 37°C), washed again and then the substrate solution was introduced (30-60 min, room temperature). The reaction results were taken into account visually or on a photometer at a wavelength of 405 nm.

To detect antibodies to the lumpy skin disease virus of cattle the ELISA was performed on a tablet with a recombinant protein of the nodular dermatitis virus of cattle. The 9 samples of blood serum were taken to analysis. Control antibodies (CC, CH) and test serums were introduced into the wells (start dilution 1:100) and incubated for (1 h, 37°C), washed, contacted with analytic serum (1 h, 37°C), washed, dried and then reacted with substrate solution. The photometric reading was performed at 405 nm (Liang *et al*., 2022). To detect the antigen of this virus or antibodies to virus after disease the diffuse precipitation reaction in 1% agar gel Difco has been performed (27 samples; blood, skin and cell cultures). Control samples with C, H, CC and CH antigens were used in the working titer, dilutions were prepared in saline solution. Wells were made in the agar using a star-shaped stencil with one central tube and 6 peripheral tubes. The central holes were filled with control samples, and the side holes – with test. The reaction kept in wet chamber for 24-48 hours at room temperature (22°C) (Colorio *et al*., 2021).

To cultivate the virus in monolayer cells, the cell cultures of lamb tests and green monkey kidneys (*Vero*) have been contaminated with skin isolates (4 samples). The cells were pretreated with benzylpenicillin and streptomycin (18 h, 4°C), infected with 0.2 ml of samples (1.5 h, 37°C), washed and grown in inactivated 2% serum (7 d, 37°C). Three passages have been performed. The test tubes were examined daily under a microscope to assess the virus (Vidanović *et al*., 2016). Samples were then taken from each vial to test for the presence of lumpy skin disease virus in a polymerase chain reaction test system (Agianniotaki *et al*., 2017). To purify the virus from cell culture, the sedimentation (10737 g, 180 min) in sucrose gradient (30%, 45%, 60%) has been performed.

Genome-wide sequencing of the virus isolate was carried out by high-performance sequencing of the next generation and subsequent genome assembly. The measure of deoxyribonucleic acid concentration was performed by spectrophotometric and fluorimetric methods (NanoDrop1000 and Qubit®.2.0 Fluorometer; Qubit dsDNA HS Assay Kits) (Nakayama *et al*., 2016). The preparation of the deoxyribonucleic acid library was carried out according to Nextera DNA Flex Library Prep Kit. The sample of deoxyribonucleic acid was subjected to tagmentation, amplification with labelling and normalisation of the library concentration (Illumina Proprietary, 2020). Sequencing was carried out using a set of reagents MiSeq Reagent Kitv3 (600 cycle), which allows sequencing 20 million readings with a length equal to 300 bp with a paired-end (Namazi & Khodakaram Tafti, 2021). The analysis of the obtained data was carried out using FastQC programmes Trimming of low-quality bases was carried out in SeqTK v1.3-r106 and Sickle v1.33. Genome assembly was carried out in SPAdes 3.13.2 programme.

Basic Local Alignment Search Tool (BLAST) software (both the web service and the local version) was used for comparative identification of the resulting contigs. The search was conducted relative to the database "nt" (nucleotide) with standard settings.

RESULTS

To confirm the identification of nodular dermatitis virus, an amplification reaction was performed with specific primers from skin and blood samples. The results PCR-screening are shown in Figure 1.

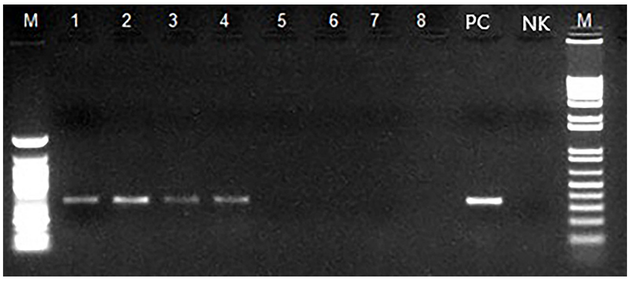

Figure 1. *Results of the polymerase chain reaction for the detection of deoxyribonucleic acid of the lumpy skin disease virus of cattle*
Note: *M – marker; from 1 to 4 affected parts of animal skin; from 5 to 8 – selected animal blood; PC – positive control (347 bp); NC – negative control*
Source: *compiled by the authors*

Four samples of affected skin taken from diseased and fallen animals were found to contain lumpy skin disease virus in this test, while no virus was detected in blood. The amplicon size of the tested samples coincided with the amplicon size of the virus of the positive control sample (347 bp). Afterwards, to determine the activity and specificity of the nodular dermatitis virus antigen, an ELISA with control antigens C and H has been performed. 18 samples of biomaterials were tested, as indicated in Table 1.

***Table 1**. Test samples*

No.	Name of the biomaterial	No.	Name of the biomaterial
1	Concentrated VCS test after the 3rd passage level on lamb testicles (Affected part of the skin, cow, black colour)	10	VCS after the 1st passage level (Affected part of the skin, heifer)
2	20% suspension of the affected part of the skin, heifer	11	VCS after the 1st passage level (Affected part of the skin, cow, red colour)
3	20% suspension of the affected part of the skin, cow, suit colour	12	VCS after the 1st passage level (Affected part of the skin, cow, white colour)
4	20% suspension of the affected part of the skin, cow, white colour	13	VCS after the 1st passage level (Affected part of the skin, cow, black colour)
5	20% suspension of the affected part of the skin, cow, black colour	14	VCS after the 2nd passage level (Affected part of the skin, heifer)
6	Blood from a cow, white colour, No. 1	15	VCS after the 2nd passage level (Affected part of the skin, cow, red colour)
7	Blood from a cow, white head, No. 2	16	VCS after the 2nd passage level (Affected part of the skin, cow, white colour)
8	Blood from a cow, white muzzle	17	VCS after the 2nd passage level (Affected part of the skin, cow, black colour)
9	Blood from a bull, white colour, No. 1	18	VCS after the 3rd passage level (Affected part of the skin, cow, black colour)

***Note:** VCS is a virus-containing suspension*
***Source:** compiled by the authors*

The results of the enzyme-linked immunosorbent assay samples for detecting the antigen of the lumpy skin disease virus of cattle are shown in Figures 2, 3. During visual evaluation, staining should be observed in wells with antigen C, weak staining is allowed in wells with antigen H, decreasing with the dilution of the antigen. There should be no staining in wells with a solution for enzyme-linked immunosorbent assay (horizontal line A). On the photometer, the reaction is indicated by the difference in optical density between the test wells and the antigen H. The result is considered positive if the optical density in the test wells is 2 or more times higher than the density in the corresponding wells of the control antigen H in two or more subsequent dilutions.

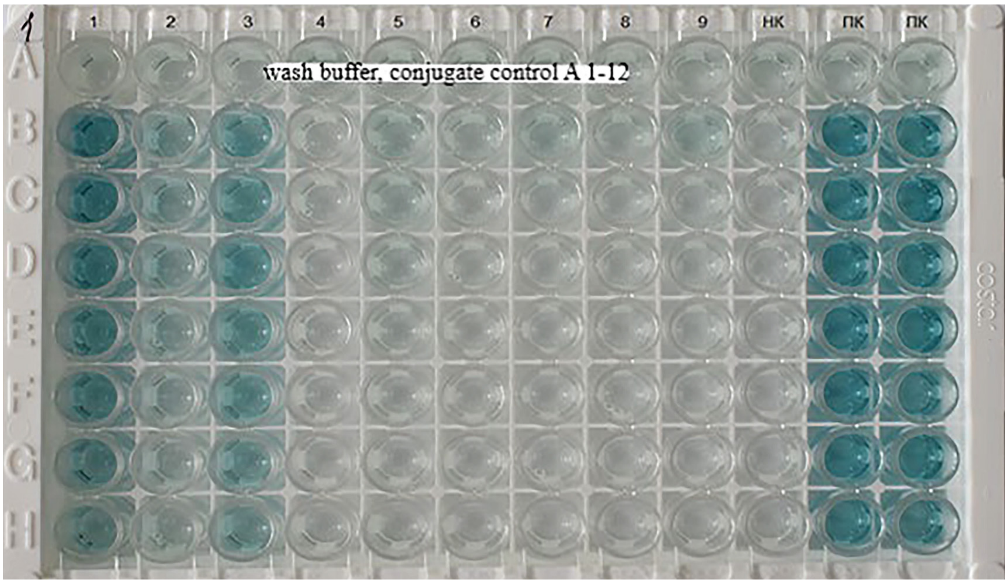

***Figure 2**. The results of tests to detect the antigen of the lumpy skin disease virus of cattle*
***Source:** compiled by the authors*

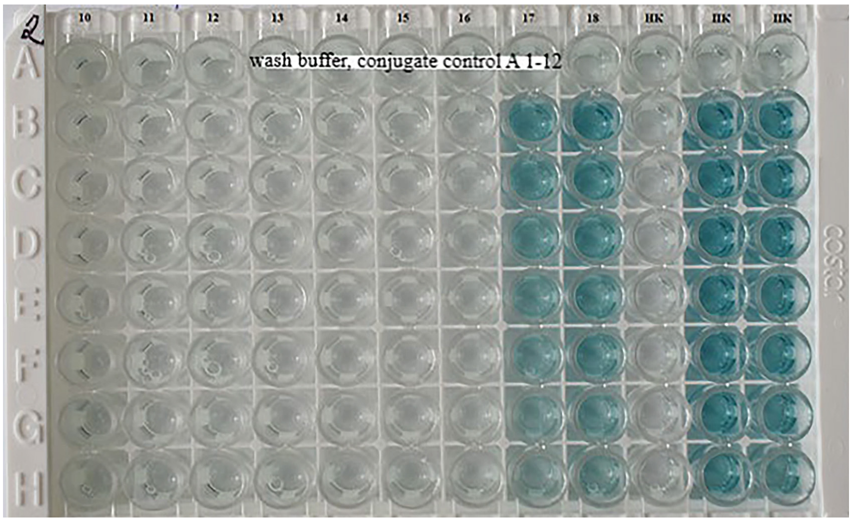

Figure 3. *The results of tests to detect the antigen of the lumpy skin disease virus of cattle*
Source: *compiled by the authors*

As a result, antigens of the nodular dermatitis virus of cattle with an activity of 1:5-1:320 were detected in the initial materials No. 2, 3 and 5, and in the blood No. 7 and 9 with an activity of 1:2. After carrying out viral isolation in monolayers of lamb testicle cell cultures, the antigen of the lumpy skin disease virus of cattle was detected only in one sample No. 1, 17 and 18 with an activity of 1:128-1:320 after the second and third passages of skin material from black cow. In other samples during cultivation, it was not possible to isolate this virus. An ELISA was also performed to detect protective antibodies to the lumpy skin disease virus of cattle after contact with provoker. 9 samples of animal blood serum were tested for enzyme-linked immunosorbent assay, as indicated in Table 2. Figure 4 shows the results of the samples.

Table 2. *The studied samples for the detection of antibodies of the lumpy skin disease virus of cattle*

No.	Name of the biomaterial	Unit of measurement	Volume	No.	Name of the biomaterial	Units of measurement	Volume
1	Blood serum No. 1	ml	0	6	Blood serum No. 6	ml	7.21
2	Blood serum No. 2	ml	3.55	7	Blood serum No. 7	ml	1.8
3	Blood serum No. 3	ml	4.8	8	Blood serum No. 8	ml	6.7
4	Blood serum No. 4	ml	6.3	9	Blood serum No. 9	ml	3.4
5	Blood serum No. 5	ml	2.45	10	Blood serum No. 10	ml	4.8

Source: *compiled by the authors*

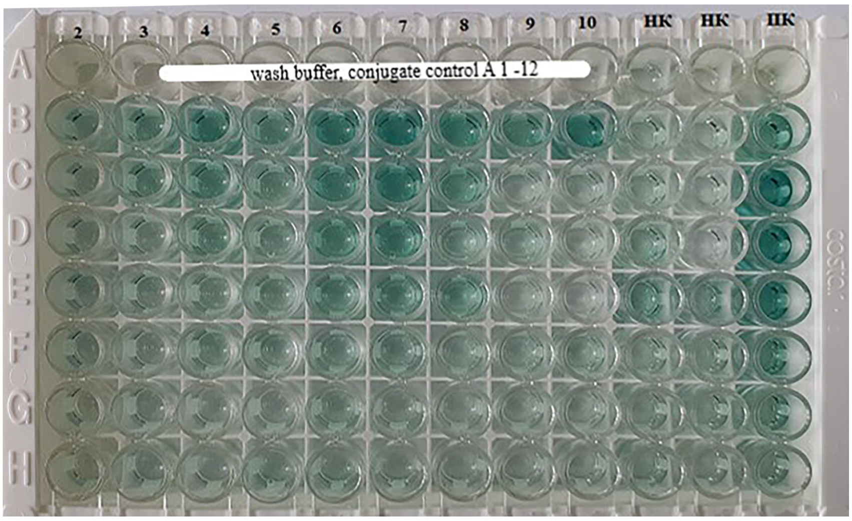

Figure 4. *Results of tests for detection of antibodies of the lumpy skin disease virus of cattle*
Source: *compiled by the authors*

During visual assessment, staining should be observed in wells with control antibodies CC, weak staining is allowed in wells with CH or there should be no staining. There should be no staining in wells with buffer solution (horizontal line A). The reaction is considered positive if staining is observed in wells with a test sample at a dilution of 1:400, with weak staining in similar dilutions in wells with the CH antigen. On the photometer, the reaction is taken into account by the difference in optical density in the wells with the tested samples and the CH antigen. The result is considered positive if the optical density in the wells with the tested samples is 2 or more times higher than in the corresponding wells with the control CH antigen in two or more subsequent dilutions. In samples No. 3, 5, 6, 7, 8, 9 antibodies to the lumpy skin disease virus of cattle with an activity of 1:100-1:400 were detected (67%). To set up the diffuse precipitation reaction, for antigens and antibodies identification, 18 samples of virus-containing suspensions and 9 samples of animal blood serum were tested, the results are presented in Table 3.

***Table 3**. Results of the diffuse precipitation reaction*

No.	Name of samples	Results	No.	Name of samples	Results	No.	Name of samples	Results
1	Concentrated VCS sample after the 3rd passage level (Affected part of the skin, cow, black colour)	+	10	VCS after the 1st passage level (Affected part of the skin, heifer)	-	19	Blood serum No. 2	-
2	20% suspension of the affected part of the skin, heifer	-	11	VCS after the 1st passage level (Affected part of the skin, cow, red colour)	-	20	Blood serum No. 3	-
3	20% suspension of the affected part of the skin, cow, suit colour	+	12	VCS after the 1st passage level (Affected part of the skin, cow, white colour)	-	21	Blood serum No. 4	+
4	20% suspension of the affected part of the skin, cow, white colour	+	13	VCS after the 1st passage level (Affected part of the skin, cow, black colour)	-	22	Blood serum No. 5	-
5	20% suspension of the affected part of the skin, cow, black colour	-	14	VCS after the 2nd passage level (Affected part of the skin, heifer)	-	23	Blood serum No. 6	-
6	Blood from a cow, white colour, No. 1	-	15	VCS after the 2nd passage level (Affected part of the skin, cow, red colour)	-	24	Blood serum No. 7	+
7	Blood from a cow, white head, No. 2	-	16	VCS after the 2nd passage level (Affected part of the skin, cow, white colour)	-	25	Blood serum No. 8	-
8	Blood from a cow, white muzzle	-	17	VCS after the 2nd passage level (Affected part of the skin, cow, black colour)	-	26	Blood serum No. 9	-
9	Blood from a bull, white colour, No. 1	-	18	VCS after the 3rd passage level (Affected part of the skin, cow, black colour)	-	27	Blood serum No. 10	-

***Note:** VCS is a virus-containing suspension*
***Source:** compiled by the authors*

The reaction results were taken into account with control antigens or antibodies, in which there must necessarily be precipitation lines between C and CC in the absence of them between C and CH or CC antibodies and antigen H. The reaction is considered positive if there are precipitation lines identical to the lines in the control between the wells with CC or C antigen and the pathological materials or sera, respectively, after the above time, there are precipitation lines in character identical to the lines in the control. In the absence of precipitation lines between these components, the reaction is considered negative. As a result, antigens of the lumpy skin disease virus of cattle were detected in samples No. 1, 3, 4 in the diffuse precipitation reaction, and antibodies of the same virus were detected in two samples with blood serum No. 21 and 24. The virus has been cultivated in animal cells monolayer. Experiments were carried out to isolate the lumpy skin disease virus of cattle from four samples of the affected parts of the skin of diseased and sick animals in monolayers of primary trypsinized cultures of lamb testicle cells and transplanted lines of green monkey kidney cell cultures (*Vero*). The results of the first passage for virus release from skin samples are shown in Figure 5 and Table 4. On day 7, in samples No. 1 and 4, changes were detected in the monolayers of lamb testicle cell cultures. Obviously, according to the PCR-signal, the titer of virus in sample 1 (from heifer cow) was lower, than in sample 4 (from black cow). Then the second passage was carried out. Its results are presented in Figure 6 and Table 5.

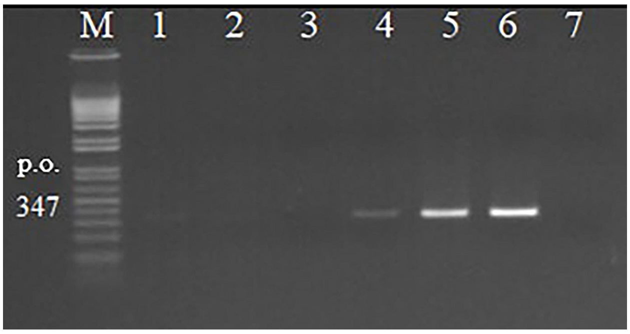

Figure 5*. Results of the first passage for virus release from skin samples*
Note: *M – marker; No. 1, 2, 3, 4 – test samples, 5 and 6 positive controls; 7 – negative control*
Source: *compiled by the authors*

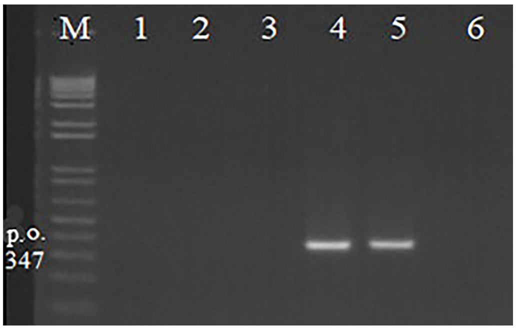

Figure 6*. Results of the second passage for virus release from skin samples*
Note: *M – marker; No.1, 2, 3, 4 – test samples, 5 and 6 positive controls*
Source: *compiled by the authors*

Table 4*. Name of samples and results*

No.	Name of samples	Results
1	VCS after the 1st passage level (Affected part of the skin, heifer)	±
2	VCS after the 1st passage level (Affected part of the skin, cow, red colour)	±
3	VCS after the 1stt passage level (Affected part of the skin, cow, white colour)	-
4	VCS after the 1st passage level (Affected part of the skin, cow, black colour)	+
5	Positive control	+
6	Positive control	+
7	Negative control	-

Note: *VCS is a virus-containing suspension*
Source: *compiled by the authors*

Table 5*. Name of samples and results*

No.	Name of samples	Results
1	VCS after the 2nd passage level (Affected part of the skin, heifer)	-
2	VCS after the 2nd passage level (Affected part of the skin, cow, red colour)	-
3	VCS after the 2nd passage level (Affected part of the skin, cow, white colour)	-
4	VCS after the 2nd passage level (Affected part of the skin, cow, black colour)	+
5	Positive control	+
6	Negative control	-

Note: *VCS is a virus-containing suspension*
Source: *compiled by the authors*

After the second passage level, the virus was detected only in one sample No. 4, the suspension, from the affected part of the skin of a black cow, contains the lumpy skin disease virus. The PCR-signal was on the level of positive control; this fact proves the active reproduction of virions in host testicular cells. A third passage was then carried out to confirm the presence of this virus. The polymerase chain reaction test was positive. Thus, it was possible to isolate a lumpy skin disease virus isolate from the biomaterial, which was named *Dermatitis nodularis bovum/2018/Kostanay/KZ*. Figure 7 shows an isolate of this virus.

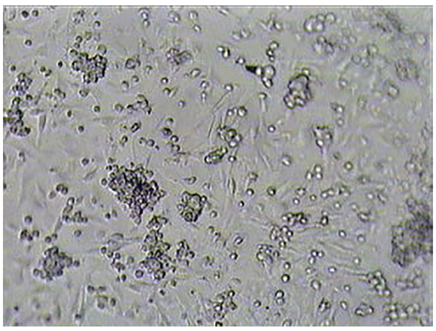

Figure 7*. Isolate Dermatitis nodularis bovum/2018/Kostanay/KZ of the lumpy skin disease virus of cattle in a monolayer of lamb testicle cell culture*
Source: *compiled by the authors*

The cultivation of resulting strain in lamb testicle cells caused destructive changes in the monolayer in the form of shiny light-refracting cells with a round shape, with clear outlines of the shells of the nucleus and cytoplasm. Virus isolation from biomaterials in monolayers of *Vero* cell cultures has not been successful. To optimize the way of virions purification, the samples were taken from each sucrose layer (at the procedure of extraction with different sucrose amount) and examined using a polymerase chain reaction to detect deoxyribonucleic acid of the lumpy skin disease virus of cattle. The results are shown in Figure 8.

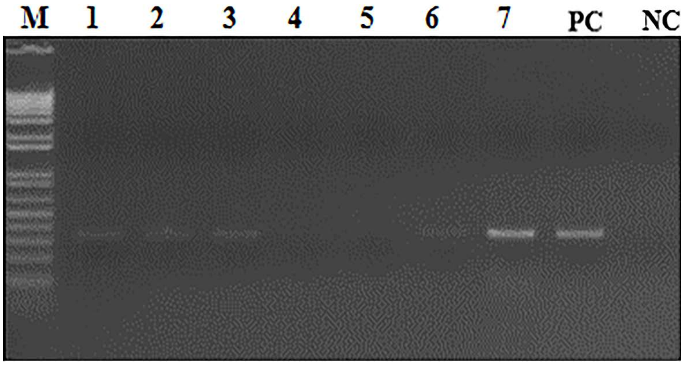

Figure 8. *Results of polymerase chain reaction after purification of lumpy skin disease virus*

Note: *M – marker; method 1 (cleaning in 30% sucrose at 10737 g for 180 min): No. 1 – sediment; No. 2 – supernatant; method 2 (clean in 30, 45 and 60% sucrose at 10737 g for 180 min): No. 3 – protein of over 30% sucrose; No. 4 – protein between 30 and 45% sucrose; No. 5 – protein between 45 and 60% sucrose; No. 6 – precipitate; No. 7 – the original virus isolate Dermatitis nodularis bovum/2018/Kostanai/KZ virus nodular dermatitis of cattle; PC – positive control; NC – negative control*

Source: *compiled by the authors*

The product of deoxyribonucleic acid isolate *Dermatitis nodularis bovum/2018/Kostanay/KZ* of this virus was detected in samples No. 1, 2, 3, 6, 7. As can be seen from Figure 8, the optimal way to clean virus particles is applying of 30% sucrose solution; at the same time, virions are equally distributed in the supernatant, sediment, and protein fraction above the sucrose solution. This pure material has been taken for sequence. Upon completion of the sequencing process, the following launch metrics were obtained, shown in Figure 9.

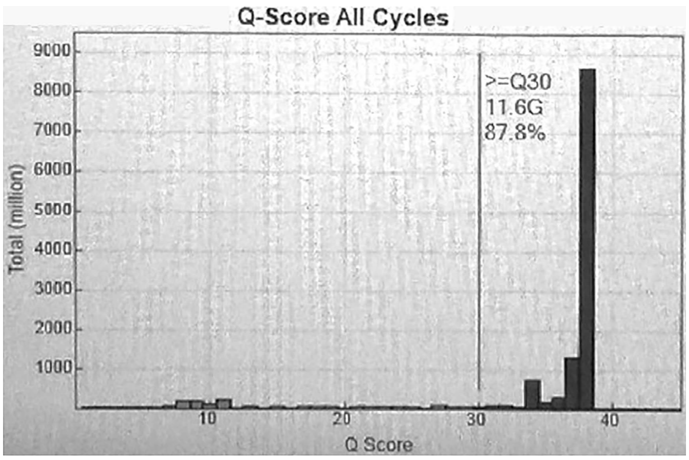

Figure 9. *Launch metrics at the end of sequencing stage*

Note: *clustering density: 1143 thousand/mm^2; number of clusters that passed the Q30 threshold: 94.4%; expected output: 16236 megabases*

Source: *compiled by the authors*

According to the metrics, the clustering density is close to the optimal value (1200-1400 thousand/mm^2), the expected yield is also close to the optimal value stated by the model manufacturer (19 million bases per cycle), the threshold Q30 indicates that 87.8% of the obtained bases have a 99.9 % probability of reliability. The GC content of the sequence was 41%. According to the comparison of BLAST analysis by the nucleotide database, this contig is identified as a LSDV with an identity percentage equal to 99.36% (Fig. 10).

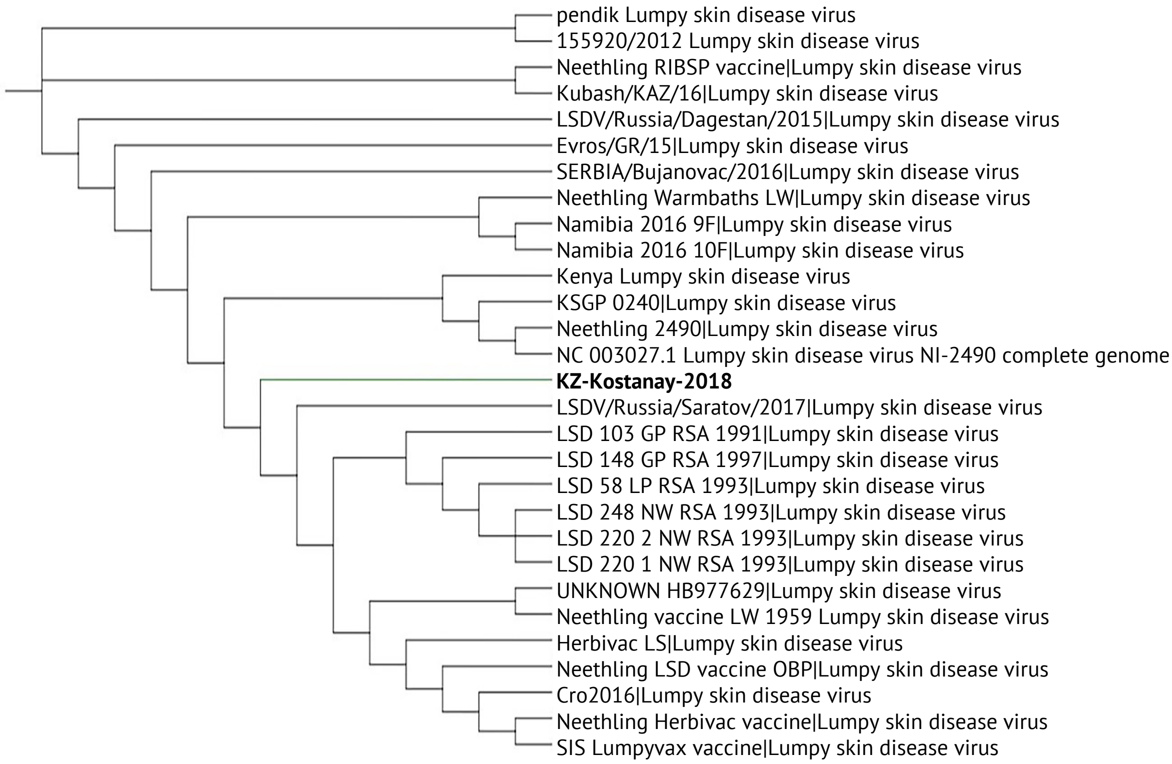

Figure 10. *Phylogenetic tree as result of BLASTN alignment*

Note: *tree scale – 0.001*
Source: *compiled by the authors*

The closest homologues of the identified nodular dermatitis virus were Kenya LSDV, KSGP 0240, NI-2490 and strains of the LSD RSA clade. The remaining assembly contigs were less than 2800 bp and according to *BLAST* analysis were both *Ovis aries* genome fragments with 99.66% identity and smaller (<1000 bp) genome fragments of nodular dermatitis virus.

DISCUSSION

The result of the work was the isolation and sequencing of the complete genome of a new strain of bovine nodular dermatitis virus derived from an epizootic in the Kostanay region. The obtained sequence has been included to the publicly available GeneBank bioinformatics data base. The strain was named *Dermatitis nodularis bovum/2018/Kostanay/KZ* M-9-21/D and was deposited in the Microorganisms Collection of the Scientific Research Institute of Biological Safety Problems.

For identification, the new generation high-throughput sequencing method with genome "collection" by Illumina's FastQC software has been performed. The advantage of this approach is the generation of hundreds of millions of sequences during one cycle. The FastQC programme makes it possible to evaluate the sequencing run process based on the generated FastQ files of individual samples for such multiple characteristics as the number and length of reads, GC% the degree of duplication, base quality, overrepresented sequences and adapters. Perhaps, existing next-generation sequencing platforms have a number of disadvantages. These include errors and assumptions in the data obtained. Such disadvantages for the Illumina platform include a declining "trend" in the base quality assessment chart and a larger range of reading lengths. Therefore, according to the generally accepted methodology for analysing next-generation sequencing data, the readings were "trimmed" to a state satisfying the reliability of accuracy (Q30). Trimming of low-quality bases was carried out in SeqTK v1.3-r106 and Sickle v1.33. The evaluation of the processed data was also carried out in FastQC. The genome was assembled in the SPAdes v3.13.2 programme with a k-measure size equal to 127 (-k 127) and a more precise assembly function (-careful). The use of a paired-end sequencing reagent with a reading length equal to 300 bp allows for correct assembly in regions of the genome with repeats less than the reading length (i.e., 300 bp) (Badhy *et al.*, 2021).

Two cell lines were used for monolayer cultivation: testicular lamb line and monkey kidney line *Vero*. If it was possible to pass three passages in the cells of the first line successfully, it was not possible to isolate the virus from the second line. Instead, N. Kumar *et al.* (2021) describe a successful method for isolating a new strain of nodular dermatitis virus from the *Vero* line. According to the phylogenetic analysis, the identified strain was a close homolog of LSDV Kenya, like in the current experiment. The researchers managed to conduct four passages without a cytotoxic effect and obtain a high titer of viral particles. The peak of viral DNA synthesis occurred at 96 hours after infection, and the peak of virion assembly – at 120 hours. For this performance, *Vero* monolayer cultivation was adapted by plating of 500 μl of inoculum after culturing the virus in goat kidney cell culture.

Instead, S. Pervin *et al.* (2023) noted the phenomenon of cytopathic effect of LSDV on the *Vero* line. Despite this, they were able to isolate the virus from pathogen-free fertilized chicken eggs in *Vero* culture. To do this, adaptation of the virus was carried out in a minimal environment in an atmosphere with 5% carbon dioxide. The cytopathic effect had been achieved in two-three days after infection, so the threshold of 75% cytolysis has been fixed microscopically, so cultivation has been stopped after 5-6 passages. Perhaps, in the future, this approach will allow the isolation of other viruses with a higher titer. Identification has been carried out by the gene encoding a binding protein with an amplicon length of 192 bp. Phylogenetic analysis was performed by the same amplicons after sequencing and uploading the sequence to the GeneBank database. Such marker is species-specific and amplifies quickly; in addition, its sequencing is performed faster and at lower costs than whole-genome sequencing. Therefore, in the future, it can be used to identify LSDV isolated from other epizootics. The identified strain (Bangladesh) had a high degree of similarity (more than 99%) with LSDV strains from outbreaks in Kenya, Serbia, Greece and Kazakhstan.

It was not possible to detect virus antigens in all analysed skin and blood samples of sick animals. According to the histopathological study of D.M. Amin *et al.* (2021), the disease was manifested by different skin lesions. In particular, in some cases, the proliferation of the epidermis with the formation of a mesh border has been observed; in others, the lesion was accompanied by a degeneration and necrotic process of the epidermis with exfoliation of cells and the formation of deep ulcers. Part of the ulcers has been characterized by immune granulomatosis: lymphocytes, macrophages, fibrocytes and plasma cells accumulated inside. These cells were also found in muscles and around blood vessels. Viral particles were localized in the cytoplasm of the basal layer of the epithelium and hair follicles. In the mentioned experiment, it was also not possible to confirm the virus in all samples: viral particles were identified by PCR in 22 samples out of 73. It is possible that the absence of viral antigens in some samples caused by either too low titer of the virus, or a late stage of the disease, or a non-specific selection of material that did not capture a sufficient amount of basal epithelium, but included more "protective" cells of the immune response (Zaviriukha *et al.*, 2024).

Contact with the virus promotes the synthesis of specific antibodies. The ones can be detected by neutralization of virions or by immunoenzymatic analysis (Korotetsky *et al.*, 2010). The ELISA method has advantages over the virus neutralization method, because it has high sensitivity, specificity, requires less time and resources, and can "process" 96 samples at the same time. At the same time, the quantitative determination is carried out spectrophotometrically (Sthitmatee *et al.*, 2023). In particular, in 6 out of 9 serum samples of sick animals, immunoglobulins against LSDV, active in dilutions of 1:400-1:100, have been detected by ELISA. As can be seen from the results, in more than 30% of cases in sick animals, the natural immune response had not activated after the initial contact with the pathogen. This fact raises the issue of vaccinating cattle to reduce mortality risks from LSDV.

CONCLUSIONS

In this research, a new strain of bovine nodular dermatitis virus was isolated and identified. It was found in Kostanay region inflammation focus. The highest titer of the virus was revealed in one of the skin samples of a sick animal. This isolate was selected for further cultivation in monolayer cells, purification and sequencing. Belonging to the species has been proven by the PCR method with the obtaining of an amplicon of 347 bp, which corresponded to the positive control band. The virus antigens have been detected in three samples of 20% skin homogenate suspension (in dilutions from 1:320 to 1:5), as well as in two blood samples (in dilutions of 1:2). In the culture of lamb testicle cells, the activity of the viral antigen was higher and was read in dilutions of 1:320-1:128. Antibodies have been identified in 6 blood samples out of 9 analysed (in dilutions of 1:400-1:100). The presence of virus antigens in the infected material as well as antibodies in blood serum has additionally been confirmed by a diffuse ELISA test. It was possible to cultivate the viral particles in a monolayer of lamb testicular cells. The virus titer increased with each subsequent passage. To purify the virus, the conditions of gradient centrifugation in a 30% sucrose solution have been optimized.

Thus, it was possible to isolate a lumpy skin disease virus isolate from the biomaterial of diseased, fallen animals, which was named *Dermatitis nodularis bovum/2018/Kostanay/KZ*. An isolate of lumpy skin disease virus was sequenced by genome-wide sequencing on the Illumina MiSeq platform. The collected sequence of lumpy skin disease virus is characterised by a length equal to 145865 bp and a coating of 80.86. BLASTN alignment resulted in a separate clade with contig identification as nodular dermatitis virus with a homologous similarity percentage of 99.36%. The resulting sequence was deposited in the GeneBank international database. At the last step strain *Dermatitis nodularis bovum/2018/Kostanay/KZ* of the lumpy skin disease virus of cattle deposited under the number M-9-21/D in the Collection of microorganisms of the Research Institute of Biological Safety Problems of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Виділення та дослідження штаму вірусу бугристої хвороби великої рогатої худоби

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Анотація. Виявлення нових штамів вірусу дозволить запобігти розвитку спалахів завдяки розробці та використанню вакцин. Метою роботи було виділення та секвенування генома вірусу бугристої шкіри з епізоотії в Костанайській області. Генетичний матеріал віріонів ідентифіковано методом полімеразної ланцюгової реакції (ПЛР); вірусні антигени та антитіла до них визначені методом імуноферментного аналізу (ІФА) або дифузійного тесту; для накопичення матеріалу вірус культивували в моноклонах клітин ячок ягняти; секвенування нового покоління виконано за допомогою системи MiSeq System та програмного забезпечення FastQ; приналежність штамів встановлено методом BLASTN-вирівнювання. Специфічні амплікони вірусу довжиною 347 п.н. були виявлені в зразках шкіри, але не виявлені в крові. У шкірному матеріалі та 2-3 пасажах моноклоналів ідентифікували антигени в розведеннях 1:5-1:320; меншу антигенну активність виявлено в крові в розведенні 1:2. У відповідь на збудника у 67 % досліджених тварин у сироватці крові синтезувалися специфічні імуноглобуліни, які виявлялися в розведеннях 1:100-1:400. Вірусний матеріал накопичували в моноклонах і виділяли в градієнті сахарози. Повногеномна послідовність отриманого матеріалу підтвердила виділення нового штаму вірусу нодулярного дерматиту з відсотком подібності до найближчих гомологів 99,66 %. Штам отримав назву *Dermatitis nodularis bovum/2018/Kostanay/KZ*; послідовність була подана до GeneBank, і об'єкт був депонований у Колекції мікроорганізмів під інвентарним номером M-9-21/D. Отримана інформація може бути використана для запобігання поширенню вогнищ інфекції великої рогатої худоби

Ключові слова: біоматеріал; секвенування; збірка; полімеразна ланцюгова реакція; вірус; дезоксирибонуклеїнова кислота

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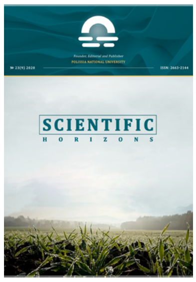

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A sporadic case of small ruminant plague in sheep: morphological and functional manifestation of cellular and humoral immunodeficiency

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Abstract. The threat of the spread of small ruminant plague to livestock in a number of countries makes the study of the mechanisms of development and prevention of this disease a priority area of research. Therefore, the purpose of the study was to investigate the changes in the blood system and organs of the immune system in small ruminant plague, which led to the development of immunodeficiency in the body of infected animals. Haematological and histological methods were used in the study. As a result of the work carried out, a decrease in the total number of leukocytes in the

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peripheral blood from 12.4 to 7.3×109/l was detected in 3 sheep that died from small ruminant plague. There was also a shift in the normal ratio of granulocytes to agranulocytes from 1:1 to 2.5:1 towards granulocytes, which was provoked by a sharp decrease in the number of lymphocytes from 49% to 25% in the leukocyte blood count. Such changes in the blood system were provoked by significant degenerative changes in the lymphoid tissue of immunocompetent organs – lymph nodes and spleen. Histological examination of the parenchyma of the above organs revealed atrophy of T- and B-dependent zones and apoptosis of lymphocytes. Taken together, these signs – haematological and histological – allow for the conclusion that both the humoral and cellular immune response systems in the body are reduced. In the affected areas of the lungs, an inflammatory reaction is observed with active migration of neutrophils and macrophages to the inflammation zone. But the active multiplication of the virus in the lung cells indicates a lack of immunity. Thus, the study revealed the mechanisms of the development of humoral and cellular immunodeficiency in small ruminant plague (SRP), which would allow developing more effective methods of combating this disease

Keywords: lymphoid tissue; histological and haematological studies; lymph node; spleen; lung tissue; differential leukocyte count

INTRODUCTION

Small ruminant plague is a dangerous transboundary disease of sheep and goats, characteristic of southern countries, and according to the Food and Agriculture Organisation of the United Nations (FAO), it poses a significant threat to agricultural and farming enterprises in more than 65 endemic countries in Africa, the Middle East, and Asia (Peste des petits…, 2024). This disease is dangerous not only for farm animals, but according to J.T. Amanova (2023), is also the cause of the death of wild, susceptible animals and threatens to reduce the biodiversity of the country's fauna. Based on such dangerous consequences of the development and spread of the disease in the Asian region, the research aimed at finding mechanisms for the protection and prevention of plague in small ruminants is an urgent area of scientific research.

According to K. Zhumakanov and A. Abdurasulov (2022), no cases of small ruminant plague have been detected in Kyrgyzstan in recent years. However, a number of researchers indicate cases of animal plague in neighbouring countries. Thus, M. Munibullah *et al.* (2022) indicate that cases of disease in sheep and goats are regularly detected in the border areas of Kazakhstan. M. Amirbekov *et al.* (2020) reported animal morbidity in Tajikistan, and S. Gao *et al.* (2021) – in China.

The outbreak of the disease among farm animals, according to J. Amanova *et al.* (2020), leads to significant economic losses, which are associated with the death or forced slaughter of infected animals, a decrease in their productivity, losses on veterinary, sanitary, and quarantine measures. Therefore, the most common method of preventing this viral disease is active immunisation of all susceptible livestock (Bayantassova *et al.*, 2023). The materials provided by M. Legnardi *et al.* (2022) indicated that USD 10.1 million was spent on animal vaccination in Kyrgyzstan only for preventive work in the period from 2017 to 2020. According to the same researchers, mass vaccination is carried out mainly in the southern regions of Kyrgyzstan and in buffer zones established on the borders with China, Kazakhstan, Tajikistan, and Uzbekistan. Since 2016, only young animals of the same year of birth have been vaccinated; to date, the vaccination coverage of the country's livestock is about 60%. All these approaches should support animal immunity and prevent the possibility of disease in the country. But a number of factors, indicated by W. Niedbalski *et al.* (2022), make it impossible to guarantee this even with this investment in prevention. These include the possibility of long term persistence of the virus in bovine cattle, migratory processes during grazing and atypical clinical signs of the disease, which complicate the diagnosis.

During the period of active replication of the virus in the body of animals, according to S. Begum *et al.* (2021), mainly the organs of the immune system are affected, which is associated with the tropism of the pathogen. The researchers point out that most of the changes concerned lymph nodes and the mucous membrane of the gastrointestinal tract with signs of immunodeficiency, due to the active breakdown of leukocytes and damage to lymphoid tissue. The development of immunosuppression in infected animals is also indicated by other researchers, in particular, O. Tenuche *et al.* (2023), but all researchers focus only on changes in regional immune organs (pharyngeal submandibular and other lymph nodes of the head and neck, mesentery), while ignoring the processes taking place in other lymph nodes and lymphoid organs. Ultimately, a decrease in the number of lymphocytes in the peripheral blood is a sign of a systemic pathological process.

Therefore, the purpose of this study was to conduct haematological studies, to investigate pathomorphological changes in parenchymal organs and organs of the immune system in sheep that died from small ruminant plague (SRP), which led to the development of an immunodeficiency condition in the body. To achieve the intended goal, the following tasks were set: determination of the total number of leukocytes and derivation of the differential leukocyte count, morphohistological studies of pulmonary and broncho-associated lymphoid tissue; investigation of the morphofunctional state of T- and B-dependent zones of lymph nodes and spleen in the body of sheep that died from the plague.

LITERATURE REVIEW

SRP is a viral disease that is caused by an RNA-containing virus belonging to the species *Morbillivirus,* family *Paramyxoviridae*. According to B. Vinayagamurthy *et al.* (2020), there are four strains of the virus in the world that differ in the nucleoprotein (N) gene, and according to R. Eloiflin *et al.* (2022), have different virulence and different degrees of clinical signs and internal organ damage. The danger of this disease is also manifested in the possibility of prolonged persistence or subclinical course of the disease in the body of non-susceptible animals – camels (Abdalla *et al.*, 2021), bovine cattle and other animals (Rahman *et al.*, 2020), which maintains a high incidence rate. Among susceptible livestock, the small ruminant plague virus maintains high virulence and mortality among infected animals. These indicators in the goat population were 51.1 and 73.9% (Shanmugavadivu *et al.*, 2023), in sheep – 88.2 and 26.7% (Abd-Elfatah *et al.*, 2022), respectively. Moreover, P. Rath *et al.* (2020) indicate that the younger the age of the affected animals, the higher the mortality rate. Thus, the incidence among weaners was 63%, and among the stock from 6 months to a year old only about 42%. Similar case results were obtained by K. Ramakrishna and P. Ramadevi (2024), noting that there were two waves of mortality, the first caused by the small ruminant plague virus and the second by the development of immunosuppression and secondary infectious agents such as pasteurellosis and enterotoxaemia. N. Sobhy *et al.* (2023) attribute a significant role in the development of immunosuppression in ruminant plague to the association with other viruses: small ruminant retrovirus (SRR), enzootic nasal tumour virus (ENTV), and Jaagsiekte sheep retrovirus (JSRV).

Authors S. Begum *et al.* (2021) indicate that, regardless of age, primary pathomorphological changes occur in the mucous membrane of the oral and nasal cavities, and the lymph nodes of the head and neck. Subsequently, according to Z. Bamouh *et al.* (2023), depending on the age of the infected animal, the disease develops in young sheep and goats with the development of respiratory symptoms and subsequent diarrhoea, and in adults – mainly mild respiratory symptoms. A. Jarad *et al.* (2022) indicate inflammatory infiltration of the surface layers of the intestinal wall with further desquamation of the epithelium and the development of necrosis. Further, G. Alwan and K. Al Saad (2023) report the development of haemorrhagic enteritis and thickening of intestinal villi walls. However, the studies by T. Jelsma *et al.* (2021) indicated that the maximum accumulation of viral particles in the intestine was observed in the mucous membrane – 4.6 $\log^{10}$ $TCID_{50}$-eq/g, and in the submucosal, muscular, and serous layer, the viral load was 3.5, 2.8 and 1.7 $\log^{10}$ $TCID_{50}$-eq/g, respectively.

But the main changes noted by such authors as A. Chota *et al.* (2020) occurred in the organs of the respiratory system. At the same time, all researchers noted histological disorders such as consolidated pneumonia in 38.1% of cases, lung congestion – 28.6%, serous fibrinous pleurisy – 28.6% on day 7 after infection. Among other pathologies, generalised lymphadenopathy with necrosis and hyperaemia of the lymph nodes was noted. The histological pattern of the lungs was characterised by infiltration of the lumen of the alveoli and bronchioles by white blood cells – neutrophils and monocytes. But the main changes occurred in the lymph nodes. According to V. Balamurugan *et al.* (2023), the main processes occur in the lymphoid tissue of mediastinal and mesenteric lymph nodes.

The tropism of the plague virus of small ruminants to lymphoid tissues was confirmed in the study by D. Adwitiya *et al.* (2022), which indicates that the main replication of viral agents occurs in regional lymph nodes. Due to intensive multiplication there is necrosis of tissues inside the lymph node and destruction of leukocytes, which leads to the development of immunosuppression. Similar conclusions were obtained by K. Schmitz *et al.* (2023), who indicate that massive infection of lymphoid tissues causes immune suppression. This leads to increased susceptibility to secondary bacterial infections. This explains the dependence of the intensity of the infectious process on the virulence of the virus strain that caused the disease, or the amount of virus that infected the animal (Niyogi *et al.*, 2023). According to A. Fayyad *et al.* (2020), if the detection of the virus in the oral cavity is taken as 100%, then from other organs the virus was isolated from lymph nodes in 100% of cases, from intestine in 30-70%, and from lung tissue in 70-100%. In addition, in recent years, a number of researchers have reported the detection of the virus from other organs – the spleen, brain, liver, and other internal organs (Sahoo *et al.*, 2020; Singh *et al.*, 2021).

Thus, the main histological studies of pathomorphological processes during the infection of animals with small ruminant plague virus were paid to the mucous membranes and lymph nodes of the head and neck, respiratory system, and gastrointestinal tract. However, the systemic action of the virus on the immune system organs of infected sheep and goats has not been given due attention.

MATERIALS AND METHODS

The research was conducted at the Faculty of Veterinary Medicine of the Kyrgyz National Agrarian University named after K.I. Skryabin The subject of the study was selected pathological material of the lungs, spleen, and lymph nodes from fallen animals and samples of relevant organs obtained from healthy sheep to investigate histomorphological changes caused by the development of viral infection. Samples of internal organs were selected for the manufacture of histological preparations and the study of pathological changes. For this purpose, the cadavers of 3 dead sheep with characteristic clinical signs of small ruminant plague were autopsied. A preliminary diagnosis for the small ruminant plague was made based on the results of a pathological autopsy of sheep. The final diagnosis was established after conducting histological studies of the affected parenchymal organs and organs of the immune system and comparing them with the characteristic pathoanatomical changes in small ruminant plague and the results of virological examination. Slices of lungs, lymph nodes, and spleen from both dead animals and 3 healthy animal analogues were selected for the study to conduct a comparative analysis. All the procedures of this study were carried out in accordance with the Regulations of the Kyrgyz National Agrarian University named after K.I. Skryabin on bioethics and were approved at the meeting of this commission.

The selection of pathological material from dead and healthy animals for histological examination was carried out in compliance with the necessary approaches to prevent the spread of pathogens in the environment and microbial contamination of feed and animal care tools. Pathological material was sampled from the spleen and lungs of the dead animals at the border of diseased and normal tissue, and lymph nodes were sampled completely followed by fixation in 10% neutral formalin solution, according to the techniques described in L.P. Gartner's (2020) manual of pathohistological techniques for delivery to the pathological anatomy laboratory. Further processing of pathological material to obtain histological preparations was carried out according to the technique with its dehydration in increasing concentration of ethyl alcohol and pouring into paraffin. Slices 5-6 μm thick were prepared from paraffin blocks on a MC-2 sledge microtome. For histological examination, histological sections were selected at the border between the affected and unaffected tissue, so that all structures of the organ under study were represented. Cross sections were made from the lymph nodes so that the capsule, cortical, and cerebral zones of the organ were represented in the preparations. Cross-cut pieces were also taken from the spleen so that the red and white pulp and the capsule were visible in the preparations. In addition, histological preparations from healthy organs – lungs, analogous lymph nodes and spleen – were prepared in the same way from the control group of sheep (3 animals).

Histological preparations were stained with a mixture of organic dyes – haematoxylin and eosin, followed by analysis under a PZO (Warszawa) light microscope with a camera and software that enables, in addition to displaying images on the screen, to keep a research journal with an archive of images for each sample. The study of histological changes in organs was carried out with both weak and large magnification. Moreover, simultaneously with the selection of pathological material, venous blood was taken from the pulmonary veins for haematological studies. Blood was collected using a sterile disposable syringe and transferred to vacuum tubes with EDTA. Blood was collected from healthy animals at slaughter in a similar manner. Haematological examinations included determination of total leukocyte count and derivation of differential leukocyte count. The result was statistically calculated in the Excel software suite from Microsoft 365.

RESULTS

Studies of the immunosuppressive effect of the small ruminant plague virus on the organism of sheep were carried out in two areas: the direct effect on the organs of the immune system – lymph nodes, spleen, and interstitial lung tissue, and the indirect effect on the level of white blood cells in the organism of animals associated with the immune response. This approach has its advantages, since it allows forming a versatile perspective on the causes and mechanism of development of the immunodeficiency state of the body in infected animals.

Haematological changes in blood in infected sheep. One of the most effective and rapid responses of the body to infection is an increase in the number of leukocytes in the bloodstream, or the body's standard immune response. This condition occurs in almost all infectious diseases, with the exception of a small part of infections in which the cycle of reproduction of infectious agents associated with immunocompetent cells of the bone marrow or immune system. It is these pathogens that include the SRP virus. The simplest and most effective method of detecting the immunodeficiency state of a sick animal is to determine the number of immunocompetent cells and, first of all, different classes of leukocytes. Therefore, to identify the degree of immunosuppression in this disease, the first stage was to compare the number of leukocytes and their main classes (differential leukocyte count) in the blood of infected and healthy sheep. The results of a comparative analysis of these indicators are presented in Table 1.

Table 1*. Haematological parameters of the blood system in healthy and SRP virus-infected sheep*

No.	Status	Number of leukocytes, 10^9/l	Granulocytes, %	Agranulocytes, %
1	Infected	7.9	68	32
2	Infected	7.6	71	29
3	Infected	6.3	76	24
Average for infected animals		7.3±0.49	71.7±2.33	28.3±2.33

Table 1. Continued

No.	Status	Number of leukocytes, 10^9/l	Granulocytes, %	Agranulocytes, %
4	Healthy	11.9	52	48
5	Healthy	12.7	54	46
6	Healthy	12.5	52	48
Average for healthy animals		12.4±0.24	52.7±0.67	47.3±0.67

Source: *compiled by the authors*

The concentration of leukocytes in the blood of infected animals was significantly lower than their level in healthy animals. Notably, the leukocyte content in the group of healthy animals corresponded to the reference values for this type of animal. The difference in the concentration of the total number of leukocytes between animals with different status was 41.1%, which already indicates inhibition of leukocyte production in infected animals. Even despite the small statistical sample of animals, the difference between the groups was highly significant (P = 0.0007). In addition to a decrease in the total number of leukocytes in the peripheral blood, a decrease in the ratio of granulocytes to agranulocytes was also noted. Thus, in healthy animals, the ratio of these groups of leukocytes was approximately 1:1. In animals infected with the SRP virus this ratio was shifted towards granulocytes. It is an indirect confirmation of the malfunction of lymphoid tissue in the body of an infected animal. However, the work of the bone marrow was not disrupted. This can be confirmed by the fact that the number and age of neutrophils in healthy and infected animals did not change, but only their concentration in infected sheep increased due to a decrease in the number of agranulocytes, whose reproduction cycle is not associated with the bone marrow. The difference between healthy and sick animals for this indicator also turned out to be significant with a level of P = 0.001. For a more detailed understanding of the processes occurring in the white blood system in the body of sheep infected with SRP, the differential leukocyte count was performed in Table 2.

Table 2. *Differential leukocyte count in healthy and sick sheep with small ruminant plague, %*

No.	Status	Leukocyte classes							
		Eosinophils	Basophils	Neutrophils				Lymphocytes	Monocytes
				M	J	B	S		
1	Infected	1	2	0	0	13	58	24	2
2	Infected	0	1	0	2	18	47	31	1
3	Infected	2	2	0	0	13	61	20	2
Average for infected animals		1±1	1.6±0.6	0±0	0.7±1.2	14.7±2.9	55.3±7.4	25±5.6	1.7±0.6
4	Healthy	0	6	0	0	3	39	49	3
5	Healthy	1	8	0	0	6	33	50	2
6	Healthy	1	7	0	1	3	36	48	4
Average for healthy animals		0.7±0.8	7±1	0±0	0.3±0.6	4±1.7	36±3	49±1	3±1

Note: *neutrophil classes: M – myelocyte; J – juvenile neutrophil; B – band neutrophil; S – segmented neutrophil*
Source: *compiled by the authors*

When analysing the indicators of the differential leukocyte count, a decrease in the percentage of lymphocytes by almost 2 times compared to healthy animals, and an increase in the number of neutrophils, are clearly visible. This increase in the number of segmented and band neutrophils was relative and was not associated with intensive synthesis of granulocytes, since the increase was observed only in the population of mature cell structures, and juvenile forms were absent. Therefore, such an increase in the concentration of neutrophils was associated not with their active reproduction, but with a relative increase in their proportion against the background of an active decrease in the level of lymphocytes, which became one of the main reasons for the development of an immunodeficiency condition.

Thus, changes in quantitative and qualitative blood parameters indicate possible functional changes in the lymphoid tissue of the immune system, therefore, further studies were aimed at investigating histological changes in the tissue of lymph nodes, spleen, and lungs of infected animals. Externally, stagnation and signs of spot bleeding under the capsule were found in the lungs and lymph nodes of sheep infected with the SRP virus. The spleen is enlarged in size, of flabby consistency with obtuse edges. But such macroscopic changes are also characteristic of many other infectious diseases, including viral diseases. Therefore, histological studies were performed in these organs as the most likely sites of localisation of the source of infection.

Pathohistological changes in the lungs. Histological examination of the SRP-affected lung revealed pathological changes in both the alveolar and bronchial parenchyma systems of the organ. The main changes were concentrated in the affected areas of the lung. They had signs characteristic of the development and progression of an inflammatory reaction. In contrast to the histological structure of the lungs in healthy animals, morphohistological signs of bronchopneumonia development were observed in infected sheep, which manifested by signs of filling the alveoli with serous fibrinous fluid, purulent necrotic tissue residues, and inflammatory cells. The signs of thickening of the interalveolar septa were also observed. Around the bronchial system, the bronchoassociated lymphoid tissue was weakly expressed and there were no secondary lymphoid follicles (Fig. 1).

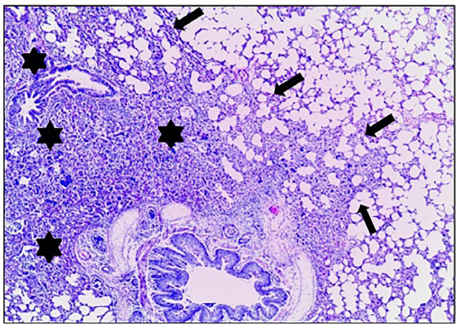

Figure 1*. Histological section of a sheep lung with SRP Serous fibrinous and interstitial bronchopneumonia*
Note*: alveoli are filled with serous fibrinous fluid (asterisks) and cells; alveolar septa are thickened (arrows). Broncho-associated lymphoid tissue is almost not expressed around the bronchus. Haematoxylin and eosin. ×25*
Source: *compiled by the authors*

When examining histological sections at high magnification (250 times or more), deeper signs of lung tissue damage were observed. They were manifested by the following morphological changes in the affected areas of the lungs: the lumen of the alveoli and bronchioles were filled with neutrophils, macrophages, desquamated epithelial cells, and necrotic mass. Among these masses, there were individual syntial cells with inclusion bodies. In contrast to the state of epithelial cells in the bronchi and lungs of healthy animals, sheep infected with SRP had numerous abnormalities at the level of cellular structure. Thus, in epithelial cells of bronchioles in infected lung sections, large eosinophilic intracytoplasmic viral inclusions were observed, whereas intranuclear eosinophilic inclusions were relatively rare. The presence of a small number of plasma cells, macrophages, lymphocytes, and neutrophils was noted around the bronchioles and vessels in the affected areas. Syntial cells with several nuclei in the cytoplasm are found in the alveoli (Fig. 2).

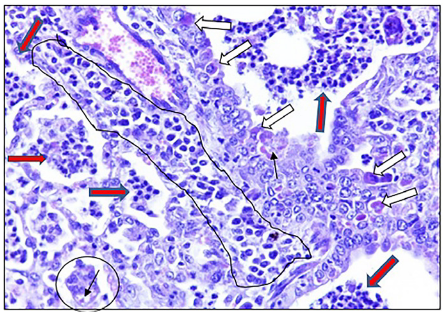

Figure 2*. Histological section of a sheep lung with SRP Bronchopneumonia*
Note*: lumen of the alveoli and bronchioles are filled with neutrophils, macrophages, exfoliated epithelial cells of the alveoli and bronchioles, and necrotic mass (red arrows). Syncytial cells with inclusion cells (inside the circle) are found in the alveoli. Numerous epithelial cells of bronchioles contained large eosinophilic viral inclusion bodies, mainly intracytoplasmic (white arrows), but rarely intracellular (black arrows). There are not many plasma cells, macrophages, lymphocytes, and neutrophils around bronchioles and blood vessels (inside the black line). Hematoxylin and eosin. ×250*
Source: *compiled by the authors*

In addition, at higher magnification, in pathologically changed areas in the epithelium of bronchi and bronchioles their hyperplasia, and the presence of intracellular eosinophilic viral inclusions were noted. These inclusions were localised both in the cytoplasm and in the nuclear structures of the cell (Fig. 3). In the bronchial lymph nodes, minor haemorrhages in the subcortical layer were observed, and atrophic changes in the cortical and cerebral ball of the node. The degree of manifestation of pathological changes in the lung tissue was proportional to the period of the disease.

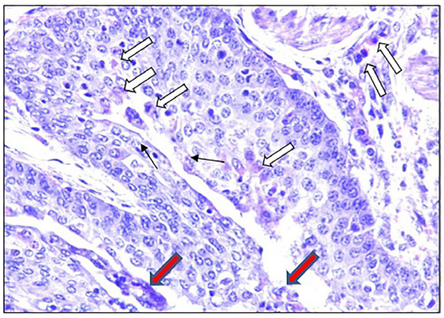

Figure 3*. Histological section of a sheep lung with SRP*
Note*: bronchial epithelial cell hyperplasia. Numerous epithelial cells placed around large bronchi contain relatively large eosinophilic viral inclusion cells, mostly intracytoplasmic (white arrows) and rarely intranuclear (black arrows). There are not many plasma cells, macrophages, lymphocytes and neutrophils around bronchioles and blood vessels. There is a necrotic mass in the lumen of the bronchi (red arrows). Hematoxylin and eosin. ×250*
Source: *compiled by the authors*

Thus, pathohistological changes in the lungs indicate the development of an inflammatory reaction in alveolar-bronchial tissues and active proliferation of cellular elements into the inflammatory zone. There is an active replication of viral particles – eosinophilic intracellular particles, which indicates a deficiency of the immune system.

Pathohistological changes in regional lymph nodes. When analysing the differences in histological changes in the tissues of the regional lymph nodes affected by the SRP virus, in comparison with those obtained from healthy animals, the presence of significant areas of lymphoid tissue atrophy in the paracortical or T-zone, and the destruction of secondary lymphoid follicles associated with the B-dependent zone was noted in the affected tissues at low magnification (Fig. 4). When analysing histological preparations obtained from lymph nodes from infected animals at high magnification, the following changes were found: progressive atrophy of lymphoid follicles in the B-zone of the cortical layer of the node, which was confirmed by the absence of signs of mitosis of lymphoblasts in their light centre, the presence of apoptoses as a result of the development of apoptosis of lymphocytes (Fig. 5). The development of lymphocyte destruction process was confirmed on histological preparations by the presence of significant atrophy areas in the paracortical zone of lymph nodes (Fig. 6). In the brain matter, the histological structures of the medullary thrust and brain matter are partially erased. In this layer of the lymph node affected by the SRP virus, areas of lymphocyte decay are also noted, identified by the presence of apoptoses (Fig. 7); in some preparations, diffuse and focal haemorrhages were found in the medulla layer of the substance (Fig. 8).

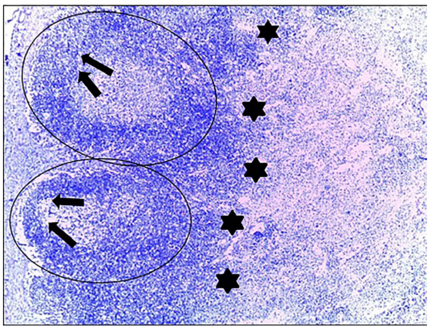

Figure 4. *Transverse histological section of the regional lymph node*
Note: *atrophy of the T-zone (asterisks) and disintegration of secondary lymphoid follicles (inside the oval and arrow). Haematoxylin and eosin. ×25*
Source: *compiled by the authors*

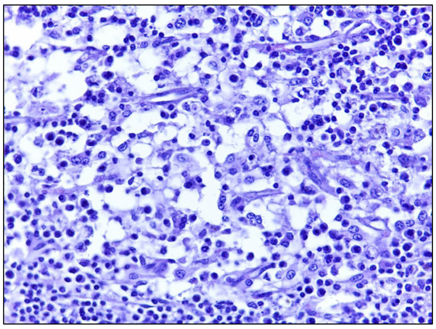

Figure 5. *Transverse histological section of the regional lymph node*
Note: *atrophy of lymphoid follicles (B-zones), in the light centre there is no mitosis of lymphoblastic cells, apoptosis (decay) of lymphocytes and apoptosomes. Hematoxylin and eosin. ×250*
Source: *compiled by the authors*

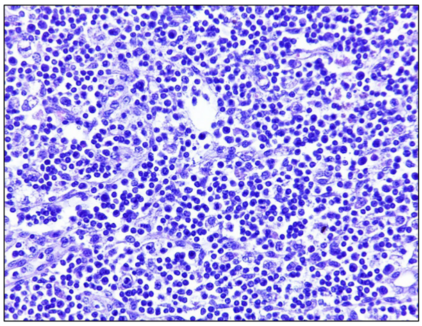

Figure 6. *Transverse histological section of the regional lymph node*
Note: *atrophy of the paracortical zone (T-zone), apoptosis (decay) of lymphocytes and apoptosomes. Hematoxylin and eosin. ×250*
Source: *compiled by the authors*

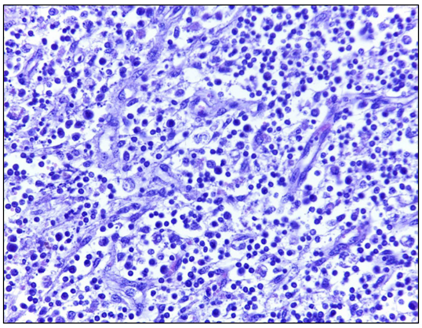

Figure 7. *Transverse histological section of the regional lymph node*
Note: *change in the histological picture of the brain matter and inability to distinguish between cerebral tracts and cerebral sinuses. Apoptosis of lymphocytes and apoptosomes in the brain matter. Hematoxylin and eosin. ×250*
Source: *compiled by the authors*

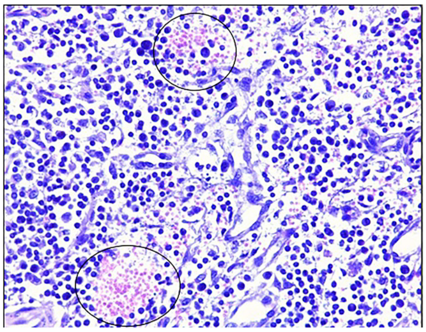

Figure 8. Transverse histological section of the regional lymph node
Note: haemorrhage in the brain matter (inside the circle). Hematoxylin and eosin. ×250
Source: compiled by the authors

Thus, in small ruminant plague, the main morphohistological changes in regional lymph nodes are partial atrophy of T- and B-dependent zones of lymphocyte reproduction, which is probably the main reason for the decrease in the level of cellular and humoral immune response to infectious agents. Thus, significant apoptosis of lymphocytes in the lymph nodes, and atrophy of lymphoid tissue, are the main reasons for the decrease in the level of leukocytes in the blood of animals and the associated development of an immunodeficiency condition.

Pathohistological changes in the spleen. In the spleen, as in other organs of the lymphoid system, for example, in regional lymph nodes, foci of diffuse atrophy of the internal structures of the organ are noted on histological preparations. In particular, degenerative processes in the pulp of the spleen, namely in the T- and B-zones, are noted in infected animals. The white pulp contains a small number of lymphocytes, which may be the result of a decrease in their concentration in the peripheral blood, and there are also no signs of lymphoblast proliferation. There are no lymphoid structures (follicles) in the spleen tissue. It is difficult to distinguish the white pulp from the red pulp on the preparations (Fig. 9). Diffuse and focal areas with capsule haemorrhages were revealed on histological preparations at high magnification. The red pulp shows the presence of erythrocytes, lymphocytes, plasma cells, macrophages, eosinophils and haemosiderin in small numbers (Fig. 10).

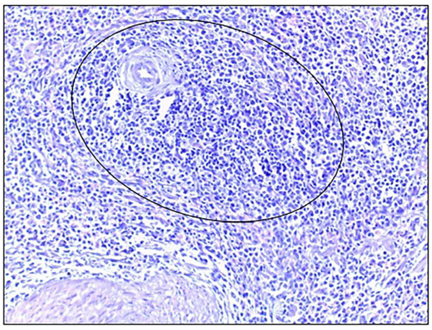

Figure 9. Histological section of the spleen Atrophy of the white pulp of the spleen
Note: atrophy of the T- and B-zones of the white pulp (inside the oval). No proliferation of lymphoblasts is observed. Lymphoid follicles are absent. Hematoxylin and eosin. ×62.5
Source: compiled by the authors

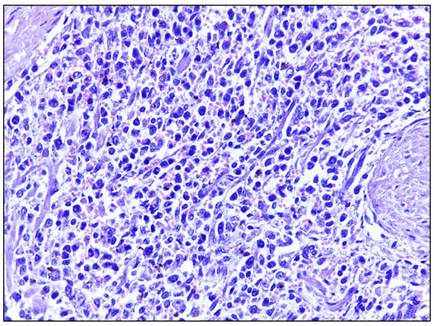

Figure 10. Histological section of the spleen
Note: red pulp. Trabeculae (arrows), erythrocytes, lymphocytes, plasma cells, macrophages, eosinophils, and haemosiderin are visible. Hematoxylin and eosin. ×250
Source: compiled by the authors

Thus, in the spleen affected by the small ruminant plague virus, and in other organs of the lymphoid system, atrophic changes are noted, which reduce the immune potential of the organism. At the histological level, this was manifested in the smoothing of the boundary between the T- and B-zones of the white pulp, responsible for the formation of cellular and humoral reactions in the body of sheep, respectively. Summing up the results of the studies and histological changes occurring in the body of small ruminants infected with plague, it should be noted specific morphofunctional and immunological changes in lymphoid tissue that cause a decrease in the number of lymphocytes and the development of an immunodeficiency state of the body. The main histological changes in lymphoid tissue cells are diffuse eosinophilic granularity in the cytoplasm and nucleus, which is associated with the active reproduction of the virus. Similar changes are characteristic of the cytoplasm and nuclei of the alveolar and bronchial epithelium, alveolar macrophages, and syncytial lung cells.

DISCUSSION

Given that small ruminant plague is characterised by the progressive development of an immunodeficiency state in infected and re-infected animals, this disease is a significant threat to sheep production. The possibility of virus persistence in immune animals and the use of vaccines on sheep and goats leads to the development of atypical forms of the disease. One of the key signs of the disease remains the development of progressive immunosuppression, which subsequently leads to secondary infection with the development of a symptom complex of signs characteristic of secondary infection (Turmagambetova *et al.*, 2017). In such cases, the primary disease is mitigated and overshadowed, and the only way to determine the presence of the plague virus is through characteristic pathohistological changes in the body of diseased animals. In addition, even in vaccinated animals, when exposed to a pathogenic strain of the SRP virus, signs of infection develop in immunocompetent organs. This, according to I. Saeed *et al.* (2022), is the cause of weakening of the immune system in some cases.

The simplest way to detect the development of an immunodeficiency condition is to estimate the total number of leukocytes and the differential leukocyte count (Turgenbayev *et al.*, 2023). The conducted studies clearly show a significant decrease in the total number of leukocytes. Their level is only 40% of the reference value. Thus, a decrease in the number of lymphocytes, which are associated with the work of the humoral part of the immune system, namely the production of antibodies, leads to the development of immunodeficiency and a decrease in the body's protective response (Namouchi, 2022). In addition, a shift in the ratio of granulocytes to agranulocytes was observed in the blood of infected animals. Similar changes in the blood system of infected goats and sheep were also observed by M. Uddin *et al.* (2022), and S. Khaliq *et al.* (2020). However, S. Maina *et al.* (2015) found no decrease in the total number of leukocytes in the blood of infected goats. Perhaps this could be due to the low virulence of the virus used in the experimental infection of animals in the experiment, since a laboratory strain was used. But despite these differences in the number of leukocytes in the peripheral blood of infected animals, all the above-mentioned researchers and experiments conducted within the framework of this study agree that when goats and sheep are infected with the SRP virus, there is a decrease in the number of lymphocytes. Considering that the processes of reproduction and differentiation of this fraction of white blood cells occur in the lymphoid tissue of the immune system, unlike other fractions of leukocytes, the development cycle of which is associated with the bone marrow, it can be concluded about the tropism of the causative agent of the small ruminant plague. The absence of changes in the number of neutrophils, eosinophils, and basophils in the differential leukocyte count from infected animals in the conducted studies indicates that the functions of the bone marrow, as the main organ of reproduction of blood cells, are not pathologically affected. Similar results were also obtained by A. Jarad *et al.* (2022). In addition, this is confirmed by the almost constant number of other blood cells, the development of which occurs in the bone marrow – red blood cells and platelets. Thus, further work was aimed at studying histological changes in the lymphoid tissue of immunocompetent organs, in particular interstitial lung tissue, regional lymph nodes and spleen, which could lead to a decrease in the number of lymphocytes in the blood.

During the pathological and anatomical autopsy of animals fallen from the small ruminant plague, it was noted that the main changes were found in the organs of the respiratory system, gastrointestinal tract, and other internal organs. However, morphofunctional changes in the lungs, lymph nodes, and spleen were the most pronounced, which is why these organs were selected for further histological studies.

During microscopy of histological preparations of the lungs, a significant part of the pathomorphological changes in tissues was characteristic of the development of an inflammatory reaction. Through this, in the affected tissue areas, the alveoli and bronchioles were filled with exudate with significant inclusion of cellular elements including neutrophils, macrophages, and other cells of the cellular immune response system. The active work of cellular immunity is confirmed in the study by S. Gautam *et al.* (2021), which indicate that there is an active accumulation of CD163+ and M2c macrophages in the affected areas of the lungs, which are involved in inflammatory reactions. This explains the development of syntial structures in the affected areas of the lungs. These histological symptoms indicate that this part of the body's protective function is working normally and adequate reactions to the ingress of an infectious agent are taking place. However, active reproduction of viral particles is observed in the epithelium of the respiratory tract and alveoli, which is diagnosed by the presence of eosinophilic inclusions, which are the centres of assembly of viruses in the cytoplasm and nuclear structures of cells (Busol *et al.*, 2023). The changes occurring in certain areas of the lungs show a different intensity of pathological processes. This is probably due to the elongation in time of infection of certain areas of organ tissue. A similar conclusion regarding the manifestation of pathological changes depending on the time after infection was made by M. Islam *et al.* (2023) when dissecting 36 fallen goats at different stages of the disease. The researcher noted a different degree of manifestation of pathohistological changes in the structures of alveolar-bronchial tissue in animals with different intensity of disease progression and the period of time elapsed between the manifestation of clinical signs and the death of animals.

However, the most characteristic changes occurred in the lymphoid tissue of the lymph nodes, where the development of lymphocytes takes place. Among the histological signs of structural abnormalities, there were point and diffuse haemorrhages in the lymph node tissue, a decrease in the clarity of the boundary between the cerebral and cortical layers, but the main changes concerned atrophy of morphological structures responsible for the differentiation of immunocompetent cells and apoptosis of already formed lymphocytes. Such changes were typical for almost all the studied lymph nodes and degenerative changes occurred in both T- and B-dependent zones of lymphocyte development with different intensity in the controlled animals. That is, as in the case of pathological processes in the lungs, the degree of manifestation of the pathological picture in the lymph nodes depended on the duration of the disease in animals (Radsikhovskiy *et al.*, 2023). The exception was the characteristic of histomorphological changes in the spleen. Regardless of the condition of the animals at the time of their death on histological preparations of the spleen, the intensity of the processes was almost at the same level – these were diffuse haemorrhages into the capsule of the spleen, significant foci of lymphoid tissue atrophy with damage to T- and B-dependent lymphocyte formation centres, haemosiderin residues. Perhaps this is due to early infection by viral particles of spleen tissues through the circulatory system during the incubation period, and at the time of manifestation of clinical signs in animals, pathological changes provoked by the SRP virus have already occurred in the spleen. Thus, given the tropism of the virus to lymphoid tissue, the damage to the organs of the immune system depends on the time interval from infection to testing. Similar findings were obtained by M. Islam *et al.* (2023).

Summing up the work performed to investigate the influence of the causes of immunodeficiency state development from pathomorphological changes in the lymphoid tissue of immunocompetent organs, it should be noted that these processes were the reason for the decrease in the number of lymphocytes in the peripheral blood, and the decrease in the humoral and cellular immune response of the organism. A significant concentration of macrophages and neutrophils was noted in the zone of inflammatory reaction in the lungs. Thus, immunodeficiency in small ruminant plague is a consequence of the destruction of lymphoid tissues of the immune system of the organism of infected animals.

CONCLUSIONS

Based on the received literature information and the results of studies on the difference between leukocytes and their fractions in the blood of healthy and infected animals, considering morphofunctional changes in the lymphoid tissue of the immune system organs of fallen animals from the small ruminant plague, the following conclusions can be drawn. In all fallen animals, a decrease in the number of lymphocytes in the blood was observed, by more than 40%, in comparison with the reference indicators characteristic of this type of animal. In addition, the ratio of granulocytes and agranulocytes shifted from haematological parameters towards cells with granules present in the cytoplasm – neutrophils, eosinophils, and basophils. Such changes in the blood system were also manifested by changes in the differential leukocyte count, where, in addition to a decrease in lymphocytes (by almost 50%), a relative increase in the fractions of rod-core and segmented neutrophils was observed.

Histological studies have shown systemic disorders in the lymphoid tissue of the organs of the immune system – lymph nodes and spleen, which manifested themselves in atrophy of T- and B-dependent zones of lymphocyte generation, and as apoptosis of already developed lymphocytes in the parenchyma of these organs. Focal and diffuse haemorrhages also indicate a violation of the functional state in the studied organs. Such morphofunctional changes in the organs of the immune system led to a decrease in the level of lymphocytes in the peripheral blood, and thus caused the development of an immunodeficiency state of the animal body.

In the alveolar-bronchial lung tissue, signs of the development of an inflammatory reaction were found, which is characterised by cellular proliferation of tissues by neutrophils and macrophages. But the active reproduction of viral particles in the epithelial cells of the bronchi and alveoli, which is confirmed by the presence of eosinophilic inclusions in their cytoplasm, indicates the insufficiency of this mechanism of immune response. These conclusions are based on the results of the study of only three fallen animals, which does not allow them to be extrapolated to all cases of the disease. Therefore, such studies should be continued with the involvement of a large number of cases and a more detailed analysis of the mechanisms of development of the immunodeficiency state in the body of sheep infected with SRP.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Спорадичний випадок чуми овець: морфофункціональний прояв клітинного та гуморального імунодефіциту

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Анотація. Небезпека поширення чуми дрібної рогатої худоби для тваринництва низки країн робить вивчення механізмів розвитку та профілактики цього захворювання пріоритетним напрямом наукових досліджень. Тому метою роботи стало вивчення змін у системі крові та органів імунної системи за чуми дрібних жуйних, які призвели до розвитку імунодефіцитного стану організму хворих тварин. При проведенні роботи були використані гематологічні та гістологічні методи дослідження. У результаті проведеної роботи у 3 овець, які померли від чуми дрібної рогатої худоби, було виявлено зниження загальної кількості лейкоцитів у периферичній крові з 12,4 до 7,3х109/л. При цьому також спостерігалося зміщення нормального співвідношення гранулоцитів до агранулоцитів з 1:1 до 2.5:1 у бік гранулоцитів, що було спровоковано різким зниженням кількості фракції лімфоцитів з 49 % до 25 % у лейкоцитарній формулі крові. Такі зміни в системі крові були спровоковані значними дегенеративними змінами в лімфоїдній тканині імунокомпетентних органів – лімфатичних вузлах і селезінці. При гістологічному дослідженні в паренхімі вище зазначених органів було виявлено атрофію Т- і В-залежних зон і апоптоз уже лімфоцитів. У сукупності ці ознаки – гематологічні та гістологічні – дають змогу зробити висновок про зниження як гуморальної, так і клітинної системи імунної відповіді в організмі. В уражених ділянках легень спостерігається розвиток запальної реакції з активною міграцією в зону запалення нейтрофілів і макрофагів. Але активне розмноження вірусу в клітинах легень вказує на недостатність імунітету. Таким чином проведені дослідження розкривають механізми розвитку гуморального та клітинного імунодефіциту за чуми дрібних жуйних тварин (ЧМР Тварин), що дасть змогу розробити більш ефективні методи боротьби з цим захворюванням

Ключові слова: лімфоїдна тканина; гістологічні та гематологічні дослідження; лімфатичний вузол; селезінка; легенева тканина; лейкоцитарна формула

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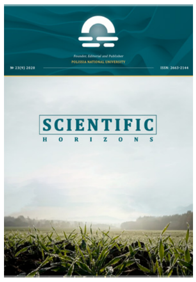

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Modes and methods of treatment of blackcurrant pomace with an enzyme preparation to increase juice yield

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Abstract. Blackcurrant is a multivitamin crop with high dietary and medicinal properties and is a source of exceptionally valuable raw materials for juice production, but its extraction is complicated by its chemical composition. The endocarp of blackcurrant fruit (the actual pulp) is formed by the slimy arylus surrounding the seeds, which prevents juice extraction. The purpose of this study was to scientifically substantiate the modes and methods of effective extraction of blackcurrant juice with preliminary enzymatic treatment of the pomace while preserving its natural chemical composition

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and biologically active substances. The study was conducted with blackcurrant fruits of the varieties Mynai Shmyrev, Belarus sweet, Novyna Prykarpattia in the laboratory of the Department of Technology of Storage and Processing of Plant Production and the Training and Production Department of the Uman National University of Horticulture. Research methods (physical, chemical, physical-chemical) and statistical processing of results (analysis of variance) are generally accepted. Blackcurrant fruit usually yields an unsatisfactory juice yield of 18-24%, which lacks its inherent colour. According to Harrington's desirability assessment, enzymatic treatment with pectofoetidine increases the juice yield from crushed fruit to satisfactory (37-44%) and very good (57-60%) for crushed fruit (pomace), which is 2-3 times higher. During the fermentation of pomace, three stages are distinguished: the first is the destabilisation of the colloidal system of the pomace (up to 1 hour); the second is the degradation of the pomace structure (up to 2 hours); and the third is the stabilisation of the process (after 3 hours). Optimum conditions for pomace fermentation are ensured by using 0.03% by weight of the preparation suspension, a temperature of 42-45 °C, and a process duration of 2 h. This results in up to 60% juice extraction, 96-102% acid conversion and up to 97% ascorbic acid content. The increase in juice yield by 6-15% correlates with the characteristics of the variety. In juices with sugar, the content of ascorbic acid is 1.7 times lower, but its preservation reaches 98-99%. Sugar and hot bottling are the factors that stabilise ascorbic acid in juices. Increasing juice yield and improving its quality is economically and technologically feasible

Keywords: enzymatic treatment; juice yield; juice quality; ascorbic acid preservation

INTRODUCTION

Blackcurrant juice has no equal in terms of nutritional and dietary value. Fruits with 88-94% cellular juice. However, it is not possible to completely separate the liquid and solid phases of the fruit during the extraction process. Natural blackcurrant juice has its own characteristics. O. Cherevko *et al.* (2020) formulated the objective reasons for the differences in juice extraction from blackcurrant fruits. The latter is conditioned by the fact that the value of the juice, namely flavour, aroma and, especially, colouring substances, is mainly localised in the cells of the integumentary tissues, which are difficult to destroy, even with varying degrees of mechanical grinding or crushing of the fruit. The juice yield of fruit depends on the cellular permeability of the fruit pulp, which has a mucilaginous consistency due to the pectin gel. Its splitting can loosen the tissues, reduce the viscosity of the juice, and facilitate its separation. Pectin substances in blackcurrant fruits are in a mobile state and to a certain extent determine the formation of physical and mechanical properties of fruit tissue.

According to O-C. Bujor *et al.* (2018), the hydrophilic biopolymer pectin can increase the water-holding capacity of plant tissue. The destruction of structural polymers that make up the middle plates of plant cells occurs, which leads to the breakdown of connections between cells and is the result of tissue breakdown into individual cells. The intercellular protopectin of the middle lamellae is connected to each other by ionic and covalent bonds. Ionic bonds are destroyed by heating at pH ⩽ 4.5. Covalent bonds are broken by a complex of enzyme preparations. The process of enzymatic hydrolysis of protopectin can occur due to the destruction of internal or external bonds of the cellulose-hemicellulose-pectin complex.

Furthermore, the shape, nature, and degree of strength of water bonds with fruit pulp affects juice extraction. Blackcurrant juice, as a biological liquid, belongs to a heterogeneous dispersed system. Adsorption processes occur on the surface of its particles, which help to stabilise the colloidal juice solution. C. Colodel *et al.* (2018) found that the total amount of bound water in blackcurrant fruits is in osmotically bound and colloidally bound forms, with a share of 78%. In currant fruits, osmotically bound water accounts for only about 46%, while in apples, cherries, pears, and other types of fruit, it accounts for 60-70%. It forms a coarse fibrous structure of pectin substances between which there is a spatial grid, the cells of which are filled with water molecules. The proportion of colloidally bound (adsorbed) water in currant fruit is 32%, while in the previously listed fruits it is 15-25%. It is formed due to the concentration of water molecules in the surface layer of colloidal particles of the product, which is part of the micelles of various hydrophilic colloidal solutions – proteins, polysaccharides. Extracting such water is quite difficult. In juice production, some of the colloidally bound water can be extracted by enzymatic treatment of the raw material and, as a result, the hydrate layer is destroyed.

D. Chettri and A. Verma (2023), K. Aaby and M.R. Amundsen (2023) substantiated that various biochemical changes occur during juice production, associated with processes such as hydrolysis, oxidation, reduction, condensation, etc. Some of these processes are necessary to form and improve the taste properties of juices, but the vast majority cause the formation of undesirable or harmful substances and, as a result, deterioration of juice quality. Namely, the presence of oxidising enzymes that cause darkening of the juice, enzymes that reduce the content or change colouring substances, enzymes that oxidise ascorbic acid, and reduce the value of the juice is undesirable in preparations.

Considering the high extractivity and acidity of natural blackcurrant juice, it is considered a semi-finished product for the production of pleasant and harmonious tasting juices with sugar. The purpose of this study was to establish optimum conditions and rational methods of preliminary enzymatic treatment of blackcurrant pulp to increase juice yield and their influence on changes in quality indicators.

LITERATURE REVIEW

One of the most promising methods of pre-treating fruits and berries for juice extraction is the use of enzyme preparations. Generally, pectolytic, cytolytic, and proteolytic enzyme preparations are used in the canning industry. According to B. Flaumenbaum *et al*. (1986), H. Schmidt *et al*. (2022), pectolytic preparations are widely used in juice production, primarily for clarification of natural juices and for depectinisation of juices before concentration. However, as pointed out by K. Fan *et al*. (2019), with the right type of preparation and treatment regimes, it is possible to increase the juice yield from fruits and berries by 5-20-30% (del Rocío Castro-López *et al*., 2016; Ninga *et al*., 2018).

The crucial factor affecting the completeness and speed of juice extraction is the presence of pectin substances. Their breakdown helps to loosen the tissues, reduce the viscosity of the juice, and facilitate its separation. However, the amount and fractional composition of pectin substances in fruits and berries is not the same. For each type of raw material, the most effective preparations with a specific enzyme complex should be selected. Pectolytic enzymes are obtained from extracts of Aspergillus avamori and Foetidus fungi. Such preparations, apart from a complex of pectolytic enzymes, contain hemicelluloses, celluloses, proteinases, polyphenol oxidases, etc. The amount of accompanying enzymes in the preparations is not the same. Some of them can negatively affect the quality of the juice, for instance, change its colour, oxidise ascorbic acid.

A. Todaro *et al*. (2016) describe the mechanism of action of enzyme preparations as follows: enzymes destroy protopectin, which cements individual cells together, resulting in cell separation and tissue loosening. Furthermore, the protopectin encrusting the cell membranes is destroyed, which weakens their mechanical strength and, as a result, the protective properties of the protoplasmic membranes underneath. Then enzymes and some non-enzymatic substances enter the cell, causing coagulation of protein-lipid colloids and cell death. This is due to the long-term effect of the temperature factor. These changes increase cellular permeability, protoplasmic membranes rupture, and juice yield increases. Notably, hydrolysis of protein compounds in pomace and juices also occurs with the help of pectolytic enzyme preparations with high proteolytic activity, such as pectofoetidine. The effect of pectolytic enzyme preparations on the juice yield from fruits and berries has been studied in research institutions and laboratories. M. Siddiq *et al*. (2018) found that when apple pomace and some types of berries were processed, depending on the enzyme preparation, the juice yield increased by 4.5-15%.

The most effective use of preparations is for raw materials that are difficult to juice. The treatment of gooseberry pulp with nigrin (0.05% and 0.1% by weight) increased juice yield by 11-19%, and additional heating of pulp to 70°C increased it by 29%. The addition of 0.02% suspension of pectavamorin, pectofoetidin, and phylazim to plum pomace at 80°C increased juice yield by 10%. Comparable data were obtained by I. Olawuyi *et al*. (2021) and A. Marsol-Vall *et al*. (2021), who claim that treated aronia, blueberry, and plum pomace increased juice yields to 61%, 87% vs. 57%, 50%, respectively. Treatment of blackcurrant fruit pomace with pectavamorin P10x (0.075% by weight) for 2 hours provided 67% juice yield (control -20%). The juice's sugar content increased by 78%, polyphenols by 2.5 times, and the amount of pectin decreased from 0.68% to 0.10%.

Analogous studies M. Siddiq *et al*., (2018) found the feasibility of 4-hour fermentation of the pomace. The juice yield was 64% (control – 16%). A sharp decrease in juice viscosity and pectin content (from 0.98% to 0.10%), an increase in polyphenols (from 0.22% to 0.55%), sugars (from 10.8% to 13.7%), and vitamin C (from 175 to 207 mg/100 g) was observed. Heating blackcurrant pomace to 45°C, adding 0.02% suspension of pectofoetidin P10x and pectavamorin P10x, and keeping for 4 hours at 40°C increased juice yield by 27-28%. The highest juice yield from blackcurrant pulp by centrifugation was obtained after treatment with pectofoetidine G20x – 75% (control – 32%). Under these conditions, A. Marsol-Vall *et al*. (2021) obtained up to 64% juice. The loss of pectin substances in the juice was 83.3%, and its viscosity was reduced by 8.3 times.

However, the use of pectin-degrading pectolytic preparations is only beneficial in weakening the protective properties of the cell membrane, which is deprived of the encrusting element, and facilitating pressing by reducing the viscosity of the flowing juice. The majority of the cell wall of blackcurrant fruit, which has completed its formation, is the secondary membrane, which mainly consists of polysaccharides – cellulose and hemicelluloses. They form a cell membrane matrix that prevents juice from leaking out. Therefore, it is advisable to use cytolytic enzyme preparations, such as cellulases, amylases, and hemicelluloses.

Experimental data showed that the maximum extraction of the liquid phase from apples (up to 85%) is provided by the cytolytic complex cellulase-100 due to the deep hydrolysis of a complex of polymers that determine the structural strength and water retention capacity of tissues. The complex treatment of raw materials with enzyme preparations pectofoetidin and celloretin increased the juice yield of currant berries by 22.5%. Furthermore, pectin substances (27% of the raw material), sugars, and colouring agents were better preserved. The source of cytolytic preparations can be enzymes of malt raw materials – cellulase, hemicellulose. They break down β-glucans and destroy the connection of pectin substances with cellulose and hemicellulose of plant tissue through the neutral polysaccharides arabinoxylans and arabinogalactans.

A complex of barley malt enzymes is used to produce a pectin extract. S. Wang *et al.* (2023) and T. Yu *et al.* (2023) confirmed the feasibility of using barley malt cytolytic enzymes for the pretreatment of blackcurrant berries (ratio of pulp: barley malt – 1:0.4, temperature – 40°C, duration – 2 hours). The juice yield was increased by centrifugation to 70%. The preservation of pectin content is 61-70% compared to 15.5% when treated with pectofoetidine. Considering the specifics of the raw materials and the features of the technological process, optimum conditions were created for the action of enzyme preparations for currant fruits: temperature – 35-40°C, pH – 2.8-3.5.

According to the technological instruction, the calculated amount of the preparation for the treatment of pomace is dissolved with a small amount of warm water (35-40°C), poured with 10 times the amount of juice heated to 45-50°C, and stirred until a homogeneous suspension is obtained. It is prepared immediately before introduction. The suspension is applied to the pomace of pome fruit immediately after grinding. 10-15% of water is added to the pulp of stone fruit, heated to 40-45°C, mixed with a slurry, and temper for 40-60 min before pressing. However, the Technological instruction (1992) does not contain any references to the specifics of processing the pomace of berry fruits and specifically blackcurrant fruits with enzyme preparations. The fermentation temperature is not specified. However, other scientific literature suggests that the pomace of crushed blackcurrant fruit is heated to 40-45°C, 0.03% of the enzyme preparation avamorin is added as a suspension in juice or water, mixed thoroughly, tempered for 4-6 h, and then pressed (Wang *et al.*, 2015).

Therefore, to increase juice extraction from blackcurrant fruits, preparations with a certain complex of enzymes should be selected: pectolytic, in which the presence of cytolytic and proteolytic enzymes is desirable, and by means of a differential approach, optimum conditions for their action should be created.

MATERIALS AND METHODS

Research on juice extraction was conducted during 2003-2006 with blackcurrant fruits of the varieties Minai Shmyrev, Belarus sweet, Novyna Prykarpattia in the laboratory of the Department of technology of storage and processing of crop production and in the Educational and Production Department of the Uman National University of Horticulture. Blackcurrant plantations are located in the central part of the Right-Bank Forest-Steppe of Ukraine. The region's climate is temperate continental with unstable moisture, uneven precipitation, and unstable temperatures.

Blackcurrant fruits of consumer ripeness were harvested at the end of June under favourable weather conditions, berries without tassels were selected in boxes-trays weighing 4-5 kg and transported to the laboratory. The weight of blackcurrant fruits of each variety is 60 kg. Juice was extracted by pressing with a screw press (pressure 0.3-0.4 MPa) after inspection, washing of the fruit, obtaining the pulp, preparation of the pulp by the following methods of pretreatment with the enzyme preparation pectofoetidine G20x (E 1500 units/g), dose 0.03%:

1. Mechanical grinding to the pomace (control).

2. Treatment with pectofoetidine, fermentation for 4 h at 42-45°C:

a) pomace of ground fruit; b) pomace of crushed fruit.

3. Pectofoetidine treatment of the pulp of crushed fruit at 37-40°C, 42-45°C:

a) a suspension of the dry preparation; b) an extract of the preparation after fermentation (1–2 h).

The suspension was prepared from the dry powder with a small amount of juice (to dissolve) before adding to the pomace. The extract of the preparation was obtained by pouring 5-10 times the amount of juice and insisting for 1-2 h.

4. Treatment of the pomace of ground fruits with pectofoetidin at 37-40°C, 42-45°C with a fermentation duration of 0.5, 1, 1.5, 2, 3, 4, 5, 6, and 8 h.

The mass of the sample for pressing – 1.5-2 kg. Threefold repetition. The juice yield was estimated using the generalised Harrington desirability function. To harmonise the taste of freshly pressed natural juices by chemical composition, they were used to prepare juices with sugar. According to the content of dry substances and acids that are regulated by (DSTU 4150:2003, 2004), they corresponded to the highest grade. According to the Technological instruction (1992), the recipe was 60% juice + 40% sugar syrup (30%).

The method of making blackcurrant juices with sugar involved mixing boiled syrup with juice, heating to 90-95°C, packaging, sealing (hot bottling). In raw materials, freshly pressed natural juices and juices with sugar (after 15 days of storage), the content of dry soluble substances, total sugar content, acid content, sugar-acid index, and ascorbic acid content were determined. Organoleptic evaluation of juices was performed. Preservation of ascorbic acid in juices was calculated according to the following formula (1):

$$P_{AA} = \frac{AA_1 \times m_1}{AA_2 \times m_2} \times 100\%, \tag{1}$$

where AA_1 is the content of ascorbic acid in the finished product, g; AA_2 is the content of ascorbic acid in the raw product, g; m_1 is the mass of the finished product, g; m_2 is the mass of raw product, g.

Determination methods:

- for the content of dry soluble substances – refractometric (Shirokov, 1974);
- for acid content – titrometric (Pleshkov, 1976);
- for total sugar content, invert sugar content, glucose – ferricyanide (Shirokov, 1974);
- ascorbic acid content - iodometric (Pleshkov, 1976);
- preservation of ascorbic acid (Flaumenbaum *et al.*, 1986).

The repetition of chemical analyses was threefold. The mass of the sample for analysis – 2 kg of fruit or 1.5 kg of product. Statistical processing of research results was performed using variance analysis. Experimental studies of plants (both cultivated and wild), including the collection of plant material, were following the institutional, national, or international guidelines. The study adhered to the standards of the Convention on Biological Diversity (1992) and the Convention on Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS AND DISCUSSION

Technochemical evaluation of pectofoetidine in the processing of blackcurrant pulp. The most effective enzyme preparation for juice extraction from blackcurrant fruits – pectofoetidine G20x (E 1500 units/g) – was used. It consists of a complex of enzymes: pectinase, polygalacturonase, pectin methyl esterase, cellulase, and amylase (Todaro *et al.*, 2016). According to the technological instruction, the dosage of the product is 0.01-0.03%. As the amount of pectin in the raw material increases, the dose of the product increases, but the excess of 0.03% is not allowed.

According to Table 1, pomace processing dramatically increased juice yield. This is due to the liquefaction of the pomace, reduction of the juice viscosity, and its easy separation. The influence of the raw material processing method on the juice yield is 98%. Juice extraction from fruit according to the Harrington desirability level (Cherevko *et al.*, 2020): very good – more than 55%, good – 48-55, satisfactory – 40-48, unsatisfactory – 35-40, very unsatisfactory – less than 35%.

Table 1. *Juice yield depending on the method of pretreatment of blackcurrant fruits of different varieties (preparation dose – 0.03%, fermentation – 4-6 h, temperature – 42-45°C), %*

Preprocessing method	Minai Shmyrev			Belarus sweet			Novyna Prykarpattia		
	2003	2004	2005	2003	2004	2005	2003	2004	2005
Mechanical grinding of fruits to pomace (control)	21.8	19.2	20.1	19.0	17.8	18.6	23.4	20.3	22.4
Pectofoetidine treatment of the pomace of ground fruit	59.2	57.3	58.8	58.2	56.7	57.5	60.3	57.2	59.6
Treatment of the pomace of crushed fruit with pectofoetidine	42.9	37.2	42.0	42.3	36.7	40.5	44.3	38.7	43.0
LSD_{05}					2.1				

Source: *developed by the authors of this study*

The physical and mechanical properties and specific anatomical structure of the raw materials adjusted the juice yield. When the preparation was used to treat the pomace of crushed fruit, the juice yield increased 3 times compared to the control and reached a very good score of 57-60%. With analogous treatment of the pomace of crushed fruits, the juice yield was estimated as satisfactory and amounted to 37-44%, which is only 2 times higher than in the control. The enzymatic maceration of crushed fruits increased the effectiveness of the preparation by 1.5 times. The result indicates that the blackcurrant pomace should be ground as much as possible when treated with an enzyme preparation. Currants have a dense, elastic skin. Grinding it increases the contact area and creates optimum conditions for the action of enzymes, as noted by L. del Rocío Castro-López *et al.* (2016). Clogging of the channels during the pressing of macerated pomace is not dangerous, as the juice flows out easily and only needs to be separated from the fruit tissue particles.

The difference in juice yield by variety is not always significant and ranged within 1.0-3.6%, which is due to the chemical composition of the fruit, tissue structure, the degree of cell damage during grinding, etc. No direct evidence of the influence of the chemical composition of the raw materials on the effect of the preparation was found, but in the pomace of different varieties of fruit, enzyme activation or inhibition processes may occur, as evidenced by T. Yu *et al.* (2023). In terms of chemical composition, freshly pressed juices obtained by treating raw materials with an enzyme preparation (Table 2) are close to fresh fruit. They differed in appearance – they lost their inherent viscosity and had a liquid, highly extractable phase. The colour of the juice is intense, the smell is well pronounced, typical of blackcurrant fruit. Whereas the juice from the control variants was devoid of natural colour.

***Table 2**. Indicators of the chemical composition of blackcurrant fruits of different varieties and juices from them (fermentation with pectofoetidine (dose – 0.03%, duration – 4-6 h, temperature – 42-45 °C)*

Preprocessing method	2003				2004				2005			
	Dry soluble substance content, %	Total sugar content, %	Acid content1), %	Sugar-acid index	Dry soluble substance content, %	Total sugar content, %	Acid content1), %	Sugar-acid index	Dry soluble substance content, %	Total sugar content, %	Acid content1), %	Sugar-acid index
Minai Shmyrev												
Fruits	16.0	7.26	3.32	2.19	15.8	7.01	3.05	2.30	16.4	8.91	2.65	3.36
Mechanical grinding of fruits to pomace (control)	15.6/21.4	7.06/16.24	3.20/1.92	2.21/8.46	15.4/21.2	6.83/16.1	2.92/1.75	2.34/9.20	16.0/21.6	8.68/17.2	2.50/1.52	3.47/11.3
Treatment of pomace of shredded fruits with the preparation	16.0/21.6	7.29/16.37	3.25/1.95	2.24/8.39	15.8/21.6	6.92/16.15	3.00/1.81	2.31/8.92	16.4/21.8	8.82/17.3	2.60/1.56	3.39/11.09
Treatment of pomace of crushed fruits with the preparation	15.8/21.4	7.20/16.32	3.20/1.92	2.25/8.50	15.6/21.4	6.92/16.15	2.95/1.77	2.35/9.12	16.0/21.6	8.63/17.17	2.50/1.50	3.45/11.45
Belarus sweet												
Fruits	16.0	8.82	2.55	3.46	14.2	6.56	2.31	2.84	16.8	9.21	1.86	4.95
Mechanical grinding of fruits to pomace (control)	15.6[2)]/21.4	8.68/17.20	2.42/1.45	3.59/11.8	13.8/20.4	6.38/15.8	2.12/1.27	3.01/12.4	16.4/21.8	9.12/17.4	1.76/1.05	5.18/16.24
Treatment of pomace of shredded fruits with the preparation	16.0/21.6	8.80/17.28	2.50/1.50	3.52/11.52	14.2/20.6	6.48/15.89	2.24/1.34	2.85/11.85	17.0/22.20	9.28/17.57	1.82/1.09	5.10/16.12
Treatment of pomace of crushed fruits with the preparation	16.0/21.6	8.76/17.26	2.48/1.48	3.53/11.66	14.0/20.4	6.38/15.82	2.18/1.30	2.93/12.17	17.0/22.20	9.30/17.57	1.79/1.07	5.19/16.42
Novyna Prykarpattia												
Fruits	14.6	7.91	2.61	3.03	13.2	6.01	2.18	2.76	13.8	8.40	1.79	4.69
Mechanical grinding of fruits to pomace (control)	14.0/20.4	7.56/16.61	2.41/1.45	3.17/11.52	12.8/19.6	5.80/15.48	2.10/1.25	2.76/12.38	13.4/20.0	8.26/16.96	1.65/1.00	5.01/16.96
Treatment of pomace of shredded fruits with the preparation	14.6/20.8	7.80/16.68	2.52/1.50	3.10/11.12	13.0/19.8	5.86/15.52	2.04/1.22	2.87/12.72	13.8/20.2	8.32/17.00	1.83/1.10	4.55/15.45
Treatment of pomace of crushed fruits with the preparation	14.4/20.6	7.78/16.67	2.48/1.50	3.13/11.11	12.8/19.6	5.76/15.46	2.00/1.22	2.88/12.67	13.8/20.2	8.26/16.95	1.80/1.08	4.59/15.69
LSD$_{05}$	0.04/0.12	0.41/0.68	0.09/0.11	0.22/0.88	0.04/0.12	0.41/0.68	0.09/0.11	0.22/0.88	0.04/0.12	0.41/0.68	0.09/0.11	0.22/0.88

***Note:** 1) in terms of citric acid; 2) above dash – natural juice, under dash – juice with sugar*
***Source:** developed by the authors of this study*

Juices with sugar met the standard's quality requirements. The positive effect of the treatment of raw materials with the enzyme preparation on the preservation of ascorbic acid was established (Table 3). The juices lost mainly 6-7% of ascorbic acid from the content in the fruit, or its retention was about 94% (control – 83-85%). That is, the ascorbic acid content in the juices of the natural experimental variants is on average 10% higher.

Table 3. Ascorbic acid content (mg/100 g) and its preservation (%) in blackcurrant fruits of different varieties and in juices from them (preparation dose – 0.03%, duration – 4-6 h, fermentation temperature – 42-45°C)

Preprocessing method	2003		2004		2005	
	Content	Preservation	Content	Preservation	Content	Preservation
Minai Shmyrev						
Fruits	182.1	-	176.0	-	172.0	-
Mechanical grinding of fruits to pomace (control)	151.1* 88.7	83.0 97.8	149.7 88.0	85.1 98.0	144.5 85.0	84.0 98.0
Treatment of pomace of shredded fruits with the preparation	171.7 101.5	94.3 98.5	156.2 92.6	88.8 98.8	162.2 96.0	94.3 98.6
Treatment of pomace of crushed fruits with the preparation	170.8 101.1	93.8 98.7	165.1 98.0	93.8 98.9	161.3 95.4	93.8 98.6
Belarus sweet						
Fruits	273.5	-	308.0	-	272.8	-
Mechanical grinding of fruits to pomace (control)	227.0 133.2	83.0 97.8	261.5 152.9	84.9 98.1	289.1 134.8	84.0 98.1
Treatment of pomace of shredded fruits with the preparation	257.1 152.0	94.0 98.5	289.5 171.3	94.0 98.6	256.4 151.7	94.0 98.6
Treatment of pomace of crushed fruits with the preparation	258.2 152.9	94.4 98.7	288.3 170.9	93.6 98.8	255.3 151.5	93.6 98.9
Novyna Prykarpattia						
Fruits	229.3	-	180.4	-	192.4	-
Mechanical grinding of fruits to pomace (control)	191.2 112.4	83.4 98.0	153.3 90.3	85.0 98.2	162.0 95.1	84.2 97.8
Treatment of pomace of shredded fruits with the preparation	216.2 128.3	93.4 98.9	170.0 101.0	94.2 99.0	151.0 89.5	78.5 98.8
Treatment of pomace of crushed fruits with the preparation	215.1 128.0	93.8 99.2	142.1 84.7	78.8 99.3	152.2 90.4	79.1 99.0
LSD_{05}	6.25 5.72	-	6.25 5.72	-	6.75 5.72	-

Note: *above dash – natural juice, under dash – juice with sugar*
Source: *developed by the authors of this study*

The high preservation of ascorbic acid in juices was facilitated by the conditions of the technological process – heat treatment of raw materials, their deaeration, and the lack of oxygen access during fermentation (Marsol-Vall *et al.*, 2021). However, in freshly pressed juices from Novyna Prykarpattia fruit, the amount of ascorbic acid was lost by 26-27% of the amount contained in fresh fruit, and its preservation in juices decreased to 79%. These results are probably not natural. They are attributed to the oxidation of the pomace by air oxygen during prolonged fermentation and a breach of the hermetic state, while other process conditions did not change.

In juices with sugar, a 1.7-1.9-fold decrease in ascorbic acid content was observed (control – 2-fold). Juices with sugar in the experimental variants contained, as a rule, 11-14% more ascorbic acid. The preservation of ascorbic acid in juices with sugar in the control variant is 98%, in the experimental variant it is about 99%. The characteristics of blackcurrant varieties had a significant impact (80%) on the ascorbic acid content in juices. Its high content in the fruits of the Belarus sweet variety had a positive effect on the indicator in juices. The level of the juice index is 1.2-1.4 – 1.5-1.7 times higher than in juices from the Novyna Prykarpattia and Mynai Shmyrev varieties.

Influence of heat treatment of raw materials and methods of application of enzyme preparation on the yield and quality of juices from blackcurrant fruits. According to I.F. Olawuyi *et al.* (2021), the accelerated action of a pectolytic enzyme preparation is provided by heat treatment of raw materials. This process partially destroys protopectin, coagulates and dehydrates colloids, as a result of which the cells lose their ability to retain juice. Such changes promote greater enzyme contact, facilitate the enzymatic hydrolysis of pectin substances, which considerably increases the effectiveness of the enzyme preparation, even though heat treatment apparently partially inactivates the pectolytic enzymes of raw materials. Furthermore, heat treatment of raw materials prevents microbiological and oxidative processes. Therefore, only with the use of complex thermal and enzymatic treatment of the pomace can one count on a high result. As already mentioned, the optimum temperature for the action of enzyme preparations for blackcurrant fruits is 35-40°C.

The effect of temperature on the efficacy of pectofoetidine G20x in the blackcurrant pomace was not found. However, it is known (Wang *et al.*, 2015) that enzyme preparations are thermostable and do not inactivate at 65-70°C for 10-15 min. Complete inactivation is achieved at 70°C in one hour. By raising the temperature to 45-50°C, the enzymatic hydrolysis of pectin substances can be significantly enhanced. However, the optimum temperature for the action of the pectinase enzyme, which is part of pectofoetidine, is 40°C. Exceeding a temperature of 45°C leads to its inactivation. The researchers found a variant that combined the most favourable conditions – heating the pomace to 50-52°C before fermentation. At the same time, the methods of applying the enzyme preparation in the form of a suspension and an extract were studied. The positive result of applying the enzyme preparation does not depend on the amount of juice applied, but on its time sustained in the juice. During this time, it is inactivated by the polyphenolic substances in the juice. With less juice, the enzyme inactivation is less.

The studies have shown (Table 4) that the method of application of the preparation and the temperature of processing the pomace of crushed fruit had a considerable effect on the juice yield. Under the same conditions, the process of applying the enzyme preparation in the form of a suspension is more efficient – the juice yield reached 58-63%, which is 11-15% more than from the pomace treated with the preparation extract. The use of the enzyme preparation for blackcurrant pomace does not require any preliminary preparation of the extract. Before adding the preparation to the pomace, the dry powder should first be dissolved in a small amount of juice.

***Table 4**. Juice yield from blackcurrant fruit pulp of different varieties depending on the method of preparation application (fermentation time – 4-6 h, dose – 0.03%), 2006, %*

Method of application of the preparation into the pomace	Minai Shmyrev		Belarus sweet		Novyna Prykarpattia	
	Pomace fermentation temperature, °C					
	37-40*	42-45	37-40*	42-45	37-40*	42-45
Suspension	60.0	60.5	57.0	58.8	62.8	63.4
Extract	51.8	53.6	48.1	50.5	54.3	55.2
LSD_{05}	1.38					

Note: ** the pomace was preheated to 50-52°C*
Source: *developed by the authors of this study*

According to the results of juice yield, depending on the fermentation temperature, the increase in juice yield from the pomace fermented at 42-45°C was not always significant. The reasons for this effect lie in the processing time and the sensitivity of enzymes to temperature. The fermentation of the pomace for 4 h proved to be long-lasting and had an ambiguous effect on juice extraction, which is confirmed by Z. Wang *et al.* (2015). The temperature probably also changed the effect of the preparation. Preheating the pomace to 50-52°C had two effects: it intensified the reactions and did not irreversibly inactivate enzymes (e.g., pectinase). However, further fermentation at 37-40°C inhibited the activity of the enzymes of the added preparation. Fermentation at 37-40°C with the integrated use of preheat treatment of the pomace can be recommended for the extraction of juice from certain varieties of blackcurrant. However, increasing the temperature of fermentation of the pomace to 42-45°C is more effective regardless of the variety.

According to Table 4, the difference in juice yield when treating the fruit of the studied varieties with the enzyme preparation ranged from 3-5% to 12-15%. In terms of chemical composition, freshly pressed juice from raw materials treated with an enzyme preparation is close to fresh fruit (Table 5). It is logical to increase the acidity of juices by 1.4-2.8% due to the accumulation of galacturonic acid due to the breakdown of pectin. Its enzymatic hydrolysis is quite complicated under the influence of a complex of enzymes, and the specific effect of various chemical compounds on their activity. Acid degradation, neutralisation, and complexation with metals as a result of the long enzymatic process are possible. This assumption can be confirmed by the fact that the pH of the juices decreases, which is in line with analogous statements by S. Wang *et al.* (2023). Comparison of freshly pressed juices obtained at different fermentation temperatures showed no difference in physicochemical parameters.

***Table 5**. Indicators of the chemical composition of blackcurrant fruits of various varieties and juices from them, 2006*

Method of application of fermented preparation to the pomace	Fermentation temperature 37–40°C						Fermentation temperature 42–45°C					
	dry soluble substance content, %	total sugar content, %	acid content (in terms of citric acid), %	sugar-acid index	ascorbic acid content, %	preservation of ascorbic acid, %	dry soluble substance content, %	total sugar content, %	acid content (in terms of citric acid), %	sugar-acid index	ascorbic acid content, %	preservation of ascorbic acid, %
Minai Shmyrev												
Fruits	13.2	7.10	2.10	3.38	169.8	-	13.2	7.10	2.10	3.38	169.8	-
Suspension	13.0 19.8	7.01 16.20	2.05 1.23	3.45 13.17	163.6 96.9	96.3 98.7	13.2 20.0	7.02 16.21	2.07 1.24	3.39 13.07	164.6 97.3	96.9 98.5
Extract	12.6 19.6	6.85 16.12	2.06 1.25	3.32 12.90	154.0 91.5	90.7 99.0	12.8 19.8	6.90 16.14	2.05 1.20	3.37 13.45	155.7 92.3	91.7 98.8
Belarus sweet												
Fruits	17.0	8.80	1.44	6.11	252.6	-	17.0	8.80	1.44	6.11	252.6	-
Suspension	16.8 22.0	8.69 17.22	1.47 0.86	5.91 20.02	242.3 143.6	95.9 98.8	16.8 22.0	8.72 17.23	1.40 0.84	6.23 20.51	242.0 144.1	96.2 98.8
Extract	16.4 21.8	8.60 17.16	13.9 0.84	6.19 20.43	224.8 133.5	89.0 99.0	16.4 21.8	8.65 17.19	1.46 0.88	5.92 19.53	226.1 134.0	89.5 98.8
Novyna Prykarpattia												
Fruits	13.2	7.80	1.41	5.53	226.2	-	13.2	7.80	1.41	5.53	226.2	-
Suspension	12.8 19.8	7.60 16.54	1.45 0.84	5.24 19.69	219.4 130.5	97.0 99.1	13.2 20.0	7.72 16.63	1.37 0.81	5.64 20.53	217.6 129.3	96.2 99.0
Extract	12.6 19.6	7.50 16.50	1.40 0.82	5.36 20.12	205.8 122.5	91.0 99.2	12.8 19.8	7.61 16.57	1.39 0.85	5.47 19.49	207.0 122.7	91.5 98.9
LSD_{05}	0.02 0.04	0.28 0.54	0.06 0.07	0.29 1.67	4.76 4.46	-	0.02 0.04	0.28 0.54	0.06 0.07	0.29 1.67	4.76 4.46	-

***Note:** above dash – natural freshly pressed juice, under dash – juice with sugar*
***Source:** developed by the authors of this study*

The characteristics of the variety had a significant impact on the chemical composition of the juices – the influence was 95%. The juices of the Belarus sweet variety have high soluble solids and sugars content, and due to their low acidity, their sugar-acid index is on average 1.8 times higher than that of the Minay Shmyrev variety. The quality of the freshly pressed juices was reflected in the finished product – juices with sugar. In terms of dry soluble solids (19.6-22.0%), the latter met the requirements of the standard. However, in terms of acid content (0.82-0.88%), juices with sugar from Belarus sweet and Novyna Prykarpattia did not meet the standard, which is due to the initial acid content of 1.4% in the fruit. The sugar-acid index of juices from the Minay Shmyrev variety was medium, and the sugar-acid index of the Belarus sweet and Novyna Prykarpattia varieties was high – 20. The organoleptic evaluation of juices with sugar revealed a natural appearance, aroma, and colour typical of blackcurrant. They had a liquid consistency and high extractability. The taste of the juices was pleasant but lacked softness and lightness due to the liquefaction of the fruit mass during fermentation and the transfer of insoluble substances not specific to juice components to the juice.

The preservation of ascorbic acid in freshly pressed juices is high – 90-97% and depended more on the method of application than on the temperature of pomace fermentation. In the juices from the pomace treated with the preparation suspension, the retention of ascorbic acid is 96-97%, and in the preparation extract – 89-92%. The preheating of the pomace to 50-52°C before fermentation at 37-40°C played a crucial role in this. Under these conditions, oxidative enzymes are inactivated, which increases the preservation of ascorbic acid. On the other hand, a higher juice yield results in a better extraction of ascorbic acid, especially from the peel. In juices with sugar, the content of ascorbic acid decreased by 1.7-1.9 times, but its preservation was high – about 99%.

Influence of the duration of enzyme treatment and pomace temperature on the yield and quality of blackcurrant juices. The treatment of pomace with an enzyme preparation, along with the hydrolysis of pectin substances (reducing viscosity), also involves biochemical and microbiological transformations. These processes do not affect the quality of the juices during short-term ageing, but in the case of prolonged fermentation, the quality of the juices deteriorates and in some cases the product may spoil. Oxidation processes often cause undesirable changes in the taste and colour of juices. Furthermore, the pomace contains the microflora of the raw material, which multiplies freely at 20-40°C and can cause undesirable microbiological changes. Heat treatment of raw materials to some extent prevents microbiological and oxidative processes. However, the effects of oxidising enzymes cannot be avoided, even in the case of heat treatment of raw materials.

The advantage of blackcurrant as a raw material for juice production is that its fruit has a low activity of oxidative enzymes. It contains ascorbic acid, which acts as a natural antioxidant. Its content of 1 mg/g already has a positive effect. Ascorbic acid simultaneously increases the activity of enzymes. In the scientific literature, there are quite opposite conclusions regarding the duration of pomace treatment – from 40-60 min to 4-6 h or more. This is due to different raw materials, different enzyme preparations and their activity. The influence of the duration of treatment of pomace with a suspension of the enzyme preparation pectofoetidin G20x at different temperatures on the yield of blackcurrant juice was studied.

A detailed analysis of the data (Table 6) showed that the duration of pomace processing affected the efficiency of juice extraction. Regularities in the increase of juice yield during the process were established. After only 0.5 h of fermentation, the juice yield was 36-42%, which was about 60-65% of the total yield. Each subsequent 0.5 h of pomace fermentation, up to a total duration of 2 h, significantly increased the juice yield: by 7-11% in 1 h, by 10-15% in 1.5 h, and by 14-16% in 2 h. If the juice extraction from the fermented pomace for 1 h was 65-72%, then for 1.5 h it was 74-81%, and for 2 h it was 82-93%.

***Table 6**. Juice yield depending on the temperature and duration of fermentation of blackcurrant pulp of different varieties, 2006, %*

Fermentation duration, h	Minai Shmyrev		Belarus sweet		Novyna Prykarpattia	
	Fermentation temperature, °C					
	37-40*	42-45	37-40*	42-45	37-40*	42-45
0.5	37.4	39.2	35.7	36.2	40.8	42.3
1.0	40.3	43.6	38.2	40.3	43.8	46.3
1.5	45.7	49.5	43.0	46.4	48.3	52.2
2.0	52.5	56.3	47.0	53.7	55.6	60.0
3.0	57.8	60.0	52.6	57.0	59.8	62.3
4.0	60.0	62.1	55.0	58.0	61.8	63.4
5.0	61.0	62.5	56.6	58.5	62.8	63.8
6.0	61.5	62.8	57.0	59.0	63.3	64.0
8.0	61.7	63.4	57.2	59.6	63.5	64.4
LSD_{05}	3.8					

Note: ** preheated to 50-52°C*
Source: *developed by the authors of this study*

Fermentation of the pomace for 2 to 4 hours increased the juice yield, but the intensity of the process was slowed down. If the juice extraction from the pomace fermented for 3 h was 92-97%, and for 4 h – 96-98%, the increase in juice yield was 4–10% and 2-5%, respectively. Juice extraction after 4 h was 98-99%, but the increase was not significant – 0.3-2%. The characteristic changes in the pomace during fermentation depended on temperature and variety characteristics (Table 6). Regardless of the process duration, the juice extraction has always been better from the pomace at a fermentation temperature of 42-45°C: the juice yield is 1-8% higher. The difference is most pronounced in the first hours of fermentation. After 2 h of pomace fermentation, juice extraction at 37-40°C was 82-87%, at 42-45°C – 89-93%, after 3 h – 94% and 95-97%, and after 4 h – 96-97% and 97-98%. Further fermentation of the pomace is not advisable.

The process of fermenting the pomace depended on the characteristics of the variety. Juice extraction from the flesh of the Novyna Prykarpattia variety was the best. Regardless of the duration of the process, the juice yield was 3-9% higher than that of the Minay Shmyrev fruit pulp, and 9-15% higher than that of the Belarus sweet fruit pulp. When fermenting the pomace for up to 8 h, the juice yield may increase.

However, this fermentation time is technologically and economically impractical. Due to the deep hydrolysis of pectin substances, the acidity of the juice increases and ethyl and even methyl alcohols can accumulate (Ninga *et al.*, 2018).

This is confirmed by the acid content of the juices. Within half an hour of fermentation (Table 7), the colloidal system was so destabilised that 94 to 99% of the acids were transferred to the juices. Then a gradual increase (by 2.7-5.0%) in the acid content of the juices was observed by 3 h. The fermentation of the pomace was 97-103%. However, the difference in the acid content of the juice between the second and third hour of fermentation is 1%. After 4 h, the accumulation of acids in the juices decreased (2.9-5.4%), and their transfer to the juice remained at 98-103%. Only after 8 h of fermentation, the acidity of the juices increased again by 3.2-5.7%, and the acid content reached 98-104%.

Table 7*. Acid content in freshly pressed natural juice from blackcurrant fruits of different varieties (in terms of citric acid), 2006, %*

Fermentation duration, h	Minai Shmyrev		Belarus sweet		Novyna Prykarpattia	
	Fermentation temperature, °C					
	37-40*	42-45	37-40*	42-45	37-40*	42-45
0.5	2.064	2.097	1.360	1.380	1.360	1.380
1.0	2.070	2.104	1.367	1.387	1.374	1.394
1.5	2.084	2.124	1.380	1.401	1.394	1.414
2.0	2.104	2.144	1.387	1.420	1.420	1.441
3.0	2.117	2.157	1.401	1.434	1.427	1.447
4.0	2.124	2.164	1.407	1.441	1.427	1.454
5.0	2.124	2.164	1.407	1.441	1.427	1.454
6.0	2.124	2.164	1.407	1.442	1.434	1.454
8.0	2.131	2.171	1.414	1.447	1.434	1.461
LSD_{05}	0.02					

Note: ** preheated to 50-52°C*
Source: *developed by the authors of this study*

The process of fermentation of blackcurrant pulp can be divided into 3 stages: the first (up to 1 h) – destabilisation of the colloidal system, characterised by a sharp decrease in pomace viscosity, mainly by the transition of raw material components into juice; the second (up to 3 h) – degradation of the pomace structure, characterised by liquefaction, a significant decrease in its viscosity and increased transition of raw material components into the juice due to the destruction of polysaccharides; third (after 3 h) – stabilisation of processes due to the completion of structural changes.

It can be assumed that after destabilisation of the colloidal system, the breakdown of polygalacturonic acids begins. Due to the formation of galacturated acids, the amount of reduced substances increases, but there is no further reduction in pomace viscosity. The acidity of freshly pressed juices increased by 0.5-3.6%. With an increase in the temperature of pomace fermentation to 42-45°C, the accumulation of acids in the juices increased by 1-2%. Furthermore, while 100% of the acid content in pomace juices at 37-40°C was recorded after 2 h of fermentation, at 42-45°C it was recorded after 1.5 h. The characteristics of the variety affected the juice yield of raw materials. The pomace from the fruits of the Novyna Prykarpattia and Minai Shmyrev varieties had the best technological properties. The extraction of acids from it into the juice was more intensive and reached 102-103%. Structural changes in the pomace from the Belarus sweet variety were slower. This affected the juice yield.

The issue of preserving vitamin C in juices during pomace fermentation is of scientific and practical importance (Table 8). After fermentation of the pomace for 0.5 h, the content of ascorbic acid in the juices decreased by 6-11%. Its safety was high – 90-94%. Its loss during pomace production was obviously to some extent overlapped with its extraction from the pomace, as already established above (Table 7). After 1-1.5 h of pomace fermentation, the content of ascorbic acid in the juices continued to increase by 2-4%, and its preservation was up to 93-97%. Fermentation of the pomace for up to 3 h caused its loss in juices, but its preservation stayed at 95-96%. With further fermentation of the pomace, the content of ascorbic acid in the juices decreased and the level of its preservation decreased sharply – from 93-94% in 4 h to 80-86% in 8 h.

***Table 8**. Ascorbic acid content (mg/100 g) and its preservation in freshly pressed natural juice from blackcurrant fruits of different varieties, 2006*

Fermentation duration, h	Minai Shmyrev		Belarus sweet		Novyna Prykarpattia	
	Fermentation temperature, °C					
	37-40*	42-45	37-40*	42-45	37-40*	42-45
0.5	154.6/91.1	156.0/91.8	227.0/89.9	230.2/91.1	208.4/92.1	213.0/94.2
1.0	159.6/94.0	163.0/96.0	234.8/93.0	240.3/95.1	214.0/94.6	219.4/97.0
1.5	163.1/96.0	164.8/97.1	240.0/95.0	244.8/96.9	219.4/97.0	218.9/96.8
2.0	163.3/96.2	164.9/97.1	245.0/97.0	245.0/97.0	219.4/97.0	219.0/96.8
3.0	162.6/95.8	163.0/96.0	239.5/94.8	240.3/95.1	217.4/96.1	214.9/95.0
4.0	159.0/93.6	159.3/93.8	234.4/92.8	234.0/92.9	212.5/93.9	212.6/94.0
5.0	157.8/92.9	157.1/92.5	230.0/91.0	231.2/91.5	210.3/93.0	211.5/93.5
6.0	153.7/90.5	151.7/89.3	225.3/89.2	222.3/88.0	206.5/91.3	205.9/91.0
8.0	145.2/85.5	140.8/82.9	210.3/83.3	204.2/80.8	195.2/86.3	190.7/84.3
LSD_{05}	5.0/1.0					

Note: ** preheated to 50-52°C*
Source: *developed by the authors of this study*

Temperature had a significant effect on the ascorbic acid content of the juices only up to 2 h of fermentation. Its preservation in juices from pomace fermented at 42-45°C is 1-2% higher, which is due to an increase in its extraction into the juice. Fermentation of the pomace for up to 6-8 h sharply reduced the content of ascorbic acid in the juices. Moreover, its losses in juices at 42-45°C are higher, and its preservation is lower – 81-84% versus 83-86% at 37-40°C. Apparently, as noted by M. Siddiq *et al.* (2018), at higher temperatures during the long process, the oxidation of ascorbic acid was more intense. The content of ascorbic acid in the juices depended on the variety, with a 92% effect. Juices from the Belarus sweet and Novyna Prykarpattia varieties contained almost 1.3-1.5 times more ascorbic acid than juices from the Minay Shmyrev variety. Its preservation had a comparable trend.

Based on the analysed data, it can be concluded that, unlike other scientists who have conducted analogous studies, it was found that the enzymatic treatment of the pomace with pectofoetidin increases the juice yield from crushed fruit to satisfactory (37-44%) and very good (57-60%) for ground fruit, which is 2-3 times higher than without treatment. Rational conditions for pomace fermentation are ensured by using 0.03% of the preparation by weight, a temperature of 42-45°C, a processing time of 2 h with up to 60% of the juice extracted, with 96-102% of acids transferred to it and up to 97% of ascorbic acid content.

Studies confirm the findings of K.A. Ninga *et al.* (2018), S. Wang *et al.* (2023) that the juice in blackcurrant fruits is firmly retained by living tissue. Mechanical destruction of the cell membrane by grinding is not sufficient. It is necessary to use additional methods of exposure, which is explained by the specific features of the structure and physiological properties of the cellular tissue of blackcurrant fruits. Their ability to juice is less dependent on the resistance of cytoplasmic membranes to mechanical stress, and more on their viscosity and elasticity, the cytological and anatomical structure of the cell tissue, and the content of pectin substances. The presence of the latter, with a high calcium content in the mineral composition of the juice, increases the stability of its cytoplasm and makes it difficult to extract the juice. Juice flow is inhibited by the high content of soluble pectin substances in blackcurrant, which increase the viscosity.

It is not enough to mash blackcurrant fruits; they must be subjected to special processing. Treatment with enzymatic pectolytic preparations, which primarily destroy soluble pectin, is quite effective. This is confirmed by I.F. Olawuyi *et al.* (2021) and A. Marsol-Vall *et al.* (2021). However, on the other hand, pectolytic enzymes also act on cells with toxic non-enzymatic substances that are part of the preparation and cause coagulation of protein-lipid membranes and plant cell death. It is as a result of such transformations that cell permeability increases, protoplasmic membranes break and juice yield increases. The disadvantage of the studies is the lack of safety indicators for blackcurrant juice.

Furthermore, a classic enzyme preparation, pectofoetidine, was used in the studies. However, there are other enzyme preparations with different composition and enzyme activity. Their disadvantages and advantages during the extraction of blackcurrant juice with a high content of ascorbic acid were not established. Blackcurrant juices are healing products for children (del Rocío Castro-López *et al.*, 2016). However, a question arises regarding their study for use in infant and dietary nutrition due to the use of enzyme preparations. No direct evidence of their negative impact on juice quality has been found. In recent years, the environment has been increasingly contaminated by mutagens of physical, chemical, and biological origin. Hormonal imbalance due to chronic stress is a source of mutagenic danger. Mutagenesis can pose a threat to human health. A range of mutagens have carcinogenic effects. It is important to establish the antimutagenic properties of blackcurrant juice. This is a priority for further research.

It should be borne in mind that scientists and breeders are working on developing blackcurrant varieties with specific consumer properties, improving technological methods of growing the crop, protecting it from pests and diseases in different weather conditions, etc. All this requires constant, ongoing research into the impact of both internal and external factors on the quality of blackcurrant as a raw material with high C-vitamin activity. Thus, the use of blackcurrant genetic resources, both today and in the future, is inexhaustible. And the study of the formation of the biochemical value of fruits, ways to increase juice yield is a theoretical and practical prerequisite for the production of high-quality juices that solve the problem of providing the population with biologically valuable products of natural origin all year round.

CONCLUSIONS

Based on the study, the high efficiency of blackcurrant pomace treatment with pectofoetidine G20x for juice extraction was established. In the absence of concrete recommendations for the extraction of blackcurrant juice in the technological instructions for the use of enzyme preparations, it was found that the yield of blackcurrant juice increases when the pomace is treated with the enzyme preparation pectofoetidine G20x. Moreover, depending on the method of mechanical impact on the fruit, from a satisfactory level of 37-44% (crushing) to a very good level of 57-60% (grinding), which is 2-3 times more than without processing. The effectiveness of the preparation is increased by 1.5 times by grinding the fruit before fermentation.

For maceration of blackcurrant pomace from the preparation, it is not advisable to prepare the extractor beforehand. Since the juice yield from pomace treated with a powder suspension (0.03% by weight) is 11-15% higher. Fermentation of blackcurrant pomace is a lengthy process, which includes three stages: the first is destabilisation of the colloidal system of the pomace (up to 1 h); the second is degradation of the pomace structure (up to 2 h); the third is stabilisation of the process (after 3 h). Optimum conditions for pomace maceration: temperature – 42-45°C, duration – 2 h. Under these conditions, the juice yield increases to 56-60%. The juice is characterised by almost complete conversion of acids, including ascorbic acid, from the raw material.

The quality and biological value of freshly pressed blackcurrant juices from fermented raw materials are close to fresh fruit in terms of physicochemical and organoleptic characteristics. The preservation of ascorbic acid in juices with sugar is 90-97% (83-85% without processing). The strength of the variety's influence on the preservation of ascorbic acid in freshly pressed juices is 92%. The production of blackcurrant juices with sugar does not reduce the C-vitamin value of the product. Therewith, the content of ascorbic acid in juices is reduced by 1.7 times due to the preparation technology, but its preservation is high – 99%. The characteristics of the blackcurrant variety substantially affect the juice yield. An increase in juice yield by 6-15% correlates with the chemical composition of the fruit. Considering that scientists are working towards the development of the latest enzyme preparations, possibly even complex ones, further research is promising.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Режими та способи обробки м'язги чорної смородини ферментним препаратом для підвищення соковіддачі

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Анотація. Чорна смородина – полівітамінна культура з високими дієтичними та лікувальними властивостями є джерелом виключно цінної сировини для виробництва соків, проте витягання його ускладнене хімічним складом. Ендокарпій оплодня чорної смородини (власне м'якоть) формується ослизненими арилусами, які оточують насінини, що перешкоджає добуванню соку. Мета роботи – наукове обґрунтування режимів та способів ефективного витягання соку чорносмородинового з попередньою ферментативною обробкою м'язги за збереження його природного хімічного складу та біологічно активних речовин. Дослідження проведено з плодами чорної смородини сортів Минай Шмирьов, Білоруська солодка, Новина Прикарпаття в умовах лабораторії кафедри технології зберігання і переробки продукції рослинництва та навчально-виробничого відділку Уманського національного університету садівництва. Методи досліджень (фізичні, хімічні, фізико-хімічні) та статистична обробка результатів (дисперсійний аналіз) загальноприйняті. З плодів чорної смородини, зазвичай, одержують незадовільний вихід соку – 18-24 %, що позбавлений властивого забарвлення. За оцінкою бажаності Харрінгтона ферментативна обробка пектофоетидином збільшує вихід соку з роздавлених плодів до задовільного (37-44 %) та дуже доброго (57-60 %) для плодів подрібнених (м'язга), що в 2-3 рази більше. Під час ферментації м'язги виділено три стадії: перша – дестабілізації колоїдної системи м'язги (до 1 год.); друга – деградації структури м'язги (до 2 год.); третя – стабілізації процесу (після 3 год.). Оптимальні умови ферментації м'язги забезпечуються застосуванням 0,03 % від маси суспензії препарату, температури 42-45°C, тривалості процесу 2 год. Це спричинює повноту витягання соку до 60%, переходом у нього 96-102% кислот та збереженістю до 97 % вмісту аскорбінової кислоти. Підвищення виходу соку на 6-15 % корелює з особливостями сорту. У соках із цукром вміст аскорбінової кислоти в 1,7 рази нижчий, проте її збереженість сягає 98-99 %. Факторами стабілізації аскорбінової кислоти у соках є цукор і гарячий розлив. Збільшення виходу соку та покращення його якості економічно та технологічно доцільне

Ключові слова: ферментативна обробка; вихід соку; якість соків; збереженість аскорбінової кислоти

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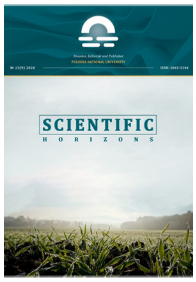

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Evaluation of collection specimens of guar (*Cyamopsis tetragonoloba* L.) by economically valuable traits

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Abstract. For effective introduction of guar into production, it is necessary to use varieties adapted to the Southern Steppe of Ukraine, the creation of which is impossible without well-researched collection material, which is relevant and important. Guar is in high demand globally in the food and oil industries, and most importantly, thanks to its ability to symbiotically fix nitrogen, it improves soil quality in a cost-effective and natural way. However, it is a completely new culture for Ukraine. The purpose of this study was to investigate the gene pool of guar plants and identify the best ones with economically valuable traits. The study used field, laboratory, and statistical methods. The results of the study conducted in 2020-2022 helped to identify the best introduced guar samples in terms of tolerance to hot temperatures and the arid climate of the Southern Steppe of Ukraine. The most adapted to this zone are IU07466 Haldi bhati and IU074658 Pusa Naubahar. They formed the maximum yields: IU07466 Haldi bhati – 262.5 g/m^2, IU074658 Pusa Naubahar – 329.0 g/m^2, which is more than 135% compared to the standard. The above-mentioned samples were characterised by a vegetation period of 133.5 days and 129.5 days, respectively, which, on average, over two years of research, exceeded the duration of the "germination – maturation" period of the standard variety IU074657 Ankur by 4.5-8.5 days. The correlation and regression analysis of the data obtained from the study of the introduced samples of the guar gene pool suggested a direct correlation between the weight of seeds per square metre and the number of beans and seeds per plant. The correlation coefficient is 0.997-0.986, respectively. It is recommended to use the identified sources of valuable traits for further effective implementation in the breeding and

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research programmes of scientific institutions to create self-pollinated lines, synthetic populations, varieties adapted to the extreme conditions of the Southern Steppe of Ukraine

Keywords: guar; plant gene pool; length of the growing season; adaptability; productivity

INTRODUCTION

The almost complete destruction of the irrigation system during the war poses a challenge for farmers to introduce crops that are not watered or require minimal irrigation water in the southern region of Ukraine. One of these annual legumes, which is gaining great popularity in the world, is guar (*Cyamopsis tetragonoloba* L.). To grow guar in the south of the country, suitable varieties are required. S. Ismail *et al*. (2019), A. Alshameri *et al*. (2019) argue that guar is relatively drought-resistant compared to many other crops and is well adapted to arid and semi-arid climates with high temperatures, tolerant of saline soils. It is grown in India, Pakistan, the USA (Texas and Oklahoma), Sudan, and Australia. Like other legumes, guar is used in the national economy. M. Shakir *et al*. (2020) indicated that guar cake, similar to soybean meal, is a separate additive to animal food due to its high crude protein content of about 480 g/kg dry matter.

Demand for the crop is growing rapidly around the world due to the use of guar gum in industrial applications such as paper and textiles, ore flotation, explosives, and the oil industry for hydraulic fracturing of oil and gas formations. Q. Zhu *et al*. (2019) note that gum is used to produce about 300 industrial products. R. Kuhns & G. Shaw (2018) report that guar gum is the main derivative of guar, it is obtained from the seeds of the plant *Cyamopsis tetragonoloba* of the *Leguminosae* family. D. Sandhu *et al*. (2021) found that it is a natural high molecular weight polysaccharide, also known as the natural stabiliser E412, an excellent emulsifier, viscosity regulator, and emulsion stabiliser, which is useful for controlling many health problems such as diabetes, heart disease, and colon cancer. Due to its gel-forming properties, guar gum reduces cholesterol and glucose levels.

Recent studies by B. Acharya *et al*. (2022) and D. Sandhu *et al*. (2022) have shown that guar gum can be produced at lower prices than imported gums and produce lower greenhouse gas emissions than existing crops. Given the widespread use of guar and its products in the national economy and the high purchase prices, Ukraine should not be dependent on its imports, considerably saving currency assets. However, this requires having varieties adapted to the region where the crop will be sown. According to Ukrainian scientists R. Vozhegova *et al*. (2022a), the main requirements that breeders have for growing guar in a particular zone are early maturity, productivity, and quality indicators of guar. F. Gresta *et al*. (2018) emphasise that the duration of the ripening period of some guar genotypes can be longer than 160 days, and therefore this should be factored in. S. Thapa *et al*. (2018) recommend sowing guar in crop rotation because of its ability to symbiotically fix nitrogen, improving soil quality in a cost-effective and natural way.

J. Singh *et al*. (2021) claim that 350-400 mm of rainfall during the growing season is sufficient for growing guar. In addition, A. Garcia *et al*. (2023) report that guar can also be implemented in regions with low precipitation (under 400 mm). According to R. Vozhegova *et al*. (2022b), the weather conditions in the southern zone of Ukraine are similar to those where guar is grown: on average, 300-350 mm of precipitation falls per year, with fluctuations over the years from 250 to 650 mm, and the sum of effective temperatures above 10°C is 3,200-3,400°C. Therefore, the study of the guar gene pool and the selection of the best economic traits will allow them to be used to create new varieties, and short-stemmed varieties are suitable for these purposes. Considering the analysis of the above sources, the purpose of this study was to investigate the adaptability of guar collection samples to the natural and climatic conditions of the Southern Steppe of Ukraine and to identify the sources of valuable traits.

MATERIALS AND METHODS

The examination of the collection of new guar samples, their in-depth research in the irrigation conditions of the Southern Steppe of Ukraine and preservation of their authenticity in the face of climate change was a planned research work during 2020-2022 under the state applied task: “To investigate guar samples to create varieties with their further implementation and use in production”. The study was conducted at the Breeding Department of the Institute of Irrigated Agriculture of the National Academy of Sciences of Ukraine (now the Institute of Climate-Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine). The subject of the study was the introduced collection samples of guar IU074657 Ankur, IU074658 Pusa Naubahar, IU074659 Maharandi, IU074660 Sheetal, IU074661 Haldi bhati, IU074662 Aryan, IU074663 Tindal, obtained for the study from the National Centre for Plant Genetic Resources of Ukraine. The best samples were submitted for registration to the National Centre for Plant Genetic Resources of Ukraine as valuable samples in terms of morphological, biological, and economic characteristics.

The soil of the experimental plot is dark chestnut medium loamy, with a topsoil containing 2.0-2.2% of total humus, 1.8 mg/kg of nitrate nitrogen, 32.3 mg/kg of mobile phosphorus and 251.0 mg/kg of potassium.

The limiting factor in technological support is insufficient rainfall during the growing season. The specificity of the zone also lies in the rather severe effects of air drought during dry days. Therefore, growing guar in the Southern Steppe zone of Ukraine is possible only under irrigation conditions.

The agrotechnical conditions of the experiments are generally accepted for southern Ukraine (Goloborodko & Kokovkhin, 2014). Its predecessor – winter wheat. 100 kg/ha of ammonium nitrate was applied for pre-sowing cultivation. Sowing was carried out in the first decade of May, when the soil temperature at a depth of 5 cm was 18-20°C. Samples of the collection nursery were sown in single-row plots 5 m long at 100 kg/ha without repetition. The guar variety IU074657 Ankur was used as a standard. After sowing, Hortus herbicide (2 l/ha) was applied to the soil surface. In June, postemergent herbicide Picador was applied (1 l/ha). Between June and September, the area was irrigated 3 times at 400 m³/ha.

To characterise the weather conditions, the study used data from the Kherson Agrometeorological Station located near the experimental field. The weather conditions during the years of research were typical for the southern region of Ukraine, which facilitated an objective assessment of the introduced material, selection of the best samples by morphological, biological, and economic characteristics (Figs. 1, 2, 3).

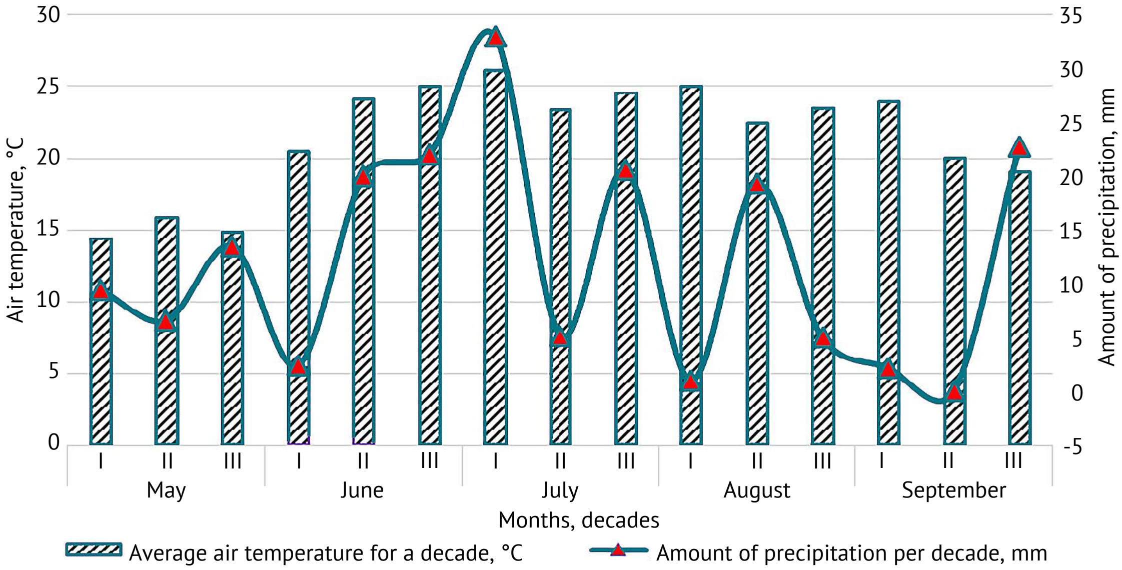

***Figure 1**. Trends in air temperature (°C) and precipitation (mm), 2020*
***Source:** compiled by the authors of this study*

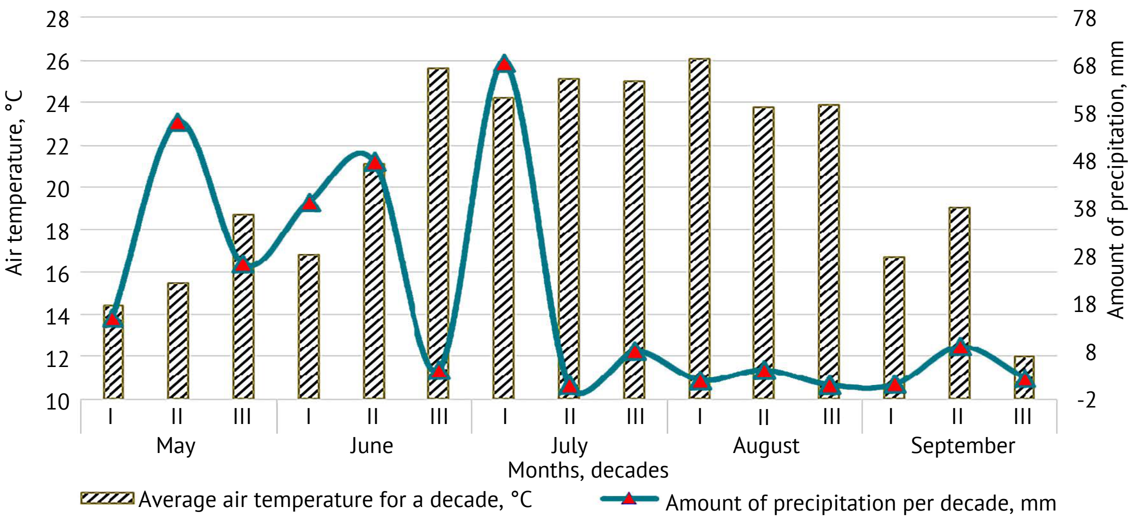

***Figure 2**. Trend in air temperature (°C) and precipitation (mm), 2021*
***Source:** compiled by the authors of this study*

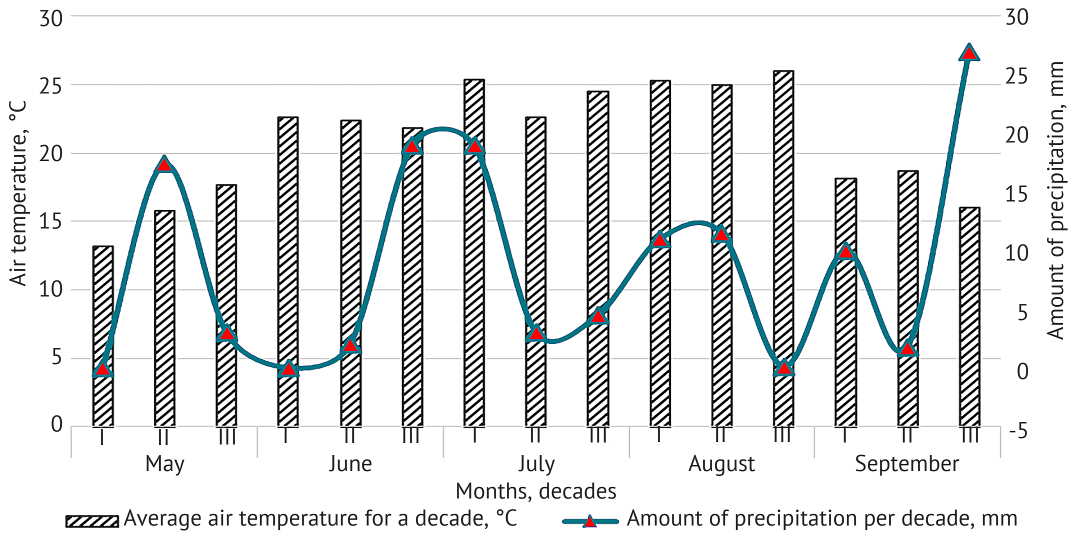

***Figure 3**. Trends in air temperature (°C) and precipitation (mm), 2022*

***Source:** compiled by the authors of this study*

Observations and records were kept during the study (Kobyzieva *et al.*, 2014). The duration of the growing season was determined according to the description of the periods and phenological phases of growth and development of guar plants. The beginning of the phase was considered to be the presence of at least 15% of plants, and 75% of plants were considered to have the full phase (Volkodav, 2001). In the phase of full ripeness in the field, 10 registered plants were measured for plant height and the height of the lower bean attachment above the soil level. The final indicator of such accounting is the average plant height and the height of the lower bean attachment. Once ripe, the guar plants were harvested by hand. Elements of the guar harvest structure were determined according to the methodology of the state variety testing by test sheaves taken from an area of 1 m^2 (Volkodav, 2001). In laboratory conditions, the structural analysis was evaluated according to the following main quantitative traits: "number of beans", "number of seeds" per plant according to the Broad Unified Classifier of the genus *Glycine max*. (L.) Merr. (Kobyzieva *et al.*, 2014).

The seed yield was measured by individual weighing from each plot at the stage of harvest ripeness. After weighing, an average sample of seeds was taken from each plot, followed by determination of their moisture content in the laboratory and conversion to 14% moisture content (Goloborodko & Kokovkhin, 2014). The harvest from each plot was brought to 14% moisture content using formula (1):

$$X = \frac{Ax(100-B)}{100-14}, \quad (1)$$

where X is the grain yield at 14% moisture content t/ha; A is the grain yield without adjustment for moisture content t/ha; B is the grain moisture content at harvest, %.

Statistical analysis of the experimental data and determination of linear correlation coefficients were performed according to the methodology of Goloborodko and Kokovkhin (2014).

RESULTS AND DISCUSSION

According to M. Mahdipour-Afra *et al.* (2021), V. Mealing and A. Landis (2021), the length of the guar growing season is 60-90 days for determinate and 120-150 days for indeterminate varieties. A study of the guar collection in 2020 showed that in the arid climate of the Southern Steppe of Ukraine, some varieties had a longer growing season than soybeans. The shortest, 120 days, was observed in samples Maharandi IU074659, Sheetal IU074660, Tindal IU074663. Under the conditions of 2020, not all the beans formed on the plant ripened in the samples Pusa Naubahar IU074658 and Haldi bhati IU07466, the duration of the growing season of which was 130 days (Table 1).

***Table 1**. Characterisation of guar samples by morphological and biological traits, 2020*

Sample name and registration number	Growing season, days	Height, cm		Quantity, pcs./plant		Seed weight, g	
		plant	lower bean attachment	beans	seeds	per 1 m^2	% to standard
IU074657 Ankur	125.0	46.7	3.0	17.0	56.5	150.0	–
IU074658 Pusa Naubahar	130.0	48.2	3.2	16.6	64.7	210.0	140
IU074659 Maharandi	120.0	43.0	2.2	6.0	23.0	78.0	52

Table 1. Continued

Sample name and registration number	Growing season, days	Height, cm		Quantity, pcs./plant		Seed weight, g	
		plant	lower bean attachment	beans	seeds	per 1 m^2	% to standard
IU074660 Sheetal	120.0	44.0	3.1	2.2	4.0	6.0	4
IU074661 Haldi bhati	130.0	42.3	3.2	23.0	102.0	324.0	216
IU074663 Tindal	120.0	33.5	4.0	2.0	13.0	48.0	32

Source: *compiled by the authors of this study*

According to the trait "plant height", almost all guar samples were at the level of IU074657 Ankur and were characterised by small values – 33.5-48.2 cm and had "very small attachment of the lower bean" – 2.2-4.0 cm. Haldi bhati IU074661 stood out for its "very high" productivity, with 102 seeds/plant, and Sheetal IU074660 – for its "very low" productivity, with 4 seeds/plant. "Very high" yields, more than 135% compared to the control, were observed in Pusa Naubahar IU07465 210 g/m^2 and Haldi bhati IU074661 324 g/m^2. As Figure 4 shows, the maximum yield was formed by samples with a longer vegetation period of 130 days, namely IU074658 Pusa Naubahar and IU074661 Haldi bhati. On the same samples, a larger number of beans and seeds per plant was observed – 16.6 and 23.0 and 64.7 and 102.0 pieces, respectively.

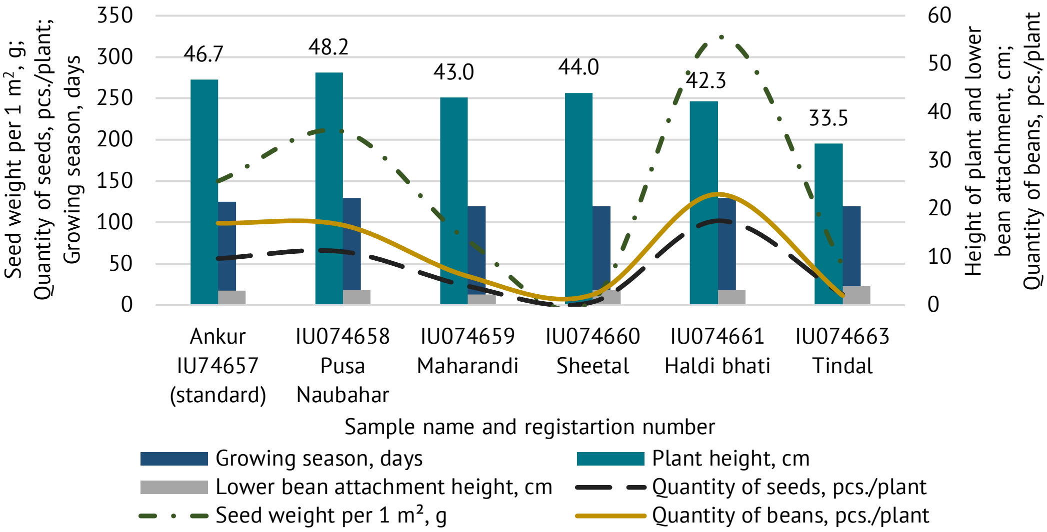

***Figure 4**. Dependence of seed weight (g/m^2) on morphological and biological traits, 2020*

Source: *compiled by the authors of this study*

Based on the correlation and regression analysis of the research results, it was found that there is a direct correlation between the seed yield per square metre and the number of beans and seeds per plant. The correlation coefficient is 0.994-0.966, respectively (Fig. 5).

The duration of the growing season in 2021 was 7-9 days longer than in 2020 (Table 2).

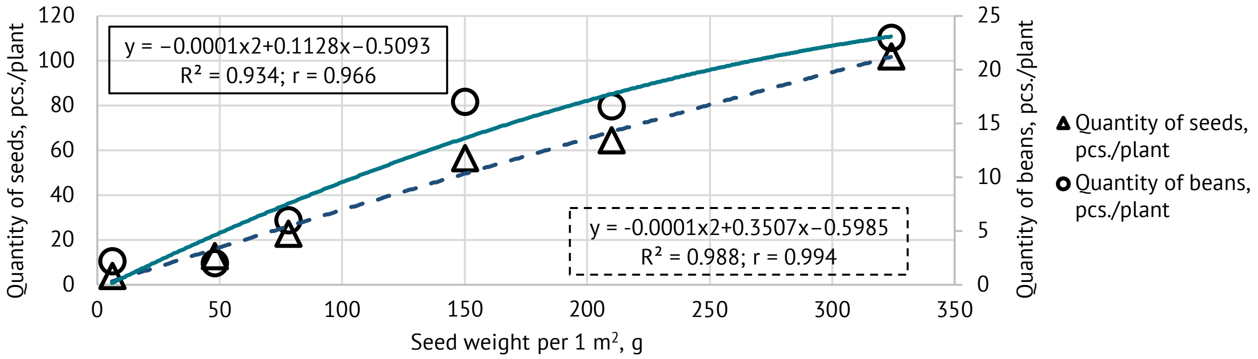

***Figure 5**. Statistical model of the dependence of seed weight (g/m^2) on the number of beans and seeds per plant, 2020*

Source: *compiled by the authors of this study*

Table 2. Characteristics of guar samples by a set of economically valuable traits, 2021

Sample name and registration number	Growing season, days	Height, cm		Quantity, pcs./plant		Seed weight, g	
		plant	lower bean attachment	beans	seeds	per 1 m^2	% to standard
IU074657 Ankur	126.0	46.8	2.9	17.0	56.6	150.1	–
IU074658 Pusa Naubahar	137.0	58.7	5.2	28.6	117.7	315.0	210
IU074659 Maharandi	129.0	49.5	3.4	18.0	59.0	178.0	119
IU074660 Sheetal	129.0	46.1	3.8	10.2	34.0	116.0	78
IU074661 Haldi bhati	137.0	45.3	4.2	29.0	132.0	334.0	223
IU074663 Tindal	129.0	43.2	5.0	17.0	48.0	148.0	99

***Source:** compiled by the authors of this study*

This was facilitated by precipitation of 68.4 mm in the first ten days of July and a drop in average daily air temperatures in September to 12.0-16.0°C. The duration of the vegetation period in the samples IU074657 Ankur was at the level of the standard variety IU074659 Maharandi, IU074660 Sheetal and IU074663 Tindal, which was 129 days. All beans formed on the plant did not ripen in the samples IU074658 Pusa Naubahar and IU07466 Haldi bhati with a growing season of 137 days, but they formed the maximum yield. Samples IU074658 Pusa Naubahar and IU074661 Haldi bhati formed 28.6 and 29.0 beans, which amounted to 117.7 and 132.0 seeds per plant, respectively, and contributed to a remarkably high yield of 315 g/m^2 and 334 g/m^2, respectively (Fig. 6).

These indicators exceeded the standard variety IU074657 Ankur by 59.9-72.9%. According to the statistical model of the dependence of seed weight/m^2 on the number of beans and seeds per plant, presented in Figure 7, there is a direct relationship between these traits: r = 0.976-0.966, respectively.

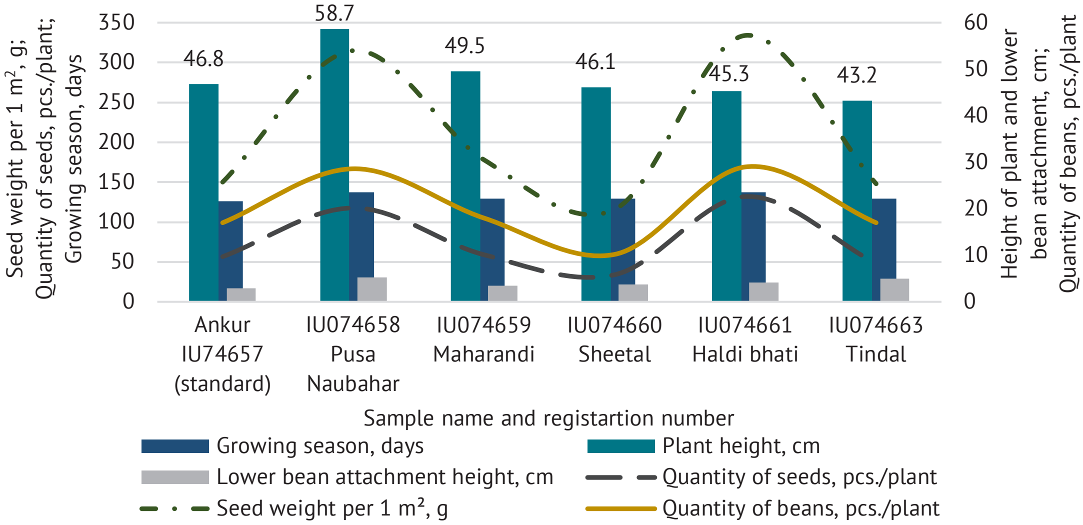

***Figure 6**. Dependence of seed weight (g/m^2) on morphological and biological traits, 2021*
***Source:** compiled by the authors of this study*

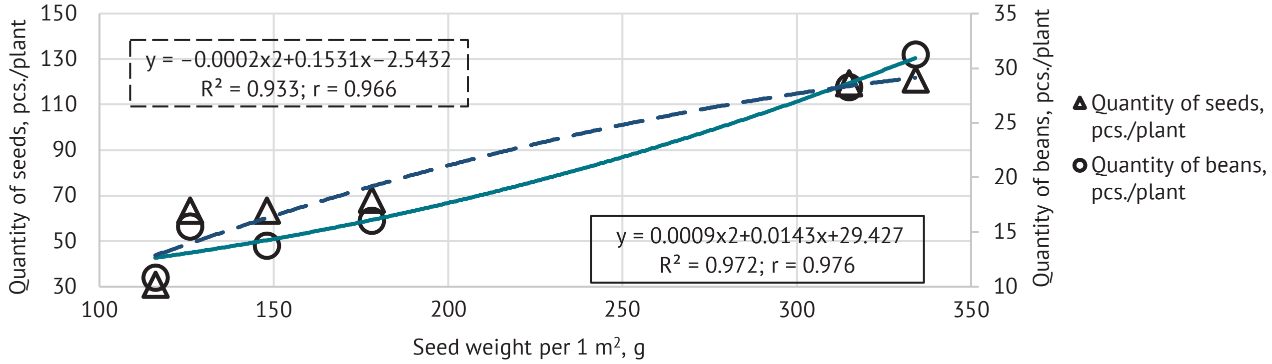

***Figure 7**. Statistical model of the dependence of seed weight (g/m^2) on the number of beans and seeds per plant, 2021*
***Source:** compiled by the authors of this study*

The studied collection samples of guar by the trait "plant height" were within 43.2-69.5 cm (IU074661 Haldi bhati, IU074663 Tindal – IU074659 Maharandi, respectively), and the height of bean attachment ranged within 3.0-4.2 cm (IU074657 Ankur – IU074658 Pusa Naubahar, respectively), which corresponds to the gradation "very small" (Table 3).

Three-year studies of guar samples have shown that the length of the plant growing season has a significant impact on the formation of seed mass/m^2. IU074659 Maharandi, IU074660 Sheetal, and IU074657 Ankur were characterised by the shortest parameters of this trait – from germination to maturation, which was 124.5-125.0 days. However, they produced very low yields. The maximum values of this trait were observed in IU07466 Haldi bhati with a growing season of 133 days and in IU074658 Pusa Naubahar, with a growing season of 129.5 days, which is more than 135% compared to IU074657 Ankur, the standard, and amounts to 262.5 g/m^2 and 329.0 g/m^2, respectively. The correlation and regression analysis of the data obtained from the research results of guar collection samples for three years of research suggests a direct correlation between the weight of seeds per square metre and the number of beans and seeds per plant (Fig. 8).

The correlation coefficient is 0.997-0.986, respectively (Fig. 9).

***Table 3** Characteristics of guar samples according to a set of economically valuable traits, average for 2020-2022*

Sample name and registration number	Growing season, days	Height, cm		Quantity, pcs./plant		Seed weight, g	
		plant	lower bean attachment	beans	seeds	per 1 m^2	% to standard
IU074657 Ankur, standard	125.0	46.7	3.0	17.0	56.5	150.0	–
IU074658 Pusa Naubahar	129.5	48.5	4.2	22.6	91.2	262.5	175
IU074659 Maharandi	124.5	69.5	2.8	12.0	41.0	128.0	86
IU074660 Sheetal	124.5	45.0	3.5	6.2	14.0	61.0	41
IU074661 Haldi bhati	133.5	43.2	3.7	26.0	116.0	329.0	220
IU074663 Tindal	129.0	43.2	5.5	17.0	48.0	148.0	99

***Source:** compiled by the authors of this study*

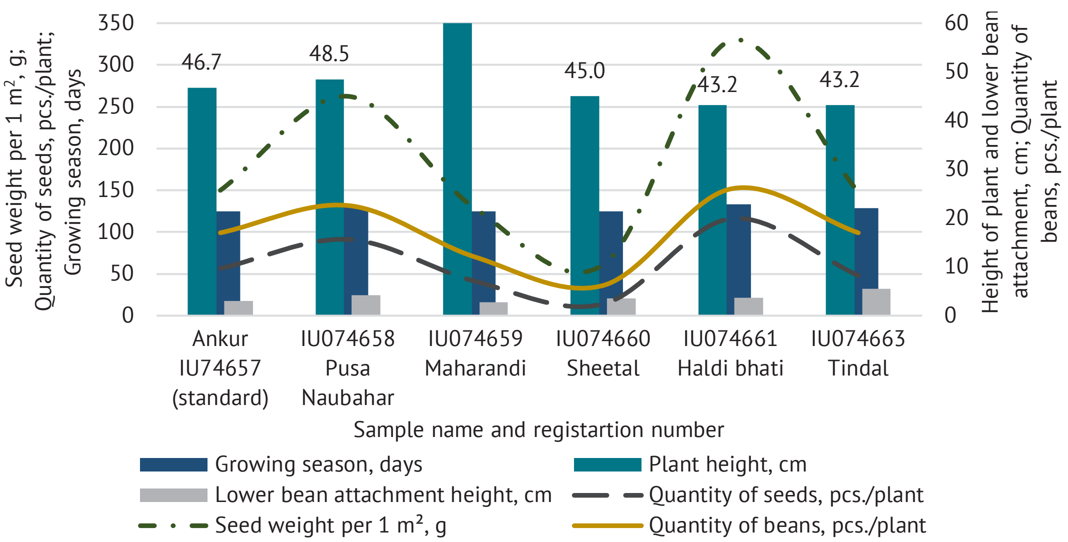

***Figure 8**. Dependence of seed weight/m^2 of the studied guar gene pool samples on morphological and biological traits, average for 2020-2022*

***Source:** compiled by the authors of this study*

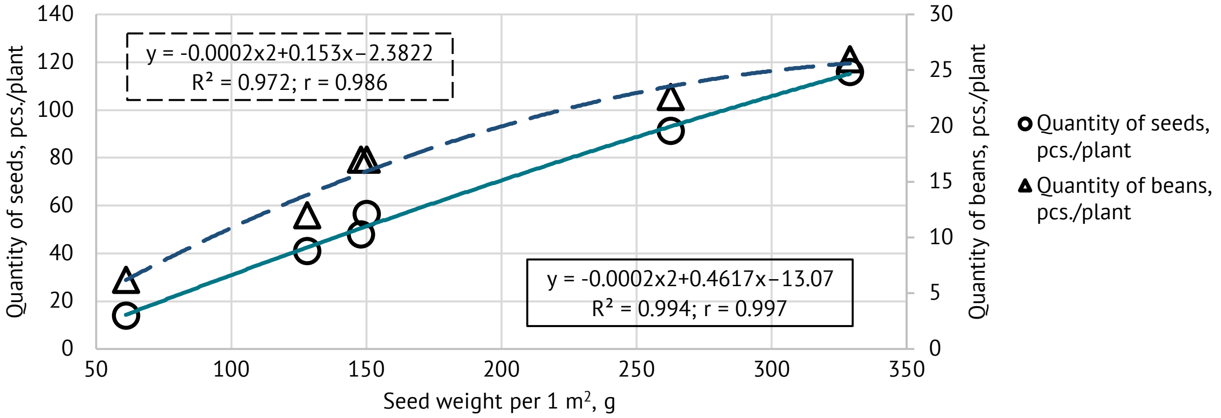

Figure 9. *Statistical model of the dependence of seed weight/m² on the number of beans and seeds per plant, average for 2020-2022*
Source: *compiled by the authors of this study*

The best samples of the guar collection IU07466 Haldi bhati and IU074658 Pusa Naubahar, which were the most adapted to the arid climate of the Southern Steppe of Ukraine, were involved in intraspecific hybridisation (Fig. 10).

Figure 10. *The guar plant on which the hybrid bean was formed*
Source: *photo taken by the author of this study*

As a result of these crosses, valuable source material was obtained – hybrid combinations Ankur/Pusa Naubahar, Ankur/Maharandi, Ankur/Sheetal, Ankur/Haldi bhati, Ankur/Tindal, Pusa Naubahar/Ankur, Haldi bhati/Ankur for further breeding work. The main producer of guar gum is India, where 80% of the world's acreage is concentrated, followed by Pakistan, which accounts for 15% of the acreage, and the United States (Texas and Oklahoma), which accounts for 5%. Clearly, very few countries produce guar. The Institute of Climate-Smart Agriculture is the only one in Ukraine that studies a crop whose plant sample collection was provided for study by the National Centre for Plant Genetic Resources of Ukraine. Since guar is a completely new crop for Ukraine, current research has focused on the study of relevant genotypes.

The guar gene pool is made up of sample numbers of different ripeness groups. According to two-year observations by R. Vozhegova *et al.* (2022a) on the growth and development of plants, it was found that in the Southern Steppe of Ukraine, where the frost-free period is 180-200 days and the growing season is 225-230 days, they can mature and form high productivity. The findings of the current study are in line with the results of G. Jaramillo *et al.* (2019), M. Amiri *et al.* (2022), who argue that the weather conditions of the year should favour the ripening of guar with a growing season longer than 160 days. However, there is some difference in this regard. Comparison of the duration of the growing season of the samples studied in the southern region of Ukraine on this basis showed that guar ripening occurred earlier than 160 days and was within 124.5-133.5 days.

IU074659 Maharandi and IU074660 Sheetal were characterised by the highest early maturity. However, these samples formed lower yields than those with a longer ripening period – on the reaction of the studied genotypes to irrigation. When growing guar in the southern region of Ukraine, three irrigations were made at a rate of 400 m³/ha. S. Hussain *et al.* (2020) share the same opinion, also recommending three waterings during the guar growing season. R. Shrestha *et al.* (2021) report that guar, as a drought-tolerant crop, can be grown without irrigation. Therewith, the plant produces slightly lower yields. At the same time, S. Ahmadi *et al.* (2017) argue that for normal growth it is necessary to optimise the irrigation regime of the crop. It was found that in the non-irrigated plots, the plants were stressed by drought, which, accordingly, slightly reduced the germination index, germination percentage, and plant height; in the irrigated plots, the above indicators were higher.

The correlation and regression analysis of the average data obtained from the results of research of guar collection samples for three years of study showed that there is a fairly close positive correlation between the indicators of seed productivity and the number of beans and seeds per plant**.** The correlation coefficient is 0.997-0.986, respectively. Comparable positive correlation values of r = 0.806 between seed yield and the number of pods per plant were obtained by Santonoceto *et al*. (2019). The analysis of literature sources and the results of studying the gene pool of guar plants indicate that guar genotypes are characterised by high drought resistance. Screening genetic resources for this trait seems to be a promising strategy for gaining access to drought-tolerant varieties. The discovery of drought-tolerant genotypes is essential to ensure sustainable guar production.

CONCLUSIONS

As a result of the conducted study, the collection samples of guar (Cyamopsis tetragonoloba *L.) were* evaluated according to economic and morphological-biological characteristics. Studies have shown that *Cyamopsis tetragonoloba* prefers dry or moist soil and can tolerate drought. Therefore, the weather conditions of the Southern Steppe of Ukraine are favourable for the introduction of this crop, which requires a minimum amount of irrigated water. This will be especially important in the post-war period when a significant part of irrigated land will need to be restored. Ukraine will become one of 35 countries in the world where guar will be grown.

According to the analysis of literature sources, it was found that for the effective introduction of guar into production, it is necessary to have varieties adapted to the region where it will be sown. The best specimens in the guar collection have been identified in terms of morphological, biological, and economic characteristics. IU074659 Maharandi, IU074660 Sheetal, and IU074657 Ankur were characterised by the shortest duration of the growing season (124.5-125.0 days). IU07466 Haldi bhati and IU074658 Pusa Naubahar had the highest yields of 262.5 g/m^{2}and 329.0 g/m^2, respectively. Furthermore, the above samples were characterised by an increased number of formed seeds per plant – 91.2 and 116.0 pcs. It was found that there is a fairly close positive correlation between the indicators of seed productivity and the number of beans and seeds per plant: r = 0.997-0.986, respectively.

The scientific enrichment on the effectiveness of involving guar samples adapted to the south of Ukraine in the breeding process was further developed. As a result of intraspecific crossing, it was possible to expand the process of forming hybrid populations of Ankur/Pusa Naubahar, Ankur/Maharandi, Ankur/Sheetal, Ankur/Haldi bhati, Ankur/Tindal, Pusa Naubahar/Ankur, Haldi bhati/Ankur. In future research, the best forms will be selected for desirable economically valuable characteristics such as early maturity, productivity, quality, and ability to fix nitrogen from the air, and further breeding work will be carried out with them.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Оцінка колекційних зразків гуару (*Cyamopsis tetragonoloba* L.) за господарсько-цінними ознаками

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Анотація. Для ефективного впровадження гуару в виробництво, необхідно використовувати сорти, адаптовані до Південного Степу України, створення яких неможливе без добре дослідженого колекційного матеріалу, що є актуальним і важливим. Гуар користується широким попитом у світі в харчовій та нафтовидобувній промисловості, а головне – завдяки здатності до симбіотичної фіксації азоту, покращує якість ґрунту рентабельним і природним шляхом. Проте є зовсім новою культурою для України. Метою наукової роботи було проведення дослідження генофонду рослин гуару та виділити кращі за господарсько-цінними ознаками. В роботі було використано польовий, лабораторний, статистичний методи. Результати проведених досліджень впродовж 2020-2022 рр. дозволили виділити кращі інтродуковані зразки гуару за толерантністю до високої температури та посушливого клімату Південного Степу України. Найбільш пристособленими до цієї зони є IU07466 Haldi bhati та IU074658 Pusa Naubahar. Вони сформували максимальні показники врожайності: IU07466 Haldi bhati – 262,5 г/м^2, IU074658 Pusa Naubahar – 329,0 г/м^2, яка, по відношенню до стандарту, становить більше 135%. Вище згадані зразки характеризувались тривалістю періоду вегетації 133,5 та 129,5 діб, відповідно, що в середньому за два роки досліджень перевищувало тривалість періоду «сходи – дозрівання» стандартного сорту IU074657 Ankur на 4,5-8,5 діб. Кореляційно-регресійний аналіз отриманих даних результатів досліджень інтродукованих зразків генофонду гуару свідчить, що існує пряма залежність між показниками маси насіння з одного метра квадратного та кількістю бобів і насінин на рослину. Коефіцієнт кореляції складає 0,997-0,986, відповідно. Виділені джерела цінних ознак рекомендується використовувати для подальшого ефективного впровадження в селекційні та дослідницькі програми наукових закладів з метою створення самозапилених ліній, синтетичних популяцій, сортів, адаптивних до екстремальних умов Південного Степу України

Ключові слова: гуар; генофонд рослин; тривалість періоду вегетації; адаптивність; продуктивність

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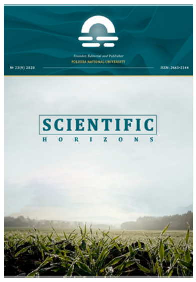

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Agrobiological substantiation of growing Hungarian vetch in mixed crops

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Abstract. The production of high-protein feed stays an integral part of the development of feed production. The purpose of this study was to investigate the biological characteristics of Hungarian vetch when grown in agrophytocenoses using different types of winter cereals. Changes in the chemical composition of dry matter at different microstages of growth and development were studied, and the influence on the yield, nutrient content, and digestibility of plant material was determined depending on the

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stages of growth and development. The study was conducted using field, laboratory, laboratory-field, and statistical methods. It was found that to reach the full flowering phase, the sum of active temperatures should be 754°C, with precipitation of about 100 mm and daylight hours of 973 h. Observations of the growing season indicate the relationship between plants and the environment, which is important to consider when growing Hungarian vetch. Vetch plants reach a height of 100-104 cm in conditions of excessive moisture. The phytocoenotic balance of such mixtures, specifically vetch of the Orlan variety and winter triticale of the Bozhych and Bohodarske varieties, ensures sustainable productivity and nutritional value of plant material. The best harvesting time for high quality vetch is from the beginning to the middle of flowering, which ensures a crude protein content of 17-20% in dry matter. The effectiveness of mixed phytocoenoses has been confirmed by ecological tests of crop combination models in different soil and climatic zones of Ukraine, where yields of 36.2-51.8 t/ha of green mass and 7.1-11.5 t/ha of dry matter were obtained over the years of use. The creation of a mixed crop allows producing high-quality plant material that is superior in energy and protein content to conventional vetch and oat mixtures. It was recommended to create such mixtures by sowing 2.5 mln/ha of cereal and 2.1 mln/ha of leguminous components after applying $N_{45}P_{45}K_{45}$ to form a crop with high digestibility and nutrition. The system of mixed cropping of Hungarian vetch is promising for the production of silage and high-quality haylage in the face of climate change and as a precursor for post-cutting sowing of maize or sorghum crops

Keywords: Hungarian vetch; winter triticale; growth and development; yield; crude protein; feed nutrition

INTRODUCTION

The ongoing transformation of agriculture in the face of climate change requires innovative solutions for efficient and sustainable feed production. One of the most promising areas is the introduction of new annual legumes into winter intercropping systems (Petrychenko *et al.*, 2020). Among them, Hungarian vetch (*Vicia pannonica* Crantz.), a frost-resistant legume species with historical roots in ancient Rome, offers promising opportunities.

Despite its ancient heritage, Hungarian vetch is grown mainly in certain regions such as the Balkans, Turkey, and Central Asia. The appeal of Hungarian vetch is not limited to its winter hardiness. This nitrogen-fixing legume boasts the ability to accumulate significant amounts of organic residues (3-5 t/ha) and nitrogen (up to 70-80 kg/ha) in the soil, meeting a significant part (60-70%) of the crop's own nitrogen needs (Klein *et al.*, 2020). This feature not only reduces the dependence on high doses of mineral nitrogen, contributing to environmentally friendly practices, but also increases soil fertility through enzymatic activity (Fernández Sosa *et al.*, 2021; Vasiljevic *et al.*, 2021). Turkish researchers have aptly identified Hungarian vetch as an organic crop due to its positive impact on soil health (Temel & Torun, 2020).

Apart from its ability to enrich the soil, Hungarian vetch has significant agronomic potential. Its high yield potential combined with an impressive protein content and notable antioxidant activity makes it a valuable addition to livestock diets. The study also highlighted the crucial role of tillage systems, seeding rates, and sowing dates in optimising the productivity of Hungarian vetch. In particular, its unique morphological characteristics allow it to be used as a high-quality feed and green manure (Veklenko *et al.*, 2020; Stein *et al.*, 2023).

In a changing climate, winter crops such as Hungarian vetch is becoming a vital resource for providing high-quality feed during the critical autumn and winter periods. The efficient use of productive moisture during this period offers a sustainable alternative to conventional approaches to feed production. Furthermore, its compatibility with winter cereals such as triticale, rye, and spelt opens the possibility of creating a variety of binary mixtures suitable for hay, haylage, or silage production throughout the year. Recognising the enormous potential of Hungarian vetch for agricultural intensification and biologisation, this study delved into its biological characteristics, focusing on crude protein accumulation during critical growth stages. Moreover, the study investigated the effect of different fertilisation regimes and seeding rates on productivity in combination with different varieties of winter triticale. Through this comprehensive analysis, the study aimed to identify the best strategies for integrating Hungarian vetch into Ukrainian and global agricultural landscapes, ultimately paving the way for sustainable and climate-resilient feed production systems.

LITERATURE REVIEW

The need for high-quality, protein-rich feed is becoming increasingly important in Ukrainian livestock production. Hungarian vetch (*Vicia pannonica* Crantz.) is a promising variant due to its high protein content, drought tolerance, and nitrogen-fixing capacity. This review analysed the existing sources of information on the potential of Hungarian vetch, focusing on its biological characteristics, suitability for mixed cropping, and overall prospects for feed production.

Hungarian vetch is a leguminous annual crop of winter and spring development, known for its rapid growth, cold tolerance, and adaptability to various soil conditions (Bovsunovska, 2018b; Hetman, 2021). The taproot system contributes to the efficient use of water, making it suitable for regions with limited rainfall, such as the Pannonian Basin in Europe, as well as steppe and dry forest-steppe regions of Ukraine (Croccetti *et al.*, 2020). Studies have shown that Hungarian vetch can accumulate more dry matter and crude protein compared to other close legume relatives, such as common vetch (*Vicia sativa* L.) since it effectively uses the reserves of productive moisture in the autumn-winter period (Bengisu, 2019; Gozubuyuk *et al.*, 2020). The presence of beneficial rhizobia bacteria in root nodules enhances nitrogen fixation, enriching the soil for subsequent crops (AVCI, 2021).

Intercropping Hungarian vetch with cereal spiked crops such as winter triticale, wheat, barley, or winter oats has become a sustainable and productive strategy for feed production in the Mediterranean and Balkans (Bozhanska, 2019; Neyestani *et al.*, 2023). Such mixed crops use resources more efficiently than monocultures, contributing to higher biomass and protein yields (Chornolata *et al.*, 2019). The drive for sustainable and high-quality feed production in Ukraine requires a focus on optimising the cultivation of mixed crops. Recent breeding research has explored the potential of combining Hungarian vetch with winter triticale (x *Triticosecale* Wittmack) in mixed crops. The main objective of this study, according to V. Tromsyuk *et al.* (2021), is the selection of the most suitable winter triticale varieties with best parameters of competition, plant habit, leafiness, overall productivity, and nutritional value of the vegetative mass. By carefully selecting triticale varieties that complement the growth characteristics of Hungarian vetch, the researchers hope to achieve a synergistic effect that will lead to higher biomass yields, improved protein content and, ultimately, higher feed quality. The ability to reduce competition within these systems not only increases overall yields, but also potentially minimises the need for external inputs such as fertilisers, thereby promoting environmentally friendly agricultural practices. Several studies in Ukraine have reported considerable increases in dry matter accumulation and crude protein yield when Hungarian vetch was sown with winter triticale, reaching 50 t/ha and 1.2 t/ha, respectively (Bovsunovska, 2018a; Hetman *et al.*, 2019). Furthermore, mixed crops demonstrate better feed quality due to increased digestibility and energy content compared to monoculture of Hungarian vetch (Önal Aşci *et al.*, 2020).

Mixed crops based on Hungarian vetch have a range of advantages for increasing the overall efficiency of feed production. Studies have shown that these systems require less fertiliser due to the nitrogen fixation by vetch, which contributes to environmental sustainability (Skamarokhova *et al.*, 2021). Furthermore, improved soil fertility has a favourable effect on subsequent crops grown in the rotation, further increasing the overall productivity of arable land. Early harvesting of Hungarian vetch allows extending the sowing period of subsequent cash crops, such as maize or sorghum, thereby optimising land use and resource allocation (Senyk, 2020).

Despite its promising potential, the introduction of Hungarian vetch in Ukraine faces certain challenges. The best sowing dates and seeding rates for different regions and soil types require further investigation. Furthermore, breeding programmes aimed at developing varieties with increased resistance to disease and stress will be useful for wider adoption (Semenov *et al.*, 2021; Turfan, 2022). Further research is also needed to improve the economic efficiency of integrating Hungarian vetch into existing farming systems.

Hungarian vetch is a promising legume crop for Ukrainian feed production. Its favourable biological characteristics, combined with its ability to increase mixed crop efficiency and protein content, make it a valuable addition to agricultural landscapes. However, overcoming challenges related to seed availability, cultivation practices, and long-term sustainability is still crucial for successful integration. By addressing these challenges through ongoing research and development, Hungarian vetch can play a significant role in improving the quality and quantity of feed production in Ukraine, ultimately contributing to sustainable livestock feeding systems.

MATERIALS AND METHODS

The research on the productivity of Hungarian vetch was conducted during 2014-2022 at the experimental field of the Department of Field Feed Crops, Hayfields and Pastures of the Institute of Feed and Agriculture of Podillia of the National Academy of Agrarian Sciences of Ukraine. The soil of the experimental plot is grey forest medium loam with a humus content of 2.18% in the topsoil, alkaline hydrolysed nitrogen – 62 mg/kg, mobile phosphorus – 149 mg/kg and exchangeable potassium – 80 mg/kg. The reaction of the soil solution is slightly acidic (salt pH 5.9) with a hydrolytic acidity of 1.14 mg-eq/100 g of soil. The climatic conditions of most years of the study were favourable for the growth and development of grass mixtures of winter triticale with Hungarian vetch. The average temperature in April-May was 11.8-13.3°C. Precipitation during the growing season was unevenly distributed. The most optimum year was 2014 (HTC 1.82) with an average daily temperature of 13.3°C and precipitation of 149 mm. The driest years were 2015 and 2017 (HTC 0.78).

The study was conducted using a two-factor field experiment with six seeding rates (factor A) and three doses of mineral fertilisers (factor B). The experiment repetition was fourfold. The placement of variants was systematic. The plot area – 24.2 m^2, the accounting area – 18 m^2. Legume-cereal mixtures of annual feed crops were used as a precursor. Commonly accepted agricultural techniques were used with basic tillage, application of $P_{30\text{-}45}K_{30\text{-}45}$ for pre-sowing cultivation and nitrogen fertilisers for sowing. The SN-16 seeder was used in combination with a T-25A tractor. The ratio of the components in the mixture was 50%:50%, 75%:75%, 50%:75%, and 75%:50% of the full quantitative seeding rate. The sowing dates depended on weather conditions and were mainly carried out in September and October.

The hydrothermal coefficient (HTC) was calculated as the ratio of the sum of precipitation to the sum of air temperatures reduced by a factor of 10 during certain periods of vegetation. Phenological observations of plant growth and development were carried out with indication of the main stages of development according to the BBCH scale according to H. Bleiholder *et al.* (2001). The height of plants was measured according to the linear method, the botanical composition of the grass stand was studied by weight analysis, and the yield of green mass was determined by weighing the entire crop from the accounting plot and then recalculating it per hectare according to the method of state variety testing (Tkachik *et al.*, 2017). The dry matter content was determined by the thermostat-weight method by drying the samples to a constant weight at 105°C. The chemical composition of the feed was analysed jointly with the Laboratory for Monitoring the Quality, Safety of Feed and Raw Materials of the Institute of Feed and Agriculture of Podillia NAAS of Ukraine (Chornolata *et al.*, 2019), following current standards, namely: sampling was carried out following DSTU ISO 6497:2005 (2008), determination of moisture and other volatile substances – DSTU ISO 6496:2005 (2006), nitrogen and crude protein – DSTU 7169:2010 (2011), crude fat – DSTU ISO 6492:2003 (2005), fibre – DSTU 8844:2019 (2020), crude ash – DSTU ISO 5984:2004 (2006), carbohydrate-lignin complex (sugars, hemicellulose, cellulose) – DSTU 7982:2015 (2017). The results were processed using Statistica 10.0.

Experimental studies of plants (both cultivated and wild), including the collection of plant material, were following the institutional, national, or international guidelines. The study adhered to the standards of the Convention on Biological Diversity (1992) and the Convention on Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS AND DISCUSSION

As part of mixed crops with cereals, Hungarian vetch can form multi-tiered plant communities in combination with other species. Such agrophytocenoses demonstrate higher efficiency of nutrient and solar energy use due to their extensive root system and significant amount of leaf mass. The interaction of legumes and cereals in the phytocoenosis improves nitrogen nutrition of plants, which contributes to higher productivity per unit area. Hungarian vetch, specifically the Orlan variety used in grass mixtures, is of interest to farmers specialising in livestock production. The use of grass mixtures as an intercrop helps to improve soil fertility and soil structure in different soil and climatic zones of Ukraine.

In the context of climate change, the temperature and rainfall in autumn determine the formation of friendly and homogeneous seedlings that appear within 13-15 days. From sowing to the winter cessation of vegetation, the average daily sum of active temperatures was 365°C, and the plants of Hungarian vetch reached the microstage of lateral shoots (BBCH 22-24) at a height of 8-10 cm. After the resumption of spring vegetation, it takes 64-65 days to reach the full flowering stage (BBCH 64-65) for vetch plants, with a sum of active temperatures of 753°C, precipitation of approximately 100 mm, daylight hours of 973 h and a hydrothermal coefficient of 1.32.

A direct indicator of the intensity of the stages of organogenesis in plants is their height, although this parameter depends on the variety. Its variation is also possible under the influence of weather conditions and technological factors. In the early stages of organogenesis, Hungarian vetch plants develop slowly, with a considerable increase in height observed from budding to flowering. At this stage, the height can increase by a factor of 1.5-2.0, which often causes the grass stand to lodge. As the stem of Hungarian vetch plants is thin and juicy, with low resistance to lodging, the presence of a supporting crop, such as winter triticale, is recommended regardless of the maturity group.

During ontogenesis, the reaction of plants to environmental conditions is observed, which is determined by plant growth and the duration of individual phases of development, especially under changed humidity. With increased moisture supply, the duration of the interphase period from vegetation renewal to flowering of Hungarian vetch was 67-69 days, while a decrease in available moisture led to a reduction of this period by 9-10 days. It was found that an intensive increase in plant height occurred with an increase in the average daily temperature in May and the passage of the phases of stem elongation (BBCH 30-39) and budding (BBCH 40-50). The height was adjusted by the level of fertilisation in single-species sowing; on the background of mineral nutrition, it increased by 24-26 cm and in the flowering phase reached 73-79 cm with a total increase of 35-38 cm (Table 1).

Table 1*. Changes in the height of Hungarian vetch plants by stages of growth and development in agrophytocenoses with winter triticale, cm (M±m)*

Culture, binary mixtures	Seeding rate, %		Fertiliser doses	Phases of growth and development		
	triticale	vetch		branching	budding	flowering
Hungarian vetch	0	100	$N_{30}P_{30}K_{30}$	14±1.14	38±4.14	73±3.12
			$N_{45}P_{45}K_{45}$	15±1.09	41±3.13	79±2.82

Table 1. Continued

Culture, binary mixtures	Seeding rate, %		Fertiliser doses	Phases of growth and development		
	triticale	vetch		branching	budding	flowering
Hungarian vetch + winter triticale	50	50	$N_{30}P_{30}K_{30}$	18±2.31	48±3.13	83±3.21
			$N_{45}P_{45}K_{45}$	18±1.81	51±3.22	86±3.87
Hungarian vetch + winter triticale	75	75	$N_{30}P_{30}K_{30}$	18±2.46	51±3.45	84±3.38
			$N_{45}P_{45}K_{45}$	17±1.90	52±6.03	88±2.98
Hungarian vetch + winter triticale	50	75	$N_{30}P_{30}K_{30}$	16±2.04	45±3.31	81±4.03
			$N_{45}P_{45}K_{45}$	16±2.12	52±5.19	85±3.48
Hungarian vetch + winter triticale	75	50	$N_{30}P_{30}K_{30}$	16±2.38	49±3.84	80±3.53
			$N_{45}P_{45}K_{45}$	16±1.53	51±3.76	87±4.33

Source: *developed by the authors of this study*

However, in grass mixtures on the background of mineral fertilisers, plant growth processes were activated in the created microclimate conditions and mutual stimulation of components. The height of Hungarian vetch from the branching phase increased by 33-36 cm to the budding phase and was within 51-52 cm, while in the flowering phase it reached 86-88 cm. Under single-species sowing, the growth and development of plants was less intense, and the height was 38-41 cm and 73-79 cm, respectively, against the background of $N_{45}P_{45}K_{45}$.

The intensive development of Hungarian vetch plants was noted under conditions of excessive moisture when the plant height was 100-104 cm at a seeding rate of 100% in monoculture. At HTC 1.05, it decreased to 74-77 cm under the influence of a compatible crop – winter triticale. Hence, it can be concluded that the created models of agrophytocenoses require a comprehensive investigation of their adaptability to extreme natural factors (lack of moisture, increased temperature during the growing season, etc.), which can be regulated by elements of cultivation technology (Table 2).

Table 2. *Changes in the height of Hungarian vetch plants in joint crops with winter triticale under different moisture supply, cm (M±m)*

Crops, seeding rate, %	Hydrothermal coefficient	
	1.60	1.05
Hungarian vetch, 100	93±2.36	76±3.63
Hungarian vetch, 50 + winter triticale, 50	96±3.92	75±3.65
Hungarian vetch, 75 + winter triticale, 75	104±2.96	74±3.12
Hungarian vetch, 75 + winter triticale, 50	100±3.78	77±3.53
Hungarian vetch, 50 + winter triticale, 75	102±3.37	77±3.52

Source: *developed by the authors of this study*

The growth of green mass in Hungarian vetch was influenced by the elements of cultivation technology. The best conditions for the formation of leaves were created on the mineral background of $N_{45}P_{45}K_{45}$. At different seeding rates, the leafiness of plants reached 44.85-50.40% and was lower by 1.17-6.72% in single-species sowing under conditions of excessive moisture. When humidity decreases, plants form a smaller leaf surface area, thereby protecting themselves from excessive evaporation and preserving them in the grass stand. In single-species crops, it was 43.47%, while in binary mixtures – 36.14-42.65% (Table 3).

Table 3. *Influence of hydrothermal conditions on the leafiness of Hungarian vetch plants depending on sowing rates and fertilisation, %*

Mixtures	Seeding rate, %	Fertiliser doses	Hydrothermal coefficient		Mean
			1.60	1.05	
Hungarian vetch	100	$N_{30}P_{30}K_{30}$	46.01	38.11	42.08
		$N_{45}P_{45}K_{45}$	51.57	43.47	47.52
Hungarian vetch + winter triticale	50:50	$N_{30}P_{30}K_{30}$	44.96	38.41	41.68
		$N_{45}P_{45}K_{45}$	50.40	40.98	45.68
Hungarian vetch + winter triticale	75:75	$N_{30}P_{30}K_{30}$	42.97	34.81	38.89
		$N_{45}P_{45}K_{45}$	48.17	42.65	45.41

Table 3. Continued

Mixtures	Seeding rate, %	Fertiliser doses	Hydrothermal coefficient 1.60	Hydrothermal coefficient 1.05	Mean
Hungarian vetch + winter triticale	50:75	$N_{30}P_{30}K_{30}$	40.01	35.88	37.94
		$N_{45}P_{45}K_{45}$	44.85	37.75	41.30
Hungarian vetch + winter triticale	75:50	$N_{30}P_{30}K_{30}$	43.15	35.67	39.41
		$N_{45}P_{45}K_{45}$	48.37	36.14	42.26

Source: *developed by the authors of this study*

The decrease in the sowing rate of the leguminous component in binary mixtures contributed to better leafiness of plants with the introduction of mineral fertilisers in the dose $N_{45}P_{45}K_{45}$. The crops competed with winter triticale for nutrients. There is a tendency for its decrease when sowing with a seeding rate of 50:75% of single-species sowing. To obtain high-protein plant material, it is better to sow Hungarian vetch with winter triticale, which has the same growth and development phases in agrocenoses. In a grass mixture, winter triticale is not only a supporting crop, but also a balancing crop in terms of the sugar-protein ratio.

The tiered placement of plants in the crop provides unequal illumination, which leads to different temperature and water conditions, especially with uneven distribution of precipitation during the growing season. Therefore, by selecting the seeding rates of legumes and cereals, the risks of plant influence on each other can be eliminated, as evidenced by the obtained dry matter yield. Although the performance of the grass mixture models varied, they stayed quite high. The highest yield of dry matter was obtained in the model where crops were sown in the ratio of sowing rates of 75:50% on the background of $N_{45}P_{45}K_{45}$, which was 8.77 t/ha with a content of Hungarian vetch of 2.90 t/ha, or a fraction thereof in the grass mixture was within 33.1%. When changing the ratio of components in the phytocoenosis, the difference in performance was within 0.62-1.10 t/ha, depending on the seeding rate. It was noted that the share of Hungarian vetch in the structure of the dry matter yield decreased to 1.95-2.22 t/ha with the application of mineral fertilisers at $N_{30}P_{30}K_{30}$. Therefore, to form a stable crop of high-protein plant material, it is advisable to apply suspended doses of mineral fertilisers (Table 4).

Table 4*. Productivity of agrophytocenosis of Hungarian vetch with winter triticale in single-species and combined crops (2014-2016)*

Binary mixtures, seeding rates, % (factor A)	Fertiliser doses (factor B)	Green mass, t/ha	Dry matter, t/ha: total	Dry matter, t/ha: incl. vetch	Dry matter content, %: total	Dry matter content, %: incl. in vetch
Vetch Hungarian vetch, 100	$N_{30}P_{30}K_{30}$	26.5	4.44	4.44	16.76	16.75
	$N_{45}P_{45}K_{45}$	29.6	4.57	4.57	15.44	15.43
Hungarian vetch, 50 + winter triticale, 50	$N_{30}P_{30}K_{30}$	35.9	6.86	1.95	19.54	17.58
	$N_{45}P_{45}K_{45}$	41.7	7.83	2.49	18.85	17.34
Hungarian vetch, 75 + winter triticale, 75	$N_{30}P_{30}K_{30}$	30.4	7.57	2.17	20.10	18.62
	$N_{45}P_{45}K_{45}$	38.1	8.15	2.61	18.86	17.54
Hungarian vetch, 75 + winter triticale, 50	$N_{30}P_{30}K_{30}$	36.7	7.20	2.04	19.20	17.74
	$N_{45}P_{45}K_{45}$	41.8	7.67	2.45	18.98	16.88
Hungarian vetch, 50 + winter triticale, 75	$N_{30}P_{30}K_{30}$	39.6	7.90	2.22	20.06	17.92
	$N_{45}P_{45}K_{45}$	46.2	8.77	2.90	19.02	17.40
$LSD_{0.05}$, t/ha: green mass A-1.50; B-1.06 AB-2.59						
$LSD_{0.05}$, t/ha: dry matter. A-0.35; B-0.25; AB-0.60						

Source: *developed by the authors of this study*

The use of different doses of mineral fertilisers led to a decrease in the accumulation of dry matter in triticale and Hungarian vetch plants over the years of research, offsetting the unfavourable weather conditions of the growing season. The difference in dry matter content between a year with favourable moisture and temperature conditions and a dry year was 2.77%, and in case of uneven distribution – 1.64%, regardless of the level of fertilisation. Therewith, the combination of different sowing rates in agrophytocenosis contributed to an increase in the dry matter content of Hungarian vetch by 0.06-0.20%, compared to the ratio of sowing rates of 50:50% with $N_{45}P_{45}K_{45}$ and 0.16-1.04% with $N_{30}P_{30}K_{30}$. Increasing the dose of mineral fertilisers contributed to a decrease in the content of dry matter in Hungarian vetch by 0.24-1.08%, compared to $N_{30}P_{30}K_{30}$. The highest dry matter yield was 8.77 t/ha with a green mass yield of 46.3 t/ha for a mixture of 50% Hungarian vetch and 75% winter triticale.

The high potential of productivity and quality of Hungarian vetch is confirmed by the study, which involved sampling of green mass from single-species sowing after the same period of 4-6 days at different microstages of crop development to identify the specific features of the production process, the intensity of interphase periods and optimisation of feed quality with the period of harvesting for feed purposes (Fig. 1).

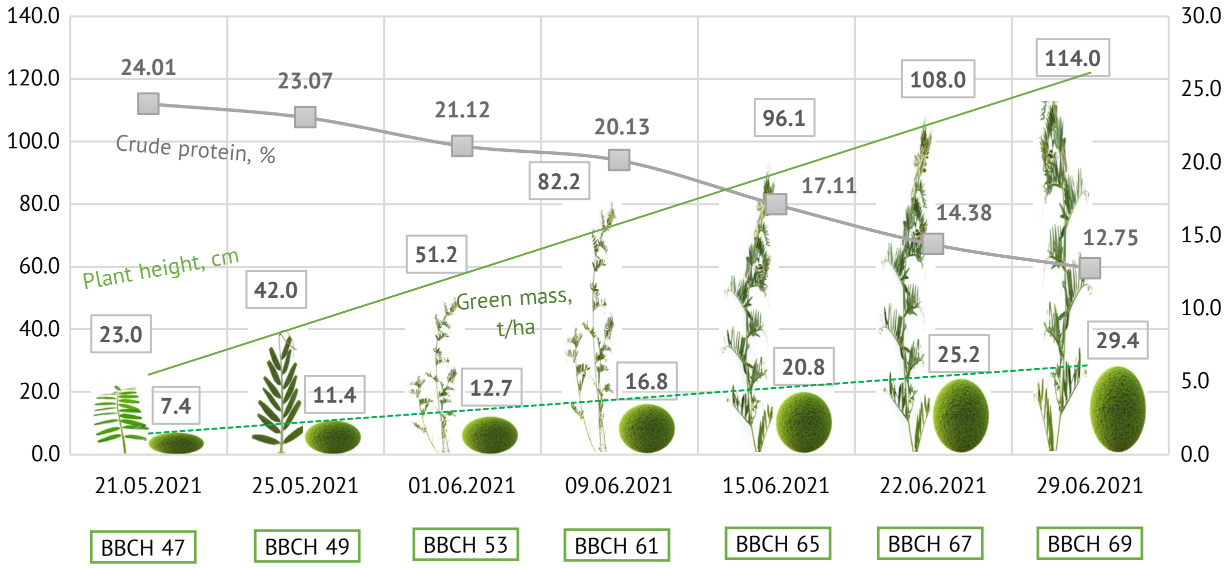

***Figure 1**. Productivity and quality potential of Hungarian vetch in single-species sowing according to BBCH development microstages, 2021*
Source: *developed by the authors of this study*

It was established that at the initial stage of the generative period of plants – BBCH 47 microstage (budding of 70% of plants), the height of vetch reached 23 cm with a green mass yield of 7.4 t/ha and a crude protein content in dry matter of 24.01%. At the onset of the BBCH 49 microstage (complete budding of plants), a yield of 11.4 t/ha of green mass was formed at a grass height of 42.0 cm and a crude protein content of 23.07%. When the BBCH 53 microstage was reached (the first flower buds appeared), the plant height reached 51.2 cm with a green mass yield of 12.7 t/ha and an even higher crude protein content of 21.12%. With the onset of the flowering phase of Hungarian vetch, the highest intensity of aboveground biomass growth and, at the same time, a sharp loss of its protein nutritional value were observed. Thus, from the completion of the BBCH 61 microstage (the beginning of flowering of 10% of flowers) to BBCH 69 microstage (the end of flowering), which lasted 20 days, the absolute values of plant height and green mass yield increased from 82.2 cm and 16.8 t/ha to 114 cm and 29.4 t/ha, and the content of crude protein in dry matter decreased from 20.13% to 12.75%. Therefore, the best harvesting phase of Hungarian vetch for preparing high-quality feed is the period from the beginning to the middle of flowering (BBCH 61-65), which lasts a week, when their height is 82.2-96.1 cm, green mass yield is 16.8-20.8 t/ha, with a crude protein content in the dry matter of the feed at 17.11-20.13%.

Studies have shown that growing Hungarian vetch in mixed crops with an accompanying cereal component considerably increases the productivity of the feed acreage compared to its single-species sowing. It was found that mixtures of Hungarian vetch with different varieties of winter triticale or Westerwolds ryegrass provided a yield of 37.6-45.0 t/ha of green mass, while only 21.8 t/ha was obtained in pure sowing. The highest yield of green mass was obtained using winter triticale of the Bozhych variety (Fig. 2).

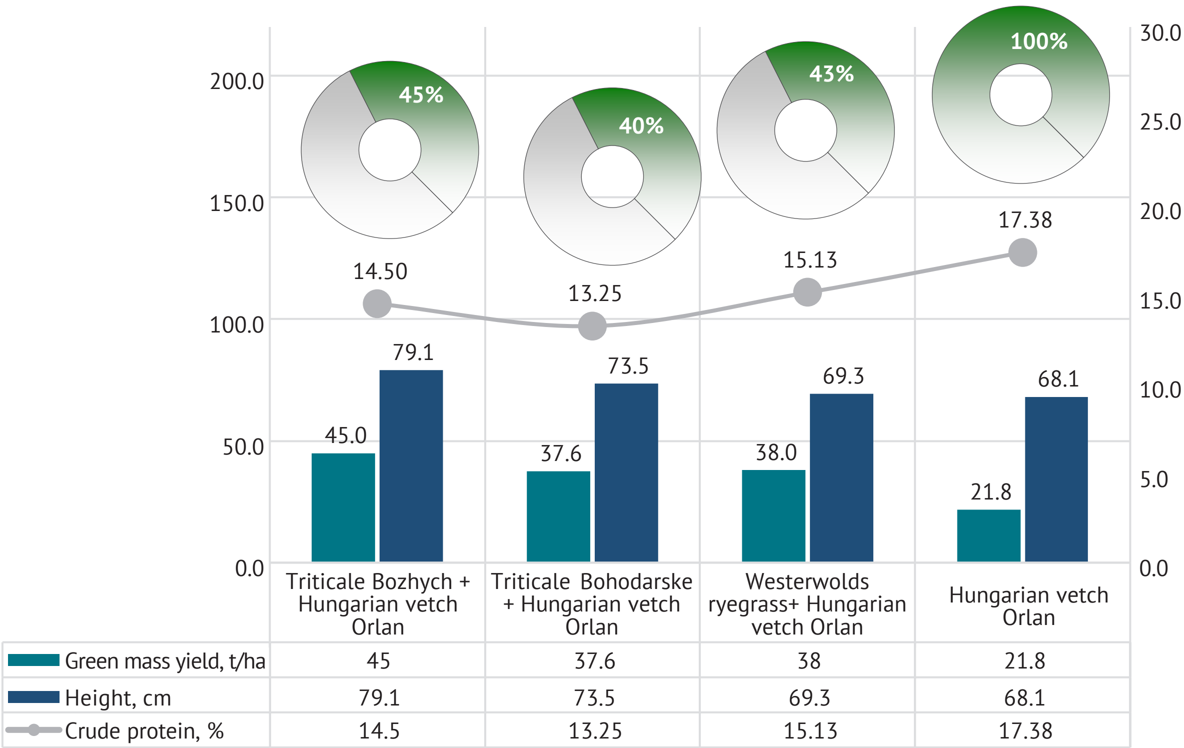

Figure 2*. Improving the productivity and feed quality of mixed crops with the inclusion of Hungarian vetch in annual winter cereal species, 2021-2022*

Source: *developed by the authors of this study*

The share of Hungarian vetch in the sowing with triticale was 40-45%, and with ryegrass – 43%. At the time of reaching mowing maturity (27 May), Westerwolds ryegrass was at the BBCH 55 stage (50% of inflorescences were ejected), and winter triticale was at the BBCH 45 stage (swelling of the ear in the axils of the flag leaf), which characterises the different maturity of these components. This resulted in their different coenotic impact on the legume component. At the time of harvesting with Westerwolds ryegrass, the Hungarian vetch was at the BBCH 55 stage of development (flower buds), while with winter triticale, the Hungarian vetch had already begun to flower (BBCH 59). Harvesting winter mixed crops at such stages of plant development allows obtaining a plant mass with a crude protein content in dry matter of 15.13% when sowing Hungarian vetch with Westerwolds ryegrass, 14.50% with winter triticale of Bozhych variety and 13.25% with Bohodarske variety. At this time, a single-species sowing of Hungarian vetch contained 17.38% of crude protein in dry matter.

Ecological testing of Hungarian vetch models with different varieties of winter triticale as the most successful variant of cenotic combination of crops in mixed sowing showed stability of productivity in different soil and climatic zones of Ukraine. On average, over the years of use, 36.2-51.8 t/ha of green mass was obtained with a dry matter yield of 7.1-11.5 t/ha. Such stability in the formation of the productivity of this mixed crop is explained by the successful annual overwintering of the components, the effective use of autumn and winter soil moisture reserves and temperature conditions. Considering that the yield of crude protein from the feed acreage did not decrease below 1.0 t/ha, the phytocoenotic balance of such a mixture was good in terms of the best proportion of the legume component in the grass stand, which contributed to sufficient nutritional value of plant material (Table 5).

Table 5*. Formation of productivity of a mixture of Hungarian vetch with winter triticale depending on soil and climatic conditions of Ukraine (average for 2016-2020)*

Mixture	Fertiliser	Ratio of seeding rates of components, %	Green mass yield, t/ha	Yield, t/ha	
				dry matter	crude protein
Forest-steppe of the Right Bank (IFAP NAAS)					
Hungarian vetch Orlan + winter triticale Polovetske	$N_{45}P_{45}K_{45}$	75+50	45.6	8.3	1.3
		75+50	42.8	7.6	1.2

Table 5. Continued

Mixture	Fertiliser	Ratio of seeding rates of components, %	Green mass yield, t/ha	Yield, t/ha	
				dry matter	crude protein
Western Forest-Steppe (Ternopil RS IVM)					
Hungarian vetch Orlan + winter triticale Polianske	Seed treatment	75+25	36.8	7.2	1.1
Southern Steppe (IIA NAAS)					
Hungarian vetch Orlan + winter triticale Bohodarske	$N_{60}P_{60}$	75+50	51.8	11.5	1.8
Southern Steppe (Mykolaiv SAES ICSA)					
Hungarian vetch Orlan + winter triticale Polianske	Without fertilisers	75+50	36.2	7.1	1.0

Source: *developed by the authors of this study*

The model of efficiency of the use of bioclimatic resources of central Podillia by mixed sowing of winter triticale of Bohodarske variety with Hungarian vetch of Orlan variety was tested, considering the intensity of the stages of development and growth of the vegetation index in the early spring growing season (Fig. 3)

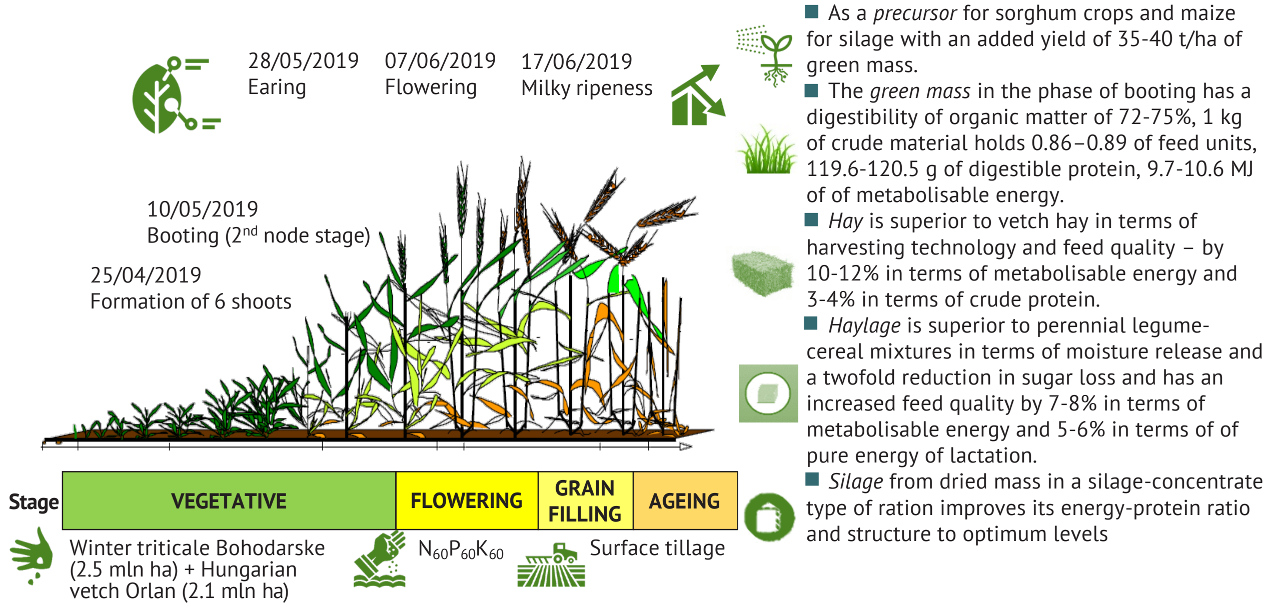

Figure 3*. Demonstration of the effectiveness of photosynthetic activity and feed productivity of a mixture of winter triticale with Hungarian vetch as an intercrop in the early spring growing season, 2019-2020*
Source: *developed by the authors of this study*

It can be noted that the creation of such a mixed crop by sowing 2.5 mln/ha of similar cereal seeds and 2.1 mln/ha of leguminous component after surface tillage and application of mineral fertilisers in a dose of $N_{60}P_{60}K_{60}$ allows forming a crop of green mass at the end of the vegetative stage of plant development (booting of a cereal species) 35-40 t/ha, which is characterised by digestibility of 72-75%, nutritional value of 1 kg of dry matter 0.86-0.89 of feed units with a content of 119.6-120.5 g of digestible protein and 9.7-10.6 MJ of metabolisable energy. Hay harvesting from such a crop is superior in terms of technology and quality to the traditional vetch-oat mixture – by 10-12% in energy content and 3-4% in crude protein. There are also substantial advantages of such a grass stand for the production of early high-quality haylage or silage, as well as a precursor for post-cutting sowing of maize or sorghum crops.

Comprehensive studies of the chemical composition of the mixture of winter triticale with Hungarian vetch at different stages of development show that during the growth and development of its plant components, the content of protein and easily soluble carbohydrates decreases and the content of fibre, i.e., structural carbohydrates, increases. As for crude fat, it tends to decrease, while the content of crude ash increases (Table 6).

***Table 6**. Nutritional value and characteristics of the carbohydrate complex of winter triticale and Hungarian vetch mixture at different stages of development of components*

Developmental stages of the mixture components	Content per 1 kg of dry matter, %					Content per 1 kg of dry matter, g/kg					
	CP	CF	CFi	CA	NFES	EHC	St	Su	HCe	Ce	Li
Triticale tillering and Hungarian vetch stemming	19.52	2.97	17.99	9.86	49.65	322.4	39.5	158.7	118.0	133.2	40.9
Triticale booting and Hungarian vetch budding	17.10	2.32	24.85	7.76	47.96	316.0	33.9	139.5	135.5	148.8	61.0
Triticale heading and Hungarian vetch flowering	16.93	1.43	28.88	11.70	41.06	251.4	31.0	92.0	152.0	161.9	71.2

***Note:** CP – crude protein; CF – crude fat; CFi – crude fibre; CA – crude ash; NFES – nitrogen-free extractive substances; EHC – easily hydrolysed carbohydrates; St – starch; Su – sugar; HCe – hemicellulose; Ce – cellulose; Li – lignin*
***Source:** developed by the author of this study based on research by Chornolata et al. (2019)*

According to the results of the study, the nutritional value of the mixture of winter triticale and Hungarian vetch in the phase of triticale tillering and vetch stemming was 0.87 feed units and 9.29 MJ of metabolisable energy per 1 kg of dry matter, which is the highest. In the phase of triticale booting and vetch budding, these values were 0.84 and 9.11, respectively, while in the phase of triticale earing and vetch flowering – 0.75 and 8.44. Thus, the most optimum time for using the mixture is the phase of cereal booting and legume budding, as the nutritional value stays quite high, and the volume of green mass is almost maximum.

To assess the economic efficiency of feed use, it is important to determine the amount of basic nutrients consumed and absorbed by the animal. This information was obtained during balance experiments on sheep fed a mixture of triticale and Hungarian vetch at different stages of growth and development. It was found that the content of dry matter increased in the phase of triticale booting and vetch budding by 8.7%, and in the phase of triticale earing and vetch flowering – by 11.4% and 2.4%, respectively, compared to the previous phases. Consumption of the mixture in the phase of triticale tillering and vetch stemming was 87%, in the phase of triticale tubing and vetch budding – 57%, and in the phase of triticale earing and vetch flowering – only 34%. In the latter two phases, the proportion of feed residues increases substantially, which leads to a decrease in the intake of nutrients in the animal body.

Based on the data on fed plant mass, feed residues, excreted urine and faeces, the content of the main nutrients in the samples was determined and the digestibility coefficients of dry and organic matter, crude protein, crude fat, crude fibre, and nitrogen-free extractive substances were calculated. It was found that the highest digestibility of all nutrients was observed in the triticale tillering and vetch stemming phases. In the following phases, there was a tendency for a gradual decrease in digestibility, with the greatest decrease in crude fat and fibre digestibility and the least decrease in crude protein digestibility.

The study of the carbohydrate-lignin complex of the samples showed that with the ageing of plants, the content of easily soluble carbohydrates decreases by more than 22% due to a decrease in starch and sugar. At the same time, the content of hemicellulose, cellulose, and lignin in the fraction of structural carbohydrates increases by 1.2-1.8 times, especially in the phase of triticale earing and vetch flowering. Generally, the content of structural carbohydrates increases by more than 30%. Consequently, the digestibility of cellulose and lignin in the animal body decreases dramatically. Thus, as plants age, the proportion of available protein decreases, and the content of hard-to-digest structural carbohydrates increases. Thus, from a livestock perspective, a mixture of winter triticale and Hungarian vetch is a valuable feed and feedstock. It is most appropriate to use it during the cereal booting and legume budding phases, when the nutrient content is still high enough and the depressant effect of fibre is not yet significantly manifested. The experimental data obtained are consistent with the results of studies by other researchers.

In the study by H. Kir (2021a), the yield and quality characteristics of a mixture of Hungarian vetch with triticale were investigated depending on the sowing time. The analysis showed that sowing time had a significant impact on all performance parameters. Specifically, delayed sowing led to a decrease in green feed yield, dry matter yield, and mixture quality. The study highlighted the first week of October as the most optimum sowing date, contributing to high dry matter yields with high quality in the continental climate of Central Anatolia in Turkey.

C. Balabanli *et al.* (2010) found that mixtures of Hungarian vetch with rye, triticale, barley, and wheat produced high hay yields and quality silage. The highest hay yield (9.08 t/ha) and crude protein yield (1.11 t/ha) were obtained from a mixture of Hungarian vetch and rye. The lowest content of NDF and ADF (50.11% and

31.92%, respectively) was observed in the mixture of Hungarian vetch with oats. The conclusion of the study is that all mixtures of Hungarian vetch with cereals can be successfully ensiled and produce high-quality silage without additional preservatives.

A. Uzun *et al.* (2004) studied the effect of four seeding rates (20, 40, 80, and 160 kg/ha) on dry matter, seed, and yield elements of four Hungarian vetch cultivars. According to the data obtained, there were no significant differences between the Hungarian vetch varieties in any of the measured traits, except for the thousand kernel weight. However, seeding rates had a substantial impact on dry matter yield, seed yield, and most yield elements. It was found that high seeding rates resulted in higher dry matter and seed yields compared to low rates in all the years studied.

S. Albayrak *et al.* (2011) investigated the effect of two row spacing (17.5 cm and 35 cm) and four seeding rates (40, 60, 80, and 100 kg/ha) on yield elements of Hungarian vetch under natural moisture conditions in Turkey. It was found that row spacing and seeding rate significantly influenced most traits and yield elements, except for feed quality. The highest dry matter yields were obtained with a row spacing of 17.5 cm and a seeding rate of 80 kg/ha. Among the combinations of sowing methods, the highest seed yield was obtained with a row spacing of 35 cm and a seeding rate of 80 kg/ha.

In the study of the effect of row spacing and seeding rates on the productivity of hairy vetch (*Vicia villosa* Roth), Hungarian vetch (*Vicia pannonica* Crantz) and common vetch (*Vicia sativa* L.) under natural moisture conditions, it was found that wider row spacing significantly increased plant height and lodging index of crops. They also resulted in an increase in the number of branches and beans per plant, seeds per bean, thousand kernel weight, and seed yield. The highest seed productivity among legume species was observed in Hungarian vetch, while the lowest was in hairy vetch. Serbian scientists have found that a wide row spacing (50 cm) increases plant height by 13.6%, the number of branches – by 57%, and the seed yield of Hungarian vetch reaches 1.560 t/ha (Karagić *et al.,* 2011).

The study of yield and feed quality of mixed crops of Hungarian vetch with barley showed a substantial influence of varietal characteristics and the ratio of components in the mixtures in Turkey. The highest content of Hungarian vetch (37.3%), green mass yield (36.3 t/ha), and crude protein content (1.510 t/ha) were obtained from a mixture of 80% Hungarian vetch (Tarm Beyazi-98 variety) and 20% barley. The highest hay yield (10.5 t/ha) was obtained from a mixture of 20% Hungarian vetch (Anadolu Pembesi-2002) and 80% barley. In terms of feed quality among the studied varieties of Hungarian vetch, the Ege Beyazi-79 variety prevailed. Even though the feed yield increased with the share of barley seeds in the mixture, the quality of the feed decreased significantly. The study concluded that the best values in terms of both yield and quality were obtained from a mixture of 80% Hungarian vetch + 20% barley (Kusvuran *et al.*, 2014).

M. Nadeem *et al.* (2010) showed that the addition of cereals to Hungarian vetch crops reduced lodging and improved harvesting. The highest yields of green mass (35.06 t/ha) and dry matter (9.29 t/ha) were recorded in the mixture of oats + Hungarian vetch. Oats provided the highest yields of green mass (31.58 t/ha) and dry matter (7.75 t/ha) in pure crops. It was concluded that the mixture of oats + Hungarian vetch can be recommended for high biomass production under natural moisture conditions in the conditions of Pakistan.

K. Alizadeh and J. Silva (2013) aimed to determine a suitable mixture of annual feed legumes and barley as a winter crop in arid climates. A 1:1 mixture of Hungarian vetch seeds (variety 2670) and other legume species with barley (variety Abidar) was tested in comparison with a monoculture. In provinces with mild winters and more than 400 mm of precipitation, this mixture demonstrated maximum green and dry mass yields, reaching 56 t/ha and 15 t/ha, respectively. The average crude protein content was 17%. Therefore, a mixture of Hungarian vetch and barley can be an effective alternative for farming in cold and semi-cold arid regions.

In a study conducted at Harran University in Turkey in 2017-2018, researcher G. Bengisu (2019) investigated the effect of single-species and mixed sowing of Hungarian vetch and barley on silage quality. When assessing the physical properties of the silage, it was found that the quality of the silage increased with the increase in the proportion of barley in the mixture. In terms of chemical properties of the silage, the lowest ADF and NDF content and the highest protein content were obtained from a silage mixture with a 75%:25% ratio of Hungarian vetch and barley.

In the study by H. Kir (2021b), the highest yields of green feed – 18.3 t/ha, dry matter – 5.7 t/ha, crude protein – 0.76 t/ha, and digestible dry matter – 3.6 t/ha, were achieved with a mixture of 25% Hungarian vetch and 75% oats. However, the highest content of crude protein, total digestible nutrients, and relative feed value was observed in single-species sowing of Hungarian vetch – 18.1%, 65.6%, 163.1, respectively. In a field study conducted in the Black Sea coastal region (Ordu, Turkey), hay yield, quality, and competition of Hungarian vetch with annual ryegrass in mixed crops were studied. It was found that ryegrass was more aggressive in terms of competitiveness in mixtures. The best mixture was 90% Hungarian vetch and 10% annual ryegrass, which provided the highest hay and crude protein yields (4.026 t/ha and 0.732 t/ha, respectively) (Önal Aşci *et al.*, 2020). Thus, the findings obtained are consistent with the data of other researchers on the positive effect of optimising seeding rates, component ratios, sowing, and harvesting dates on the productivity and quality of feed of Hungarian vetch in mixed crops.

CONCLUSIONS

As a result of comprehensive research, it was found that Hungarian vetch is a valuable feed crop that produces a high yield of green mass and seeds due to the intensive growth of the aboveground part. Due to their high productivity and nutritional value, Hungarian vetch can be successfully used as a component of high-performance feed mixtures. It was found that for Hungarian vetch to reach the full flowering phase, 754S°C of the sum of active temperatures, 100 mm of precipitation, and 973 h of daylight hours are required. The duration of the period from vegetation renewal to flowering depends on the moisture supply – with sufficient moisture, it is 67-69 days, with insufficient moisture – 58-59 days. It was proved that in the face of climate change, Hungarian vetch successfully overwinter and form a stable yield due to the efficient use of moisture. This suggests that Hungarian vetch is well-adapted to adverse weather conditions and can be considered a promising feed crop for arid regions.

The advantages of growing Hungarian vetch in mixtures with winter triticale, specifically Bozhych and Bohodarske varieties, were proved – the yield of green mass was 37.6-45.0 t/ha against 21.8 t/ha in pure sowing. Such mixtures are characterised by high performance stability. It was found that the highest yield of dry matter (7.8-8.8 t/ha) and crude protein (1.2-1.3 t/ha) is provided by sowing Hungarian vetch with winter triticale in the ratio of sowing norms of 50:75%. Therefore, it is recommended to create mixtures of Hungarian vetch and winter triticale by sowing 2.5 mln/ha of similar cereal seeds and 2.1 mln/ha of leguminous component on the background of $N_{45}P_{45}K_{45}$ with high quality indicators. The high productivity and stability of the mixture of Hungarian vetch with winter triticale was also proved in different soil and climatic zones of Ukraine.

Consumption and assimilation of the main nutrients by animals when using a mixture of triticale and Hungarian vetch in different growth stages indicates a significant influence of the stage of plant development on their nutritional potential. The results also indicate a decrease in crude protein and easily soluble carbohydrates and an increase in structural carbohydrates as plants age, leading to poorer digestibility. The best harvesting phase of Hungarian vetch for harvesting high-quality feed is the beginning to the middle of flowering (BBCH 61-65), when the content of crude protein in dry matter is 17.11-20.13%.

Further research should be aimed at investigating the possibility of using Hungarian vetch as a component of multispecies grass mixtures, developing varietal agricultural technology and further improving the elements of cultivation technology to maximise its productive potential.

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None.

CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Агробіологічне обґрунтування вирощування горошку паннонського в змішаних посівах

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Анотація. Виробництво високобілкових кормів залишається невід›ємним елементом розвитку кормовиробництва. Основна мета дослідження – вивчення біологічних особливостей горошку паннонського при вирощуванні в агрофітоценозах із застосуванням різних видів злакових озимих культур. Досліджено зміни хімічного складу сухої речовини на різних мікростадіях росту і розвитку, визначено вплив на урожайність, вміст поживних речовин і перетравності рослинної сировини в залежності від фаз росту і розвитку. Дослідження проведено з використанням польових, лабораторних, лабораторно-польових та статистичних методів. Встановлено, що для досягнення фази повного цвітіння, сума активних температур повинна бути 754 °С, із сумою опадів близько 100 мм та тривалістю світлової доби 973 год. Спостереження за вегетаційним періодом вказують на взаємозв’язок між рослинами та довкіллям, врахування якого важливо при вирощуванні горошку паннонського. Рослини горошку досягають висоти 100-104 см в умовах надмірного зволоження. Фітоценотичний баланс таких сумішей, зокрема горошку сорту Орлан та тритикале озимого сортів Божич і Богодарське, забезпечує сталу продуктивність та поживність рослинної сировини. Оптимальною фазою збирання горошку для високоякісного корму визначено період від початку до середини цвітіння, що забезпечує вміст сирого протеїну в сухій речовині 17-20 %. Ефективність змішаних фітоценозів підтверджена екологічними випробуваннями моделей поєднання культур у різних ґрунтово-кліматичних зонах України, де за роками використання було отримано врожаї 36,2-51,8 т/га зеленої маси та 7,1-11,5 т/га сухої речовини. Створення змішаного посіву дозволяє отримати високоякісну рослинну сировину, що переважає за енергетичним вмістом та вмістом протеїну традиційні горошко-вівсяні суміші. Рекомендовано створювати такі сумішки шляхом висіву 2,5 млн/га злакового та 2,1 млн/га бобового компонентів після внесення $N_{45}P_{45}K_{45}$ для формування врожаю з високою перетравністю і поживністю. Система змішаних посівів горошку паннонського є перспективною для виробництва силосу та високоякісного сінажу в умовах зміни клімату та в якості попередника для післяукісних посівів кукурудзи або соргових культур

Ключові слова: горошок паннонський; тритикале озиме; ріст і розвиток; урожайність; сирий протеїн; поживність корму

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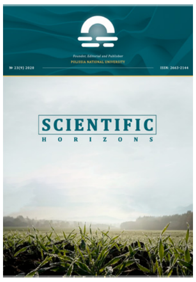

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Formation of the photoassimilation apparatus of pea (*Pisum sativum* L.) crops under biostimulants in arid conditions of the Southern Steppe of Ukraine

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Abstract. The Southern Steppe of Ukraine is characterised by a range of unfavourable agro-climatic conditions, which leads to inhibition of growth processes, disruption of physiological processes and loss of crop yields. The use of environmentally safe bioregulators stimulates growth processes, optimises nitrogen nutrition, increases the adaptability of legumes to adverse conditions and increases yields. The purpose of this study was to find out the specific features of the influence of biostimulants on

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the formation of the photoassimilation apparatus of pea of the Oplot variety in the Southern Steppe of Ukraine. A 3-year small-plot study was conducted to determine the leaf area index, total chlorophyll content, and net photosynthetic productivity in pea crops. The biostimulants were used for pre-sowing and foliar treatment of pea crops in the budding phase at concentrations described in the methodology. It was found that Stimpo (25 ml/t + 20 ml/ha) increased the leaf surface index in different stages of vegetation of pea variety Oplot by 1.12-1.54 times and Regoplant (250 ml/t + 50 ml/ha) increased the leaf surface index by 1.18-1.38 times compared to the control. A significant increase in the chlorophyll content in pea stipules was recorded after foliar treatment of crops with biostimulants and exceeded the value of control plants by 9.0-10.4% in the BBCH stages 61(65) – 75(79). Based on 3-year results, the positive impact of biostimulants on net photosynthetic productivity was proven. Thus, Stimpo and Regoplant increased the net photosynthetic productivity of pea crops by 35% and 22%, respectively, in the budding (BBCH 51-61) and flowering (BBCH 55-65) phases. The obtained scientific results contribute to the widespread use of the studied biostimulants in the technology of pea cultivation in the Southern Steppe zone of Ukraine to increase crop productivity and obtain a high-quality crop

Keywords: legumes; plant growth regulators; Regoplant; Stimpo; net photosynthetic productivity; leaf area index; chlorophyll

INTRODUCTION

Legumes rank third in the global crop production after grains and oilseeds and are an important source of food and feed. Legume protein accounts for 33% of the human diet, and legumes can fix atmospheric nitrogen, increasing soil fertility. Ukraine is distinguished by the fact that peas are the most widespread crop capable of producing high and sustainable grain yields compared to other types of pulses. In 2018, the sown area under peas amounted to 431 thousand hectares and almost half of them are in the steppe zone. In a certain number of farms, its yield is 3-5 t/ha.

Pea (*Pisum sativum* L.) exhibit high requirements for light, moisture, and soil, and therefore they often fail to fully realise their genetic productivity potential under unfavourable environmental conditions, especially in risky farming areas. Abiotic factors are the main factors that limit the yield of crops and cause up to 70% of crop losses. The Southern Steppe zone of Ukraine is characterised by a range of unfavourable abiotic factors that negatively affect the growth and development of crops and considerably reduce their productivity. Therewith, as noted by V. Mazur *et al.* (2021), the introduction of such a high-protein crop as peas into crop rotations contributes to the greening of production and improves the state of agrocenoses. Drought has become one of the most uncontrollable and unpredictable factors that constantly limits crop production and has a negative impact on pulses. M. Nadeem *et al.* (2019) showed that drought conditions reduce seedling emergence, photosynthetic activity, and disrupt the transport of photoassimilates in pulses, and in study M. Kolesnikov *et al.* (2023) showed the inhibition of growth processes in the early stages of plant ontogeny.

Bioregulators play a significant role in plant responses to environmental factors in shaping plant resistance to extreme conditions. In their review studies, B. Kumar (2021) and A. Dubey *et al.* (2020) show the role of biostimulants in the mechanisms of protection of *Fabaceae* plants from abiotic stresses, stimulation of growth processes, nutrient recycling, improvement of grain quality, and increase of crop yields. A lot of evidence has been accumulated that demonstrates the positive results of using growth regulators in soybean (Shepilova *et al.,* 2021), pea (Nebaba, 2023), bean (Pyda *et al.,* 2021), and cereal grains (Lemishko *et al.*, 2022), which contribute to a significant increase in the activity of symbiotic and associative nitrogen fixation and increase yields. N. Khan *et al.* (2020) showed that chickpea plants treated with growth regulators (salicylic acid, putrescine) had a higher height and weight of the aerial parts of plants, increased chlorophyll, protein, and sugar content, and increased yield.

O. Onyshchenko *et al.* (2023) report the effect of an antioxidant-type growth regulator on increasing the leaf surface index, accumulation of dry matter, chlorophyll content in leaves, and growth of photosynthetic potential in sunflower crops. M. Kolesnikov *et al.* (2019) showed that pre-sowing treatment of maize seeds with methyluracil promoted field germination, increased the leaf area index of maize crops, optimised net phototyping performance, and the preparation increased yields by 16-27%. O. Khodanitska *et al.* (2019) showed that the use of treptolem (a complex of auxin, gibberellin, cytokinin compounds) during the budding period of *Linum usitatissimum* L. leads to an increase in the yield of oil flax due to the enhancement of the morphogenesis of vegetative organs with the simultaneous restructuring of the anatomical structure of the stem and leaves. The stimulant enhanced the development of the photosynthetic apparatus: the formation of more leaves, prolongation of their active functioning, increase in the size of chlorenchyma cells and improvement of chloroplastogenesis.

S. Pyda *et al.* (2021) studied the effect of pre-sowing seed treatment with biostimulants Stimpo and Regoplant on the accumulation of oil in seeds and carbohydrates in the leaves of legumes, activation of growth processes and formation of the leaf apparatus of beans. S. Makogonenko *et al.* (2019) and V. Tsygankova *et al.* (2021) found that these biostimulants had bioprotective properties, enhanced growth processes, crop salt tolerance, and contributed to a significant increase in nitrogen fixation by forming a more powerful legume-rhizobial symbiosis. According to H. Kulyk *et al.* (2020), complex pre-sowing and foliar treatments with Stimpo and Regoplant growth regulators intensified the growth and development of sugar beet, increased the weight of roots, which provided an increase in root yields up to 6 t/ha in the central part of Ukraine. In experiments with yellow sorghum, V. Karpenko and S. Shutko (2018) proved that the greatest increase in the concentration of green pigments (a and b chlorophylls) and the increase in the net photosynthetic productivity index in leaves was observed when using the herbicide Pik 75 VG in combination with Regoplant. However, V. Pidlisnyuk *et al.* (2022) found no significant effect of Stimpo and Regoplant on the growth and development of *Miscanthus × giganteus* when grown on depleted soils.

The agrobiological effects of biostimulants Stimpo and Regoplant on the formation of the photoassimilation apparatus of legumes in the arid conditions of southern Ukraine are understudied. Therefore, the purpose of this study was to establish the specific features of the influence of biostimulants (Stimpo, Regoplant) on changes in leaf area index, net photosynthetic productivity, and chlorophyll content in *Pisum sativum* L. Oplot variety under the ecological conditions of the Southern Steppe of Ukraine.

MATERIALS AND METHODS

The study was conducted in 2016-2018 on an experimental field in the Melitopol district of Zaporizhzhia region. Mid-season peas (*Pisum sativum* L.) of the Oplot variety with leafless morphology were sown in the experimental plots. The Oplot pea variety was included in the Register of Plant Varieties of Ukraine in 2011 and recommended for cultivation in the Steppe zone of Ukraine. The originator is the V.Y. Yuriev Institute of Plant Industry of the National Academy of Agrarian Sciences of Ukraine. The territory of Melitopol district is characterised by a temperate continental climate with high temperatures. According to data from the weather station, the average long-term air temperature in the region is 9.9°C. Over the years of the study, during the period of pea cultivation, the average daily air temperatures exceeded the long-term average. Based on the data from the local weather station, Gossen-Walter climadiagrams were constructed in the modification (Cobon *et al.*, 2020), reflecting the hydrothermal regime during the three years of research (Fig. 1).

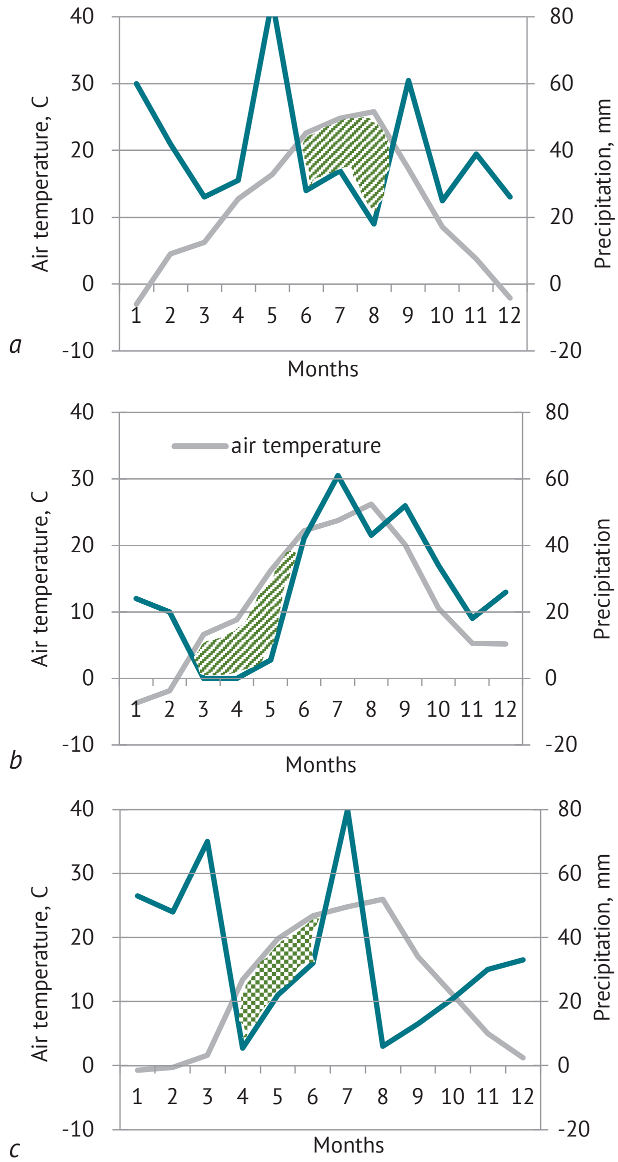

***Figure 1**. Gossen-Walter climate diagrams for 2016 (a), 2017 (b), 2018 (c)*
***Source:** Melitopol weather station data*

During the three years of research, a characteristic precipitation deficit was observed against the background of high temperatures with low hydrothermal potential in spring or summer. According to long-term observations, the annual precipitation in the region was 475 mm, while in 2016, 168 mm fell during the 90-day growing season, in 2017 – 48 mm, and in 2018 – 56 mm. Generally, spring climatic conditions favoured the growth and development of early spring crops, and therefore the efficiency of the production process during the years of research mainly depended on the availability of soil moisture, soil maintenance system, and other agrotechnical methods.

The soil of the experimental plots is a southern alluvial chernozem, characterised by the following indicators: humus content (according to Tyurin) – 2.6%, easily hydrolysable nitrogen (according to Kornfield) – 111.3 mg/kg, mobile phosphorus (according to Chirikov) – 153.7 mg/kg and exchangeable potassium (according to Chirikov) – 255 mg/kg of dry soil. The reaction of the soil solution was neutral (pH water/salt 7.0/7.3). The soil profile was not saline, but was slightly saline (exchangeable sodium 7% of CEC). The soil was of high quality, sufficiently saturated with nutrients and had favourable physical, chemical and agrophysical properties, making it suitable for growing pulses (DSTU 4362:2004, 2006). However, the use of plant growth regulators is justified to obtain high yields of quality products.

Biostimulants Stimpo and Regoplant are complex multicomponent preparations with diverse properties that are explained by the synergistic effect of the interaction of components (a mixture of carbohydrates, polysaccharides, amino acids, antioxidants, and microhormones), fatty acids, amino acids, phytohormones, trace elements) of the vital activity of the microfungus *Cylindrocarpon obtusiucuilum 680*, which is obtained from ginseng roots, and aversectins, which are metabolic products of the soil *Streptomyces avermitilis*. The influence of biostimulants on the formation of photoassimilation apparatus of pea crops in experimental microplots was studied. The seeding rate was 110 pcs/m^2. The variants in the experiment were arranged systematically with four replications. The research design included three variants. The pea seeds of the control group (variant 1) were treated with Liposam solution (5 g/l) before sowing and foliar treatment of crops was carried out with Liposam solution (5 g/l). In variant 2, the following were used: pre-sowing seed treatment – Stimpo (25 ml/t) in Liposam solution (5 g/l); foliar treatment – Stimpo (20 ml/ha) in Liposam solution (5 g/l). The pre-sowing treatment of pea seeds of variant 3 was carried out with Regoplant (250 ml/t) in Liposam solution (5 g/l), and the foliar treatment was carried out with Regoplant (50 ml/ha) in Liposam solution (5 g/l).

Before sowing, the seeds of the experimental variants were treated with biostimulants in the concentrations recommended by the manufacturers, and after drying, they were sown within a day. The first foliar treatment of pea crops was carried out at the stage of 5-6 stipules, and the second foliar treatment was carried out at the stage of budding to flowering. Plant samples were collected during the growing season of pea plants in 5 phases: BBCH 12-13, BBCH 15-16, BBCH 51-55, BBCH 61-65, BBCH 75-79. The working solution consumption was 300 l/ha during foliar treatment of pea crops.

The beginning of each phase of growth and development of pea was established after its onset in 10% of plants, complete phase – in 75% of plants, and the BBCH scale was used (Meier, 2018). At the end of the germination phase (BBCH 09), the field germination of pea seeds was determined. The number of seedlings per 1 m^2 was counted and field germination was calculated in relation to the number of seeds sown in a given area as described in V. Yeshchenko *et al.* (2005). The leaf surface area of pea plants was determined gravimetrically by the notching method. The gravimetric method relates the weight of leaves from 10 plant samples to the weight of leaf cuttings (D = 10 mm). Knowing the area of the leaf notches, the leaf surface area was calculated, and the leaf area index (LAI) was determined in m^2 per 1 m^2 of sown area.

The total chlorophyll content was determined by the fluorometric method using an N-tester (Yara, Japan). The measurement point was chosen in the middle of the first developed leaf. Based on the results of 30 random measurements performed according to the usual "W" scheme, the average value was obtained, and the results were presented in conventional units according to the methodology of I. Rodriguez and G. Miller (2000). The net photosynthetic productivity (NPP) was calculated as the ratio of the growth of plant dry matter mass over a certain period to the half sum of the leaf surface areas at the beginning and at the end of the period. The NPP was calculated using the following formula:

$$NPP = \frac{B_2 - B_1}{0.5(S_1 + S_2)n}, \tag{1}$$

where *NPP* is the net productivity of photosynthesis, g/m^{2}*day; B_1 and B_2 are the dry mass of plants at the beginning and end of the period, g; (B_2-B_1) is the growth of dry weight of plants for n number of days, g; S_1 and S_2 are the leaf surface area at the beginning and end of the period, m^2; 0.5 (S_1+S_2) is the average area of the leaf surface during the experiment; *n* is the period between two observations, days.

The dry mass of the substance was determined gravimetrically according to generally accepted methods (Ram, 2023). In the statistical analysis of the research results, the arithmetic mean, the average error of the arithmetic mean (± m), Student's t-test, and the least significant difference ($LSD_{0.95}$) were determined at a level of confidence of 95% (Yeshchenko *et al.,* 2005). The results were statistically processed using Microsoft Office Excel 2013 and Agrostat. The study adhered to the standards of the Convention on Biological Diversity (1992) and the Convention on Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS AND DISCUSSION

The study found that the biostimulants Stimpo and Regoplant during pre-sowing treatment of pea seeds already in the phase of 2-3 stipules increased the LAI of crops by 12% and 17%, respectively, under growing conditions during the study years (Table 1).

***Table 1**. Change in leaf area index (m^2/m^2) of crops of Pisum sativum L. variety Oplot under the effect of biostimulants*

Development phases	Years	Variants			LSD_{05}
		control	Stimpo	Regoplant	
BBCH 12-13	2016	0.10±0.04	0.16±0.05*	0.18±0.05*	-
	2017	0.21±0.01	0.21±0.01	0.22±0.01	-
	2018	0.19±0.01	0.20±0.01	0.19±0.01	-
	mean	0.17	0.19	0.20	0.05
BBCH 15-16	2016	0.39±0.06	0.45±0.05*	0.46±0.07*	-
	2017	0.45±0.01	0.77±0.01*	0.62±0.01*	-
	2018	0.56±0.01	0.83±0.02*	1.03±0.02*	-
	mean	0.47	0.68	0.70	0.21
BBCH 51-55	2016	2.01±0.11	2.30±0.12	2.76±0.13*	-
	2017	2.26±0.07	3.27±0.07*	3.05±0.05*	-
	2018	1.21±0.03	1.30±0.03	1.45±0.03*	-
	mean	1.82	2.29	2.42	0.44
BBCH 61-65	2016	3.80±0.25	5.70±0.31*	5.20±0.35*	-
	2017	4.16±0.04	6.49±0.09*	5.65±0.10*	-
	2018	2.51±0.08	3.93±0.12*	3.58±0.11*	-
	mean	3.49	5.37	4.81	0.75
BBCH 75-79	2016	4.03±0.31	6.10±0.29*	4.95±0.35	-
	2017	5.96±0.04	7.65±0.13*	7.47±0.09*	-
	2018	3.58±0.12	4.78±0.15*	4.20±0.14*	-
	mean	4.52	6.18	5.54	0.88

***Note:** * – difference is significant compared to the control at $P \leqslant 0.05$*
***Source:** compiled by the author of this study*

The positive effect of biostimulants on the formation of leaf surface during the vegetative phase of pea plants development was established. The LAI value of pea crops under Stimpo and Regoplant increased, respectively, by 15% and 18% in 2016, by 71% and 38% in 2017, and by 48% and 84% in 2018 in the BBCH phase 15-16. During the further vegetative development of pea plants (BBCH phase 15-16), a significant increase in LAI was observed in 1.45 times with Stimpo and in 1.48 times with Regoplant compared to control values.

During the transition to the generative stage of development, an active formation of the leaf surface area of pea plants treated with biostimulants was also noted, as indicated by the growth of LAI in the experimental variants compared to the control values. Thus, in the budding phase, the LAI of pea crops increased on average by 1.26 times under Stimpo and 1.32 times under Regoplant. The most effective increase in the LAI of pea crops was recorded by 1.54 times when using Stimpo (for three years of observations) and by 1.38 times when using Regoplant (for three years of observations) in the flowering phase (BBCH 61-65). The leaf area of pea crops treated with biostimulants stayed significantly increased until the phase of bean formation compared to the control.

The effect of biostimulants on changes in the content of total chlorophyll in pea stipules was characterised by some ambiguity. Thus, at the stages of vegetative development of pea plants, Stimpo led to a slight increase in the chlorophyll content in the stipules, which increased from an average of 1.6% to 2.7% compared to the control. However, during the years of research, a significant increase in chlorophyll content from 4.1% to 10.4% under Stimpo was recorded compared to control values, starting from the budding phase (BBCH 51-55) to the phase of bean formation (BBCH 75-79) (Table 2).

***Table 2**. Changes in the content of total chlorophyll (unit) in the stipules of Pisum sativum L. plants of Oplot variety under the influence of biostimulants*

Development phases	Years	Variants			LSD_{05}
		control	Stimpo	Regoplant	
BBCH 12-13	2016	483±4	505±8*	496±7	-
	2017	402±4	415±2*	405±2	-
	2018	560±5	565±5	568±4	-
	mean	482	495	490	9
BBCH 15-16	2016	459±6	466±5	480±6*	-
	2017	489±7	502±7	480±3	-
	2018	566±6	570±6	579±7	-
	mean	505	513	513	9

Table 2. Continued

Development phases	Years	Variants			LSD_{05}
		control	Stimpo	Regoplant	
BBCH 51-55	2016	549±11	540±10	534±9	-
	2017	489±6	555±8*	562±9*	-
	2018	583±6	590±7	595±8*	-
	mean	540	562	564	15
BBCH 61-65	2016	661±9	676±8	679±9	-
	2017	562±4	619±10*	606±10*	-
	2018	610±9	627±9*	623±9*	-
	mean	611	641	636	18
BBCH 75-79	2016	368±4	385±3*	374±4	-
	2017	624±11	741±12*	721±11*	-
	2018	650±10	685±9*	693±7*	-
	mean	547	604	596	24

Note: ** – difference is significant compared to the control at $P \leqslant 0.05$*
Source: *compiled by the author of this study*

The biostimulant Regoplant did not have a substantial effect on the chlorophyll concentration in pea stipules at the stages of vegetative growth and plant development. In the experiment conducted in 2016, there were no statistically significant changes in chlorophyll levels under the influence of Regoplant in the generative period of plant growth. At the same time, in 2017, after foliar treatments of crops with Regoplant, an increase in chlorophyll concentration by 7.8-15.5% from the budding phase to the bean formation phase was recorded compared to control values. The same results were obtained in the 2018 research, when the content of total chlorophyll significantly increased by 2.1-6.6% under the influence of Regoplant in the phases of budding, flowering, and bean formation. It was found that the studied biological preparations increased the efficiency of photosynthesis in the initial stages of pea vegetation due to an increase in the net productivity of photosynthesis (Table 3).

Table 3*. Changes in net photosynthetic productivity (g/cm^2*day) of Pisum sativum L. plants of Oplot variety under the effect of biostimulants*

Variants		Interphase periods (according to BBCH)			
		12(13) – 15(16)	15(16) – 51(55)	51(55) – 61(65)	61(65) – 75(79)
control	2016	6.5±0.2	8.4±0.4	8.3±0.4	1.6±0.1
	2017	6.4±0.3	8.8±0.4	11.2±0.5	3.8±0.3
	2018	4.6±0.2	12.7±0.6	15.3±0.7	6.3±0.4
	mean	5.8	10.0	11.6	3.9
Stimpo	2016	5.8±0.3	8.4±0.3	9.4±0.5*	1.7±0.1
	2017	7.3±0.3*	9.2±0.5	12.6±0.8	1.9±0.2*
	2018	5.2±0.3*	12.6±0.7	25.1±0.8*	6.2±0.3
	mean	6.1	10.1	15.7	3.3
Regoplant	2016	6.9±0.2*	8.5±0.3	9.7±0.4*	3.3±0.2*
	2017	7.5±0.4*	9.3±0.5	13.0±0.7*	2.0±0.3*
	2018	5.4±0.3*	13.9±0.6	19.7±0.8*	8.3±0.4*
	mean	6.6	10.6	14.1	4.5
LSD_{05}		0.4	0.6	1.9	0.5

Note: ** – difference is significant compared to the control at $P \leqslant 0.05$*
Source: *compiled by the author of this study*

From the data presented in Table 3, the NPP exceeded by 6.2% (2016), 17.2% (2017), and 17.4% (2018) its value in the control variant of pea plants between the phases 2-3 and 5-6 stipules under the influence of the biological product Regoplant. Therewith, the NPP was reduced by 10.8% in 2016 when Stimpo was applied and significantly exceeded the NPP value in control crops in 2017 by 14.0% and in 2018 by 13.0% during the periods of vegetative growth. During the next ontogenetic phase of budding, there were no statistically significant changes in the NPP in pea crops under the influence of biostimulants.

During the interphase period of budding – flowering of peas BBCH 51(55) – 61(65), there were substantial changes in the NPP between the experimental and control variants of pea crops. During this period, the average NPP of pea crops over the years of research exceeded the control values by 1.35 times under Stimpo and 1.22 times under Regoplant. The NPP of pea crops treated with the biostimulant Stimpo did not exceed the control values during the period of flowering – bean formation, while under the action of the biostimulant Regoplant, the average NPP value over the years of research exceeded this indicator in control pea crops by 15.4%. Thus, with intensive pea cultivation technologies, the use of plant biostimulants derived from natural raw materials allows for better formation of the leaf surface of crops, stimulation of photosynthetic processes, efficient use of PAR energy and a direct impact on crop yields in the Ukrainian Steppe zone.

The main advantage of modern plant growth regulators, which include a balanced complex of metabolically active substances, is their ability to target key processes of plant growth and development, as well as to effectively implement the potential of a particular variety or hybrid. The organic biological products under study are third-generation plant growth regulators with a broad spectrum of action and bioprotective effect. The mechanism of action of these preparations is based on the synergy of microbial fungi and aversectin, which has a positive effect on the physiological and biochemical parameters of plants and increases their resistance to stress (Medkov *et al.*, 2021). This is confirmed by the findings of studies of the influence of these biostimulants on the formation of the photoassimilation apparatus of different varieties of legumes in the different climate zones of Ukraine (Pyda *et al.,* 2021; Makogonenko *et al.*, 2019).

The analysis of the Gossen-Walter climate diagrams over the years of research shows that during the growing season of peas, there is a steady trend for changes in agroclimatic parameters towards warming and increasing aridity. Weather conditions have a substantial impact on the production process of pea and, specifically, the formation of the photoassimilation surface and its efficiency, such as ambient temperature, amount and uniformity of precipitation, which is confirmed by some researchers (Kolosovskaya, 2019; Vozhegova *et al.*, 2021).

Modern methods of growing crops are based on the concept of crop formation as a photosynthetic system and are developed with the biological characteristics of a particular crop in mind. The size of the assimilation surface of crops directly affects the yield of legumes and is an important diagnostic indicator.

Scientific data shows that the optimum leaf area of crop plants is 40 thsd m^2/ha, which allows for maximum crop productivity. Other studies indicate that for modern varieties and hybrids grown using intensive technologies and widely introduced into agricultural production, the optimum leaf area is within 50-60 thsd m^2/ha (Kim & Narimanov, 2021). The leaf area of pea crops over the 3 years of research increased during the growing season and the LAI reached its maximum values at the stage of bean formation. It was during this period that the most effective effect of the biostimulant Stimpo on the increase in leaf surface was noted, compared to the effect of Regoplant. The positive effect of growth biostimulants on the leaf area of pea crops is confirmed by the findings of K. Nebaba (2020).

Notably, O.S. Titarenko and L.M. Karpuk (2022) confirmed the increase in the efficiency of photosynthesis of grain sorghum hybrids with the foliar application of these biostimulants. The effect of the biostimulants under study on the accumulation of chlorophyll in pea stipules was effective only after the application of foliar treatments carried out in the BBCH phases 51-55. Presumably, according to N.V. Novytska and O.V. Ponomarenko (2022), the improvement of symbiotic nitrogen fixation under biostimulants increased the effective nitrogen assimilation, which had a positive effect on chlorophyll accumulation. The positive effect of the biostimulants under study is associated with the stimulating effect of cytokinins on chlorophyllogenesis (through increased synthesis of its precursor, protochlorophyllide) and on the intensity of photosynthesis and respiration (Khan *et al.*, 2020).

It is well-known that not all the leaf area formed in crops can be effectively used for the synthesis of dry matter. This is mainly due to the different tiering of the leaves and the problems that arise in ensuring efficient access to solar energy. Although it is difficult to estimate the illumination of individual leaves, changes in net photosynthetic productivity can still serve as a basis for some assumptions. The productive process of crops is correlated to a certain extent with photosynthetic parameters (Wu *et al.*, 2019). However, finding a quantitative correlation between plant productivity in agrocenoses and photosynthetic intensity is often difficult, as these aspects are mainly influenced by environmental conditions. The increase in NPP up to the interphase period of BBCH 51(55) – 61(65) was noted in all variants under study and was replaced by a sharp decrease in the interphase period of BBCH 61(65) – 75(79) in pea crops.

During these observations, only pre-sowing treatment of seeds with biostimulants generally did not have a substantial effect on the growth of LAI, while the NPP indicators increased during the vegetative phase of pea plants ontogeny. The biostimulants under study improved the parameters of net photosynthesis of pea plants when applying foliar treatments, which is confirmed by the findings of 3-year studies and the increase in NPP noted in other studies (Kulyk *et al.*, 2020; Pyda *et al.*, 2021; Netwal *et al.*, 2022). The biostimulant Regoplant more effectively contributed to the formation of dry biomass through the photosynthetic activity of the pea leaf apparatus compared to Stimpo during the entire growing season. Thus, the findings of this study confirm the data of other scientists on the influence of growth regulators on the formation of the photosynthetic apparatus of legumes and their effectiveness in the arid conditions of southern Ukraine.

CONCLUSIONS

The findings of the conducted study suggest that the use of biostimulants Stimpo and Regoplant in the cultivation of sowing peas is one of the effective agricultural measures to increase the efficiency of the production process through the influence on the functioning of the photoassimilation apparatus. Thus, based on the obtained 3-year results of the study, it can be concluded that the use of biostimulants Stimpo (25 ml/t + 20 ml/ha) and Regoplant (250 ml/t + 50 ml/ha) increased the leaf area index in different stages of vegetation of pea variety Oplot by 1.12-1.54 and 1.18-1.38 times, respectively, compared to the control.

The biostimulants Stimpo and Regoplant under the conditions of foliar treatments maximally increased the NPP of pea crops by 1.35 and 1.22 times, respectively, during the interphase period budding – flowering. The effect of biostimulants on the concentration of total chlorophyll in pea stipules was ambiguous and varied depending on the years. The probability of changes in the chlorophyll content was observed only after foliar treatment of crops with biostimulants. Thus, the study recorded an average increase in chlorophyll content by 4.0% under the action of biostimulants during the period of budding-flowering (BBCH 51(55) – 61(65)) and by 10.4% under Stimpo and 9.0% under Regoplant during the interphase period of budding-bean formation (BBCH 61(65) – 75(79)).

The data obtained confirm the prospects of further research on the efficiency of legume-rhizobial symbiosis, analysis of the structure of crops, elements of biological yield of peas and other legumes and product quality under the influence of biostimulants in the arid conditions of the Ukrainian Steppe zone, which will provide the population with high-quality food raw materials.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Формування фотоасиміляційного апарату посівів гороху (*Pisum sativum* L.) за дії біостимуляторів в посушливих умовах Південного Степу України

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Анотація. Південний Степ України характеризується рядом несприятливих агрокліматичних умов, що призводить до гальмування ростових процесів, порушення фізіологічних процесів та втрати врожаю сільськогосподарських культур. Застосування екологічно безпечних біорегуляторів стимулює ростові процеси, оптимізує азотне живлення, підвищує адаптивність зернобобових культур до несприятливих умов і збільшує урожайність. Метою роботи було з'ясувати особливості впливу біостимуляторів на формування фотоасиміляційного апарату гороху посівного сорту Оплот в умовах Південного Степу України. Було проведено 3-х річні дрібноділянкові дослідження в ході яких визначали індекс листкової поверхні, вміст загального хлорофілу та чисту продуктивність фотосинтезу в посівах гороху. Біостимуляторами проводили передпосівний та позакореневий обробіток посівів гороху в фазі бутонізації у концентраціях, описаних в методології. Було встановлено, що Стимпо (25 мл/т + 20 мл/га) збільшував індекс листкової поверхні у різних фазах вегетації гороху сорту Оплот в 1,12-1,54 рази та Регоплант (250 мл/т +50 мл/га) збільшував індекс листкової поверхні в 1,18-1,38 рази порівняно з контролем. Вірогідне збільшення вмісту хлорофілу в прилистках рослин гороху було зафіксовано після фоліарної обробки посівів біостимуляторами та перебільшувало значення контрольних рослин на 9,0-10,4 % в період BBCH 61(65) – 75(79). За 3-х річними результатами доведено позитивний вплив біостимуляторів на чисту продуктивність фотосинтезу. Так, Стимпо та Регоплант збільшували чисту продуктивність фотосинтезу посівів гороху на 35 % та 22 % відповідно у фазі бутонізація (BBCH 51-61) і цвітіння (BBCH 55-65). Одержані наукові результати сприяють широкому використанню досліджуваних біостимуляторів в технології вирощування гороху в зоні Південного Степу України з метою підвищення продуктивності культури та отримання врожаю високої якості

Ключові слова: бобові; регулятори росту рослин; Регоплант; Стимпо; чиста продуктивність фотосинтезу; індекс листкової поверхні; хлорофіл

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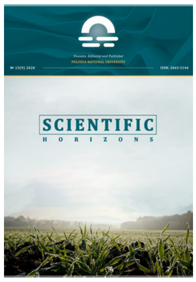

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Number of melanin-synthesising microorganisms under mineral fertilisation and liming of Albebeluvisoil

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Abstract. The study of the main patterns of distribution of microorganisms that synthesise melanins, which are precursors and components of humus molecules in agrocenosis soils is an urgent scientific task. The purpose of this study was to determine the influence of agrochemical factors on the number of melanin-synthesising microbial

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species and bacteria in grey forest soil. Microbiological, laboratory and analytical, and statistical methods were employed in the study. It was found for the first time that the number of melanin-synthesising micromycetes is minimal in the soil of the variant without fertilisers, liming with one dose according to hydrolytic acidity contributes to an increase in their number by 86.8%, application of mineral fertilisers in a dose of $N_{30}P_{30}K_{45}$ – 2.0 times, compatible use of lime and mineral fertilisers – 2.94 times. A 1.5-fold increase in the dose of mineral fertilisers leads to a 2.54-fold increase in the number of colony-forming units (CFU) of melanin-synthesising microbial species compared to a single dose of fertilisers, and a 2.0-2.62-fold increase in the dose of fertilisers, which coincides with the results of the analysis of the humus content in the soil of these variants – it exceeds the humus content in the variant with a single dose of fertilisers by 26.5% and 16.3%, respectively (correlation coefficient is 0.811). The number of melanin-synthesising bacteria in grey forest soil is 2-3 orders of magnitude higher than the number of melanin-synthesising micromycetes. However, the patterns of influence of anthropogenic factors on their number coincide with those established for micromycetes: liming with one dose of hydrolytic acidity leads to an increase in the number of melanin-synthesizing bacteria by 26.1%, application of mineral fertilizers in a dose of $N_{30}R_{30}K_{45}$ – 2.03 times, combined use of lime and mineral fertilisers – 2.48 times. A 1.5-fold increase in the dose of mineral fertilisers leads to a 5.8-fold increase in the number of melanin-synthesising bacteria compared to a single dose of fertiliser, while a 2.0-fold increase in the dose of fertiliser leads to a 13.3-fold increase, respectively. The correlation coefficient between the number of melanin-synthesising bacteria and the humus content in the soil is 0.417. The findings of the presented research can be used to develop recommendations for measures to increase the humus content of agricultural soils

Keywords: melanin-synthesising microorganisms; micromycetes; bacteria; azotobacter; liming; mineral fertiliser; humus

INTRODUCTION

Humus plays a fundamental role in the existence of biogeocenoses and agrocenoses. Its quantity and qualitative properties affect the soil's accumulation capacity, ensuring the level of effective fertility, and it accumulates carbon dioxide for a long time, reducing the negative impact of agriculture on climate change, etc. Therefore, the study of all processes that affect the accumulation of humus, and specifically, the spread of melanin-synthesising microorganisms, is relevant and practically significant. The importance of melanins for the synthesis of humus is underlined by the fact that a separate class of substances, haematomelanic acids, is now being identified as part of humus.

Melanins are the oldest biological pigments synthesised by representatives of all kingdoms of life (Zhdanova & Vasilevskaya, 1988). They are known for their ability to absorb broad-spectrum radiation, serve as an antioxidant, a photoprotector that effectively absorbs and dissipates solar radiation in the form of heat, a sorbent that chelates metals and binds organic compounds, and an organic semiconductor (Barreto *et al.*, 2023; El-Zawawy *et al.*, 2024). Specifically, A.S. Abd-El-Aziz *et al.* (2024), for melanin from the black fungus *Curvularia soli* AS21 ON076460, found antimicrobial, antiviral, and antitumour activity. The zones of growth inhibition of *Klebsiella pneumoniae ATCC 13883* and *Pseudomonas digitatum* were 37.51 ± 0.012 and 44.25 ± 0.214 mm, respectively. *Curvularia soli AS21* melanin demonstrated significant antiviral efficacy (77% inhibition) of herpes simplex virus (HSV1), as well as cytotoxic effects against human breast cancer and skin cancer cell lines (Mcf7 and A431), while causing a low percentage of cell death in normal human skin cell lines (Hfb4).

Fungi, especially ascomycetes, synthesise melanin-molecules in their cell walls to protect against fungal pathogens and harsh abiotic conditions (Baskaran *et al.*, 2019). A range of soil microorganisms of the genera *Bacillus*, *Aeromonas*, *Mycobacterium*, *Azotobacter*, *Streptomyces*, *Stachybotris*, *Aspergillus*, etc. synthesise melanins, which are analogous in their characteristics to humus molecules (Gerke, 2018). Model experiments have shown that these pigments are incorporated into humus molecules. The absorption spectra of melanin pigments of streptomycetes are comparable to those of natural humic acids. Their acidic properties are also analogous. In terms of optical characteristics and molecular weight, melanoproteins of streptomycetes are comparable to young humic acids. Humic compounds are rich in phenoxyl radicals, which can bind various organic and inorganic molecules, including amino acids, peptides, sugars, and melanins (Kulikova & Perminova, 2021).

In Ukraine, studies are currently underway to investigate the antioxidant or prooxidant properties of haematomelanic acids in radical chain oxidation processes, depending on the nature of the oxidative substrate (Efimova *et al.*, 2022). Specifically, it was shown that the addition of haematomelanic acids to the coumol and ethylbenzene oxidation system causes a slowdown in oxidation, with the observed effect increasing with increasing concentration of haematomelanic acids. It was also shown that the opposite effect is observed in the oxidation of ascorbic acid – acceleration of oxidation with the addition of haematomelanic acids, and the effect of acid concentration dependence was also established.

However, the ecology of melanin-synthesising microorganisms – what anthropogenic and natural factors and how they affect the number of melanin-synthesising microbes and bacteria (including azotobacter) – is still unexplored. This is especially important for agrocenosis soils, as the process of melaninogenesis directly affects the accumulation of humus and the level of potential soil fertility.

MATERIALS AND METHODS

The study was conducted in the stationary experiment of the Department of Agrosoil Science and Soil Microbiology of the National Research Centre "Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine" "Development and Improvement of Intensive Crop Growing Technologies Based on Extended Reproduction of Soil Fertility". The soil of the experimental plots is grey forest coarse-dusty light loam. The initial parameters of the humus state of the studied soil in the layer 0-20 cm were as follows: total humus content – 1.44%, type of humus formation – humate-fulvate – Cha – 31.9, Cfa – 40.4, Cnhr – 27.7%; Cha:Cfa = 0.79. The composition of the humus was dominated by the aggressive group of fulvic acids of fractions 1a and 1, which resulted in acidic, easily mobile soil humus, as fractions associated with mobile half-acids predominated. According to the classification of soils by granulometric composition, the soil of the experimental site belongs to the coarse dust-light loamy type: the coarse dust fraction prevails in the arable layer – 52.4%, the content of physical clay is 20.5%, and silt – 12.9%. The high content of the dust fraction and low content of the silt fraction determines a range of physicochemical and agrochemical properties of the soil under study, among which the key are low absorption capacity and low humus content, which causes low natural fertility of grey forest soil.

The object of research was the soil of the variants of the stationary experiment: 1 – without fertilisers (control); 2 – liming according to the indicator of hydrolytic acidity with a full dose of 1.0 Hg; 3 – introduction of mineral fertilisers in a dose of $N_{30}P_{30}K_{45}$; 4 – $N_{30}P_{30}K_{45}$ + $CaCO_3$ (1.0 Hg); fertilisation variants against the background of ploughing by-products of crop production (winter wheat) and biomass of green manure crops (fodder radish): 6 – $N_{30}P_{30}K_{45}$; 7 – $N_{30}P_{30}K_{45}$ + $CaCO_3$ (1.0 Hg); 8 – $N_{45}R_{45}K_{68;}$ 9 – $N_{60}R_{60}K_{90}$. Lime (limestone and dolomite powder) was applied in 1992 and again in 2005 in 1.0 and 1.5 doses according to hydrolytic acidity, 1/7 dose annually for each crop of the crop rotation and to neutralise the acidity of physiologically acidic mineral fertilisers (according to the value of hydrolytic acidity, the full dose of 1.0 Hg was 4.5-6.0 t/ha $CaCO_3$).

In 2019, white lupine of the ultra-early variety "Serpnevyi" was grown in the experimental variants, with winter wheat as the predecessor. The repetition of the experiment was fourfold, the area of the sowing plot was 60 m^2 (10×6), the accounting plot was 24 m^2 (6×4). Soil samples were taken from the root zone of plants on the day of sowing the soil suspension on the nutrient media. The number of microorganisms of the main ecological, trophic, functional, and systematic groups was assessed by sowing a water-soil suspension on the appropriate general, selective, and special nutrient media in three replicates (Alef & Nannipieri, 1995; Paul, 2015): ammonifiers consuming nitrogen from organic compounds – on meat-peptone agar, mineral nitrogen immobilisers – on starch-ammonia agar, pedotrophs – on soil agar (based on soil extract), oligotrophic microorganisms – on starvation agar, autochthonous microorganisms – on Vinogradskyi nitrite starvation agar, and azotobacter – on Ashby's medium. The number of colonies was counted for 21 days, depending on the growth rate and physiological characteristics of microorganisms of a particular ecological and trophic group. Indicators of the intensity of the processes of mineralisation of nitrogen compounds, soil organic matter, humus, probability of bacterial colony formation (BCF), and total biological activity (TBA) were determined using the method of relative values as described previously (Malynovska *et al.*, 2023). Generalisation of materials, calculation of research results, reliability of differences between samples were assessed using analysis of variance with subsequent assessment of least significant differences (LSD_{05}) using Statistica 5 software.

RESULTS

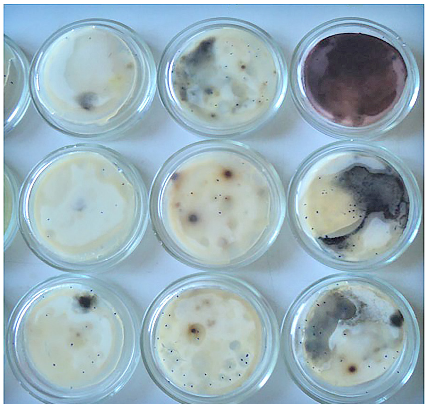
The results of the authors' research on the distribution of melanin-synthesising microorganisms under the influence of agrochemical factors in grey forest soil are presented in Table 1 and Figure 1. At the current stage of scientific development, the synthesis of melanoid pigments is considered to be a protective reaction of microorganisms to anthropogenic pollution of ecotopes, specifically, by radionuclides and heavy metals (Malynovska, 2017). It was shown that with an increase in the level of soil contamination with heavy metals from 5 to 100 MPC, the number of melanin-synthesising microbial species increases, while nitrogen-bacteria decreases: at a dose of heavy metals of 5 MPC – by 90.8%, at 10 MPC – 83.3%, at 100 MPC – 114.9%. The number and proportion of melanin-synthesising micromycetes in the total number of fungi can be used as a diagnostic indicator for grey forest soil contamination only if the contamination period does not exceed 24 months. It was shown that the nature of heavy metals' impact on the number of microorganisms depends on the presence of phytocoenosis in the monitoring system, the dose of heavy metals, the period of contamination, and the type of previous soil use.

These results confirm the following thesis: in the control variant of the experiment, the number of melanin-synthesising microbial species is minimal, since the soil of the control variant is the least contaminated with pollutants (Table 1). Liming leads to an increase in the number of melanin-synthesizing micromycetes by 86.8%, application of mineral fertilisers ($N_{30}P_{30}K_{45}$) – by 2.0 times, combined application of lime and mineral fertilisers – by 2.94 times. As a result of the application of agricultural practices, not only the number of melanin-synthesising micro-mycetes increases, but also their share in the total number of fungi. Thus, with liming in one dose, the proportion of melanin-synthesising microbial species increases by 73.7% in terms of hydrolytic acidity, with mineral fertilisation – by 1.9 times, and with the combined use of agricultural practices – by 3.99 times. A 1.5-fold increase in the dose of mineral fertilisers leads to a 2.54-fold increase in the number of melanin-synthesising microbial communities, and a 2.0-fold increase leads to a 2.62-fold increase. There is an increase not only in the number of melanin-synthesising microbial cells, but also in their proportion: at a one-and-a-half dose of fertiliser, it is 2.22 times higher, and at a double dose, it is 2.38 times higher. This confirms the assumption that the synthesis of melanoid pigments is a protective reaction not only to soil contamination by radionuclides, but also to general contamination of agrophytocenoses, which includes various pesticides, impurities in mineral fertilisers, residues of ameliorants, heavy metals, etc.

***Table 1**. Influence of agrochemical measures on the number of microorganisms in grey forest soil, mln CFU*/g of dehydrated soil*

Variant		Azotobacter, % fouling soil clod	Pedotrophs	Autochthonous	Micromycetes	Melanin-synthesising micromycetes	The share of melanin-synthetic micromycetes in the total amount, %	Melanin-synthesising bacteria	Proportion of melanin-synthesising bacteria in the total number, %	Total number of microorganisms
Without fertilisers (control)		69.3	48.6	17.1	0.448	0.0068	1.52	1.15	0.110	1,047.5
$CaCO_3$ (1.0 Hg)		92.6	129.8	14.8	0.483	0.0127	2.64	1.45	0.120	1,206.5
$N_{30}P_{30}K_{45}$		12.0	85.9	26.0	0.470	0.0136	2.89	2.33	0.182	1,283.3
$N_{30}P_{30}K_{45}$ + $CaCO_3$ (1.0 Hg)		98.7	51.0	15.8	0.337	0.0204	6.05	2.85	0.249	1,144.1
$N_{30}P_{30}K_{45}$	Green manure + by-products	15.3	22.5	22.5	0.340	0.0034	1.00	2.05	0.177	1,154.9
$N_{30}P_{30}K_{45}$ + $CaCO_3$ (1.0 Hg)		94.0	15.9	15.9	0.432	0.0103	2.38	2.12	0.205	1,031.9
$N_{45}P_{45}K_{68}$ + $CaCO_3$ (1.0 Hg)		73.3	22.7	22.7	0.496	0.0262	5.28	12.3	0.820	1,491.6
$N_{60}P_{60}K_{90}$ + $CaCO_3$ (1.0 Hg)		80.7	52.7	52.7	0.476	0.0270	5.67	28.2	1.63	1,733.7
$LSD_{0.5}$		2.84	6.85	0.85	0.85	0.002	0.11	1.04	0.02	10.2

***Source:** compiled by the authors of this study*



1 2 3

***Figure 1**. Synthesis of melanoid pigments by microorganisms of grey forest soil under ploughing of by-products of predecessor in crop rotation and green manure biomass*

***Note:** 1 – $N_{30}P_{30}K_{45}$ + $CaCO_3$ (1.0 Hg); 2 – $N_{45}P_{45}K_{68}$ + $CaCO_3$ (1.0 Hg); 3 – $N_{60}P_{60}K_{90}$ + $CaCO_3$ (1.0 Hg)*

***Source:** compiled by the authors of this study*

The patterns of distribution of bacteria capable of synthesising melanins and melanin-like pigments were also investigated. As Table 1 shows, the number of melanin-synthesising bacteria is 2-3 orders of magnitude higher than the number of melanin-synthesising fungi. However, the patterns observed for microorganisms of this group almost completely coincide with those obtained for melanin-synthesising micromycetes, specifically, it was shown that liming leads to an increase in the number of melanin-synthesising bacteria by 26.1%, the introduction of mineral fertilisers ($N_{30}P_{30}K_{45}$) – by 2.03 times, the combined use of lime and mineral fertilisers – by 2.48 times. As a result of the use of agricultural practices, not only the number of melanin-synthesising bacteria increases, but also their share in the total number of microorganisms. Thus, with liming with a single dose of hydrolytic acidity, the proportion of melanin-synthesising bacteria increases by 9.09% compared to the control, with mineral fertilisation with a dose of $N_{30}P_{30}K_{45}$ – by 65.5%, with the combined use of agromeasures – by 2.26 times.

A 1.5-fold increase in the dose of mineral fertilisers leads to a 5.8-fold increase in the number of melanin-synthesising bacteria compared to a single dose of fertiliser, and a 4.0-fold increase in their share (Table 1, Fig. 1). A 2.0-fold increase in fertiliser dose contributes to a 13.3-fold increase in the number of melanin-synthesising bacteria and a 7.95-fold increase in their share in the total number of microorganisms.

In lupine cultivation, liming enhances the mineralisation of soil organic matter without mineral fertilisation by 95.3%, against the background of ploughing exogenous organic matter (EOM) – by 13.9%, and with mineral fertilisation – does not weaken it (Fig. 2). These results are substantially different from those obtained in previous years, when liming weakened the mineralisation of soil organic matter and hindered the preservation of the pool of organic and organic-mineral substances. This may have been due to a decrease in mineralisation in the soil of the no-fertiliser variant, against which the results of the other variants are compared. Thus, in 2012, the pedotrophicity index in the control was 0.523 (winter wheat), in 2013 – 0.124 (soybean), in 2014 – 0.325 (spring wheat), in 2015 – 0.296 (buckwheat), which indicates that the content of organic matter in the soil of the control variant is gradually decreasing and, as a result, the intensity of organic matter mineralisation is decreasing. The index of oligotrophicity as a result of liming without mineral fertilisation increases by 80.4%, and against the background of mineral fertilisation and ploughing of green manure biomass and by-products of the predecessor crop in the crop rotation – decreases by 69.7% and 229.4%, respectively. A different trend was revealed when studying the mineralisation-immobilisation of nitrogen compounds: when liming without fertilisers, the mineralisation intensity increases by 71.4%, when using mineral fertilisers – by 14.3%, when ploughing green manure and by-products of the predecessor – by 66.9%.

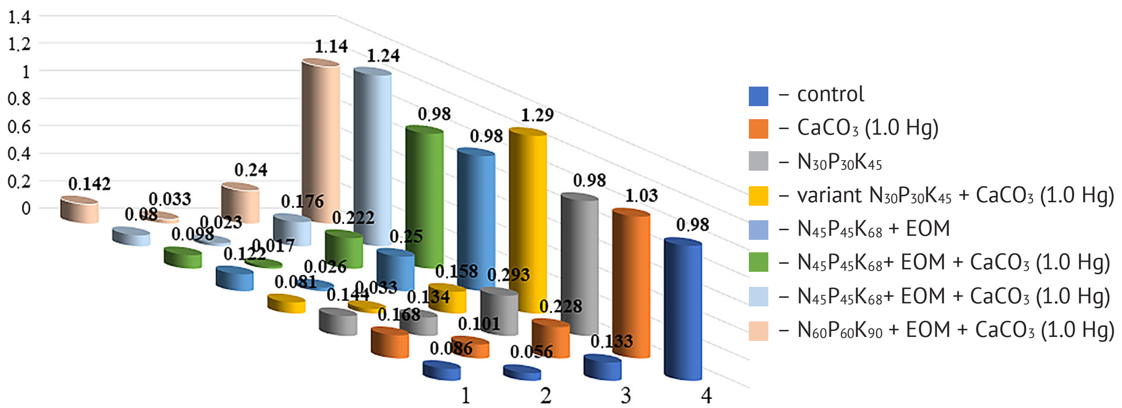


Figure 2*. Indicators of the intensity of mineralisation processes and humus content in grey forest soil under different agrochemical measures*

Note: *1 – pedotrophic index; 2 – oligotrophic coefficient 3 –nitrogen compounds mineralisation coefficient, 4 – humus content %*

Source: *developed by the authors of this study*

The regularities obtained in previous years regarding the effect of liming on the activity of humus mineralisation were also confirmed: it decreases as a result of liming the soil with a dose of 1 Hg without mineral fertiliser by 9.32%, with mineral fertiliser – by 13.5%, and against the background of ploughing green manure biomass and by-products of the predecessor – by 15.8% (Fig. 3). The application of mineral fertilisers in a single dose also slows down the mineralisation of humus compared to the control by 16.2%, together with liming – by 13.5%, against the background of ploughing by-products – by 19.3%; for the application of 1.5 doses of fertilisers against the background of ploughing by-products – by 9.66%; for the application of a double dose of fertilisers against the background of ploughing by-products, the mineralisation of humus is activated by 4.9% (Fig. 3).

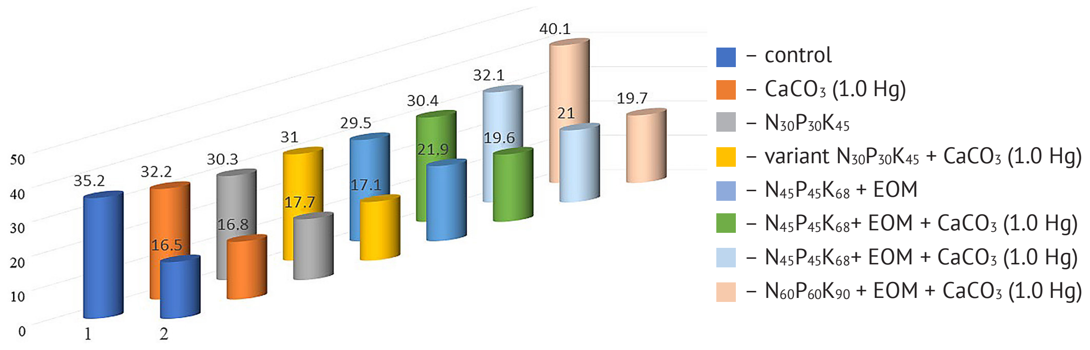


***Figure 3**. Indicators of humus mineralisation activity and phytotoxic properties of grey forest soil under different agrochemical measures*

***Note:** 1 – activity of humus mineralisation, %; 2 – weight of 100 plants of the test crop – winter wheat, g*

***Source:** developed by the authors of this study*

Neutralisation of excessive acidity of the soil solution allows increasing the total biological activity of the soil of the variant without mineral fertilisation by 31.3%, with mineral fertilisation – by 7.41, and against the background of ploughing exogenous organic matter (EOM) – by 6.79% (Fig. 4). Liming helps to reduce the level of soil phytotoxicity in all variants of application: without mineral fertiliser, with mineral fertiliser and with mineral fertiliser in the background of EOM application. The positive effect of liming is enhanced in variants with the introduction of organic matter (by-products of the predecessor and green manure) into the soil.

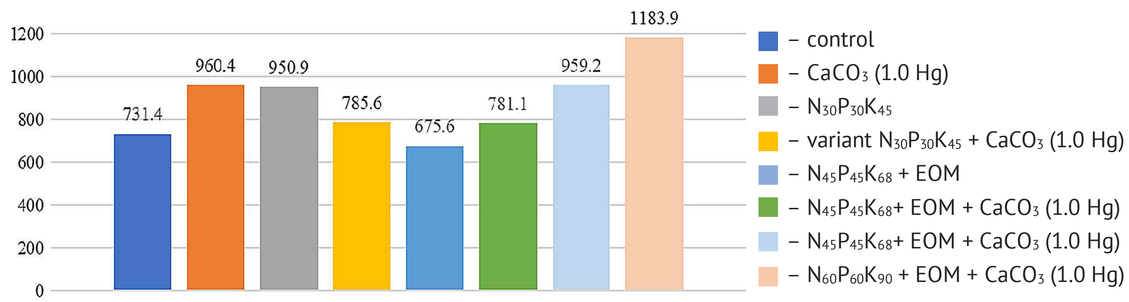
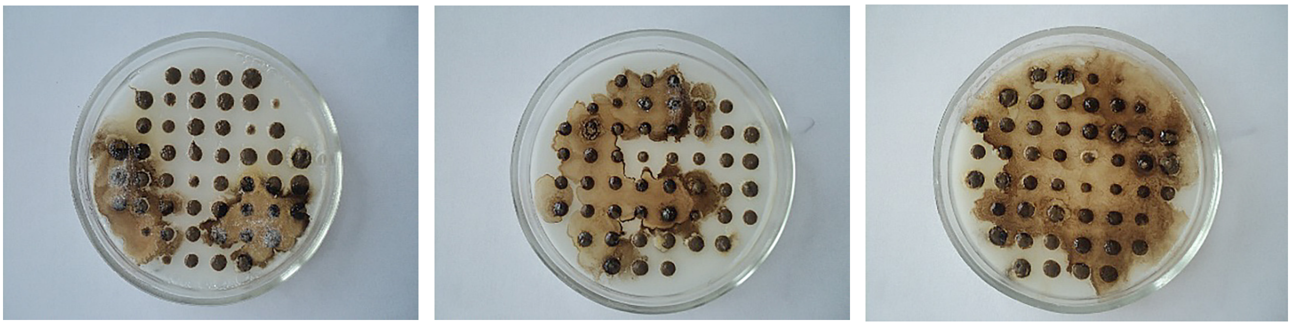


***Figure 4**. Indicators of total biological activity of grey forest soil under different agrochemical measures*

***Source:** developed by the authors of this study*

The total biological activity also increases under the influence of mineral fertilisation. The application of mineral fertilisers in a single dose leads to a 30.0% increase in TBA; in combination with liming – by 7.41%; against the background of ploughing by-products in combination with liming – by 6.80%; with the application of 1.5 dose of fertilisers against the background of ploughing by-products – by 31.1%; with the application of a double dose of fertilisers against the background of ploughing by-products – by 61.9% (Fig. 4). This highlights the overall impact of mineral fertilisers as a source of nutrients on the functioning of the phytos and microbiome.

Microorganisms of the genus *Azotobacter* also can synthesise melanin (Fig. 5). As Table 1 shows, the number of nitrogen-bacteria is closely related to the application of lime, its number reaches a maximum in the soil of the variants where the pH of the soil solution was neutralised. Liming without mineral fertilisers exceeds the control figures by 33.6%, while liming in combination with mineral fertiliser exceeds the control figures by 42.4%. The application of mineral fertilisers in a single dose without liming leads to a 5.78-fold reduction in the number of nitrogen-bacteria, while ploughing by-products reduces the number of nitrogen-bacteria by 4.53 times. An increase in the dose of fertiliser, even with simultaneous liming, helps to reduce the number of nitrogen-bacteria: a dose and a half of fertiliser inhibits the viability of this microorganism by 28.2%, and a double dose – by 16.5%.



a *b* *c*

***Figure 5**. Synthesis of melanins by bacteria of the genus Azotobacter under ploughing of by-products of the predecessor in crop rotation and green manure biomass*

***Note:** a – $N_{30}P_{30}K_{45}$; b – $N_{45}P_{45}K_{68}$ + $CaCO_3$ (1.0 Hg); c – $N_{60}P_{60}K_{90}$ + $CaCO_3$ (1.0 Hg)*

***Source:** developed by the authors of this study*

To establish the relationship between the indicators under study, a correlation analysis was conducted, which showed that the humus content in grey forest soil under white lupine cultivation is closely related to the number of melanin-synthesising micromycetes (r = 0.811) and their share in the total number of fungi (r = 0.915) (Table 2). Melanin-synthesising bacteria, despite being much more numerous than micromycetes, have a medium level of significance with humus content (r = 0.417), as does their share in the total number of microorganisms (r = 0.450). Therewith, the number of melanin-synthesising bacteria has a prominent level of correlation with the number of melanin-synthesising microbial species (r = 0.761), which indicates, in our opinion, that the distribution of melanin-synthesising fungi and bacteria is controlled by the same environmental factors. Notably, the number of melanin-synthesising bacteria is closely related to the activity of humus mineralisation (r = 0.805) and the number of autochthonous bacteria (r = 0.701), which are responsible for humus mineralisation. In general, all indicators of melanin synthesis (the number of producers, their share in the total number of microorganisms in this kingdom) are closely related.

Despite the high activity of melanin synthesis (Figure 5), the correlation between the number of nitrogen-bacteria and humus content has an average level of significance (r = 0.482) (Table 2). The determination of the physiological and biochemical activity of producer cells, in this case, the colony formation probability (CFP) of producers on agarified medium, is important for understanding the patterns of synthesis of any product or the intensity of the process in the soil. To determine the metabolic activity of microorganisms directly in the soil, the method of analysing the dynamics of colony formation was used, which makes it possible to simultaneously determine the number and composition of the complex of chemo-organoheterotrophic bacteria in the soil. Colony formation probability (CFP) reflects the physiological and biochemical activity of microbial cells in the natural environment. As Table 2 shows, only nitrogen-bacteria and autochthonous microorganisms have a medium level of significance with humus content. The correlation between the CFP of microbial species and humus content is insignificantly negative, possibly because the physiological and biochemical activity of all microbial species, not just melanin-synthesising ones, was determined. This is also clear from the fact that the physiological and biochemical activity of microbial cells depends on a range of factors: the moisture content of the soil in which they exist, the availability of substrates and energy sources, the phase of the population development cycle, etc. Therefore, the probability of colony formation can vary substantially during the growing season and generally does not correlate with the humus content of the soil.

Table 2. Correlation coefficients between indicators of microbial cenosis and humus content in grey forest soil for growing white lupine

Indicator	Azotobacter, % fouling soil clod	Autochthonous	Micromycetes	Melanin-synthesising micromycetes	Share of melanin-synthetic micromycetes in the total amount, %	Melanin-synthesising bacteria	Proportion of melanin-synthesising bacteria in the total number, %	Total number of microorganisms	Humus mineralisation activity, %	PCF of micromycetes	PCF of azotobacter	CFP of autochthonous microorganisms	Humus content, %
Azotobacter, % fouling soil clod	1.000												
Autochthonous	0.097	1.000											
Micromycetes	0.132	0.489	1.000										
Melanin-synthesising micromycetes	0.418	0.431	0.390	1.000									
Share of melanin-synthetic micromycetes in total number, %	0.482	0.274	0.116	0.948	1.000								
Melanin-synthesising bacteria	0.183	0.701	0.378	0.761	0.628	1.000							
Share of melanin-synthesising bacteria in the total number, %	0.195	0.669	0.372	0.783	0.652	0.998	1.000						
Total number of microorganisms	0.007	0.743	0.483	0.806	0.643	0.931	0.932	1.000					
Humus mineralisation activity, %	0.322	0.673	0.434	0.492	0.377	0.805	0.782	0.658	1.000				
PCF of micromycetes	0.044	0.141	0.138	-0.158	-0.123	-0.496	-0.512	-0.262	-0.336	1.000			
PCF of azotobacter	0.525	-0.364	-0.469	0.164	0.393	-0.104	-0.085	-0.285	-0.193	-0.185	1.000		
PFC of autochthonous microorganisms	0.586	-0.291	-0.248	0.134	0.360	-0.241	-0.239	-0.361	-0.018	0.200	0.785	1.000	
Humus content, %	0.482	0.034	-0.102	0.811	0.915	0.417	0.450	0.445	0.176	-0.069	0.360	0.334	1.000

Source: *compiled by the authors of this study*

Thus, melanin-synthesising microorganisms of grey forest soil, synthesising melanins, have a substantial impact on humus accumulation. The spread of melanin-synthesising microorganisms depends on the agronomic practices used in a given agrocenosis, soil type, and the level of soil pollution by pollutants.

DISCUSSION

Experimental data showing that increasing the dose of mineral fertilisers by 1.5 and 2.0 times leads to an increase in the number of melanin-synthesising bacteria and fungi confirms the assumption that with the deterioration of environmental conditions: pollution by agrochemical residues and an increase in the dose of mineral fertilisers, the synthesis of melanoid pigments is enhanced by both the bacterial and fungal components of the soil microbiota (Malynovska, 2017). The increase in the synthesis of melanoid pigments, especially with the introduction of one and a half and double doses of mineral fertilisers, coincides with the results of the analysis of the humus content in the soil of these variants – it exceeds the humus content in the soil of the variant with the introduction of a single dose of mineral fertilisers by 26.5% and 16.3%, respectively (Fig. 1, 2). However, it is possible that the increase in melanin synthesis in this case is associated not only with the deterioration of environmental conditions, but also with the optimisation of mineral nutrition of the phytocoenosis of this ecotope and, as a result, an increase in the number of soil microorganisms in the root zone, including melanin-synthesising ones (Jacoby *et al.*, 2017; Dang *et al.*, 2022). Addressing this issue is a task for the development of a methodological framework and further research in this area.

The fact that the number of azotobacter decreased with increasing fertiliser doses, even with simultaneous liming, indicates the inhibitory effect of nitrogen fertilisers on the number of CFU of freely existing nitrogen-fixing organisms, which include azotobacter. This is consistent with the well-known fact that high doses of nitrogen-containing substances inhibit the development of nitrogen-fixing microorganisms (Noar & Bruno-Bárcena, 2018; Knutson *et al.*, 2018). Thus, the patterns of distribution of azotobacter as a melanin producer largely contradict those observed for melanin-synthesising bacteria and micromycetes, whose numbers increase with increased doses of mineral fertilisers. The authors of this study believe that azotobacter is one of the microorganisms-indicators of ecological cleanliness of soil by pollutants of anthropogenic origin, its number decreases with increasing pollution levels (Malynovska, 2017) and, accordingly, melanin synthesis is more active in ecologically safe ecotopes (variants of agrocenosis). Thus, the environmental and agrochemical properties of the soil affect the synthesis of melanins by nitrogen bacteria and melanin-synthesising bacteria and micromycetes in opposite ways, which makes it difficult to assess the contribution of individual components of the soil microbiota to humus accumulation.

The high activity of melanin synthesis by azotobacter cells (Fig. 5) is not accompanied by a close correlation between the number of nitrogenobacteria and humus content (r = 0.482). This may be due to the fact that a large number of nitrogenobacteria with high melanin synthesis activity are observed in the soil under lupine cultivation, while other crop rotation crops have the opposite effect on the number and activity of nitrogenobacteria cells, and the overall contribution of nitrogenobacteria to the accumulation of melanins, which are precursors of humus substances, should be investigated and evaluated during the crop rotation under all crops.

Organic matter of plant origin is known to have a high sorption capacity for various pollutants (Duarte *et al.*, 2017; Tran-Ly *et al.*, 2020a; 2020b) and protects soil biota and plants from the negative effects of pollutants, which is reflected in the reduction in the number of melanin-synthesising microbes and bacteria in the experimental variants with the introduction of exogenous organic matter. The thesis about reducing the content of toxic substances in the soil as a result of ploughing exogenous organic matter is confirmed by the results of the study of soil phytotoxicity: it decreases in variants with the introduction of EOM by an average of 20.9% (Fig. 3). Liming has a multifaceted effect on humus accumulation. Firstly, due to a decrease in the number of autochthonous bacteria (by 15.5%), their physiological and biochemical activity (by 10.4%), a decrease in the activity of humus mineralisation (by 9.32%), and an increase in the number of melanin-synthesising microorganisms: azotobacter, micromycetes, and bacteria.

Earlier, for typical chernozem, it was found that the relationship between humus content and the number of melanin-synthesising bacteria (r = 0.528) and their share in the total number of microorganisms (r = 0.470) is of medium significance, which is much higher than the level of the relationship between humus content and the number of melanin-synthesising microbial communities (r = -0.052) (Malynovska *et al.*, 2023). This suggests that in black soil, typical for sunflower cultivation, humus is formed mainly by melanins of bacterial origin. For grey forest soil, the opposite trends are observed: it is with the involvement of micromycetes melanins that humus molecules are synthesised in this type of soil when growing white lupine (r = 0.811). In typical chernozem, a low inverse correlation coefficient (r = -0.099) was found between the humus content and the number of azotobacter, and for grey forest soil the corresponding indicator was 0.482 (with the PFC of Azotobacter r = 0.360), which suggests that melanins of bacteria of the genus *Azotobacter* are insignificantly involved in the synthesis of humus molecules when growing sunflower on typical black soil, but this trend is reversed when growing white lupine on grey forest soil (Feszterová & Hudec, 2022).

For grey forest soil, a correlation of medium significance was found between the number of polysaccharide-synthesising bacteria and humus content (r =0.417). For the typical chernozem, the existence of a direct average relationship between the physiological and biochemical activity of polysaccharide-synthesising bacteriacells and humus content (r = 0.532) was confirmed, which reflects the increased resistance of humus molecules to mineralisation by autochthonous microorganisms in soils of both types.

Thus, the agricultural practices used in modern agriculture – neutralising excessive acidity of the soil solution, applying mineral fertilisers, especially in high doses – contribute to the growth of microorganisms that synthesise the constituent components of humus molecules, such as melanoid pigments. Ploughing up crop by-products and green manure biomass leads to de-intensification of melanin synthesis due to a decrease in the distribution of their producers.

CONCLUSIONS

The investigated agrochemical measures affect the process of melaninogenesis in the soils of agrocenoses by representatives of the main kingdoms of life – prokaryotes and fungi (micromycetes). The nature and direction of the impact depend on the type of agricultural measure, the crop being grown, and the systematic, as well as functional, affiliation of the group of microorganisms producing melanins. Liming with one dose according to hydrolytic acidity leads to an increase in the number of melanin-synthesising micromycetes and bacteria by 86.8% and 26.1%, respectively; application of mineral fertilisers in a dose of $N_{30}P_{30}K_{45}$ – 2.0 and 2.04 times, combined application of lime and mineral fertilisers – 2.94 and 2.48 times, respectively. A 1.5-fold increase in the dose of mineral fertilisers leads to a 2.54-fold increase in the number of CFU of melanin-synthesising micromycetes compared to a single dose of fertilisers, and a 5.8-fold increase in bacteria; a 2.62-fold and 13.3-fold increase in the dose of fertilisers doubles the number of melanin producers, respectively.

It was shown that when growing white lupine on grey forest soil, the humus content is closely related to the number of melanin-synthesising microbial species (r = 0.811) and their share in the total number of fungi (r = 0.915), the correlation coefficient with the number of melanin-synthesising bacteria and nitrogen-bacteria is 0.417 and 0.482, respectively. The distribution of melanin-synthesising fungi and bacteria is controlled by the same environmental or agronomic factors, as evidenced by the existence of a high level of correlation between the number of melanin-synthesising bacteria and the number of CFU of melanin-synthesising micromycetes (r = 0.761).

The study of the influence of agrochemical factors on the processes of melanin synthesis by microorganisms of grey forest soil has the prospect of continuing with the involvement of other types of soils and soils with different levels of anthropogenic pollution affecting the synthesis of melanoid pigments.

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None.

CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Чисельність меланінсинтезувальних мікроорганізмів за мінерального удобрення і вапнування сірого лісового ґрунту

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Анотація. Дослідження основних закономірностей розповсюдження мікроорганізмів, що синтезують меланіни, які є попередниками і складовими гумусових молекул, в ґрунтах агроценозів є актуальним завданням науки. Метою представленої роботи було встановлення впливу агрохімічних чинників на чисельність меланінсинтезувальних мікроміцетів і бактерій у сірому лісовому ґрунті. В роботі було використано мікробіологічний, лабораторно-аналітичний, статистичний методи. Вперше встановлено, що чисельність меланінсинтезувальних мікроміцетів є мінімальною у ґрунті варіанту без добрив, вапнування однією дозою за гідролітичною кислотністю сприяє збільшенню їхньої чисельності на 86,8 %, внесення мінеральних добрив у дозі $N_{30}P_{30}K_{45}$ – в 2,0 рази, сумісне застосування вапна і мінеральних добрив – у 2,94 рази. Збільшення дози мінеральних добрив в 1,5 рази призводить до зростання кількості колонієутворювальних одиниць (КУО) меланінсинтезувальних мікроміцетів порівняно із одинарною дозою добрив у 2,54 рази, збільшення дози добрив у 2,0-2,62 рази, що співпадає із результатами аналізу вмісту гумусу у ґрунті цих варіантів – він перевищує вміст гумусу у варіанті із одинарною дозою добрив на 26,5 та 16,3 % відповідно (коефіцієнт кореляції становить 0,811). Чисельність меланінсинтезувальних бактерій в сірому лісовому ґрунті на 2-3 порядки вища за чисельність меланінсинтезувальних мікроміцетів, однак, закономірності впливу антропогенних чинників на їхню чисельність співпадають із встановленими для мікроміцетів: вапнування однією дозою за гідролітичною кислотністю призводить до збільшення чисельності меланінсинтезувальних бактерій на 26,1 %, внесення мінеральних добрив у дозі $N_{30}P_{30}K_{45}$ – в 2,03 рази, сумісне застосування вапна і мінеральних добрив – у 2,48 рази. Збільшення дози мінеральних добрив у 1,5 рази призводить до зростання чисельності меланінсинтезувальних бактерій порівняно із одинарною дозою добрив у 5,8 рази, збільшення дози добрив у 2,0 рази – відповідно у 13,3 рази. Коефіцієнт кореляції між чисельністю меланінсинтезувальних бактерій і вмістом гумусу у ґрунті складає 0,417. Результати представлених досліджень можуть бути використані при розробленні рекомендацій щодо заходів підвищення вмісту гумусу в ґрунтах сільськогосподарського використання

Ключові слова: меланінсинтезувальні мікроорганізми; мікроміцети; бактерії; азотобактер; вапнування; мінеральне удобрення; гумус

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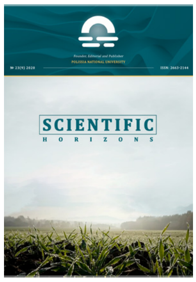

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Restoration of soil fertility and improvement of phytosanitary condition of soil in short rotation of crops in Polissia of Ukraine

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Abstract. With the intensification of agricultural land use and changes in natural conditions, soil quality in Ukraine is deteriorating, with a decrease in humus content, the average annual loss of which is about 0.6 t/ha. Therefore, its reproduction is now becoming increasingly important through the use of organic raw materials and the introduction of legumes into the crop rotation, which leads to a partial replacement of nitrogen from mineral fertilisers with biological nitrogen. The purpose of this study

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was to find ways to provide the topsoil with organic residues, develop soil microflora, and improve the activity of nitrogen-fixing bacteria in a short grain legume crop rotation. The study was conducted in 2019-2023 using the following methods: visual – to determine the stages of organogenesis; field – to determine the interaction of abiotic factors; physiological – to determine the symbiotic fixation of atmospheric nitrogen. It was found that due to mineralisation of organic residues of legume crop rotation, the amount of macro- and microelements in the soil layers 0-10 cm, 10-20 cm, and 20-30 cm increased by 45.1-46.9-43.1 mg/kg, respectively. It was found that saturation of short crop rotation by 50% with legumes, straw residues, and green manure contributed to the reduction of pathogenic microorganisms and the growth of suppressive ones. It was found that in a short rotation of crops, 10.3 t/ha of organic matter in the form of stubble and root residues, straw and green manure enters the soil, which contributed to the cultivation of environmentally friendly agricultural products with the restoration of soil fertility. The biological activity of the soil during the growing season in the layer of 10-20 cm reached 47.4%, and the content of alkaline hydrolysed nitrogen in the rhizosphere of the root system increased by 43.9 mg/kg. The value of the study lies in the fact that the introduction of short organic crop rotations in farms of various forms of ownership is an innovative approach to providing light grey soils with organic raw materials, restoring and maintaining their fertility, improving their phytosanitary condition, promoting biodiversity, achieving environmental sustainability and high yields

Keywords: crop rotation; crop residues; green manure; biological activity; soil; phytopathogens

INTRODUCTION

To obtain high and sustainable crop yields in the Polissia region of Ukraine, all elements of cultivation technology must be aimed at preserving and restoring soil fertility through the accumulation of organic residues and the formation of humus, which improves the water-physical, thermal, and agrochemical properties of the soil, increases its absorption capacity and buffering capacity, and optimises its phytosanitary condition and nutritional status.

In the system of modern intensive crop production, increasing importance is gained by the scientific research on restoring soil fertility and its biological activity, where the main component is the biological cycle of substances and soil microorganisms; investigation of soil biological activity in short legume crop rotations using post-harvest residues and green manure, establishing the processes of organic matter transformation and improving soil fertility in the biodynamic processes of humus formation (Adamchuk *et al.*, 2019; Kliuchevych *et al.*, 2020).

To provide the soil with organic raw materials, it is necessary to introduce scientifically based soil conservation crop rotations, including the expansion of perennial legumes and pulses, and the use of organic fertilisers. According to M. Torbati *et al.* (2021), the tendency to increase the volume of mineral fertilisers and pesticides, reduce soil fertility, and over-compact it is a cause for concern. There is a positive trend towards an increase in the volume of crop by-products (stubble and root residues, straw) and post-harvest green manure crops. Generally, applying 4-6 t/ha of straw and using 11-12 t/ha of green manure is equivalent to applying 12 t/ha of organic fertiliser.

Soil biological activity is the principal indicator of monitoring the intensity of organic residue decomposition, providing scientific justification and assessment of the impact of organic and mineral fertilisers on their use in managing the growth process and developing intensive elements of the technology for growing environmentally friendly crops. Determining the biological activity of the soil helps to optimise the regime of soil restoration and preservation of its fertility. The conventional practice was to use long-lasting energy crops and pesticides to ensure high yields. However, this approach led to a decrease in soil fertility, loss of beneficial mycoflora, and, as a result, the accumulation of pathogens of various aetiologies and environmental pollution.

Currently, the humus content in the ploughed soils of Ukraine's Polissia region, according to their particle size distribution, is insufficient to optimise the root layer in terms of agrophysical and agrochemical properties. Therefore, the search for ways to reproduce the soil organic matter, control and maintenance of the optimum humus status is extremely relevant. Normal humus recovery requires organic matter contained in crop residues. That is why the purpose of this study was to find ways to provide the topsoil with organic residues, develop soil microflora, and improve the activity of nitrogen-fixing bacteria in a short grain legume crop rotation.

As a result of non-compliance with scientifically based farming systems, the natural soil fertility of agricultural land is declining (the humus content of Ukrainian soils has decreased by 20%). The main reason for these negative changes is the lack of organic fertiliser application. The widespread use of mineral fertilisers and chemical plant protection products against pests has increased the threat of chemical degradation and pesticide contamination of crop production (Richard *et al.*, 2022). The main challenge of transitioning to biological farming is to ensure enhanced reproduction of soil fertility and increase crop yields. It is noted that organic fertilisers are particularly important on light sod-podzolic soils (Lisovyi *et al.*, 2021).

Crop rotation, the use of by-products and green manure positively affect the ecological state of the soil and are essential elements of agriculture to improve soil fertility, provide and preserve moisture, microbiological processes and increase crop yields (Pisarenko & Pisarenko, 2022; Voitovyk, 2023). In the research of E.G. Degodyuk *et al*. (2019), the use of straw of predecessors shows a deterioration in the nitrogen regime, immobilisation of mobile forms of nitrogen occurs during humification of organic residues (straw), which have a low nitrogen content. The use of green manure in the form of fodder radish activates the nitrification process.

The green manure incorporated into the soil before sowing is only 25% decomposed and provides energy for nitrogen-fixing microorganisms during the growing season. Green manuring of soil with a short growing season (fodder radish) suppresses weeds in maize crops, improves microbiological processes, phytosanitary condition of the soil, and activates the destruction of organic raw materials. Chaika *et al*. (2019) report an increase in sugar beet yield after vetch-oat mixture and fodder radish by 5.0-5.7 t/ha. The combined use of green fertilisers and straw creates optimal conditions for the decomposition of organic raw materials, as it occurs at a carbon to nitrogen (C:N) ratio of 20-25:1 and provides a high humification factor. Therefore, nitrogen fertilisation is not recommended under such conditions (Sydletsky *et al.*, 2021).

V.F. Petrychenko and V.V. Lykhochvor (2020) believe that the phytosanitary condition of the soil in agriculture plays an important role in ensuring plant health, resistance to pests, and increasing yields. I.V. Honcharuk *et al*. (2020) believe that biological farming, as the main environment for plant growth and development, is a factor in the phytosanitary regime of the soil-plant system when saturated with crops with similar biological characteristics. Placing crops in such rotations helps to restore soil fertility and improves the phytosanitary condition of crops. Thus, considering the relevance of the scientific research, the purpose of this study was to identify ways to restore the fertility of nutrient-poor soil in short rotation of crops, improve its phytosanitary condition to obtain high and sustainable crop yields.

MATERIALS AND METHODS

The study was conducted in a stationary short grain-legume crop rotation with saturation of cereals and legumes (50 + 50) in the conditions of the educational and research field of Polissia National University during 2019-2023. The soil is grey forest light loam.

A four-field crop rotation scheme:

1. Perennial grasses for one mowing (clover).
2. Winter wheat using nutritious residues and green manure (fodder radish).
3. Soybeans using leaf and stem mass for fertiliser.
4. Barley with clover undersowing.

The system of using crop residues and green manure:

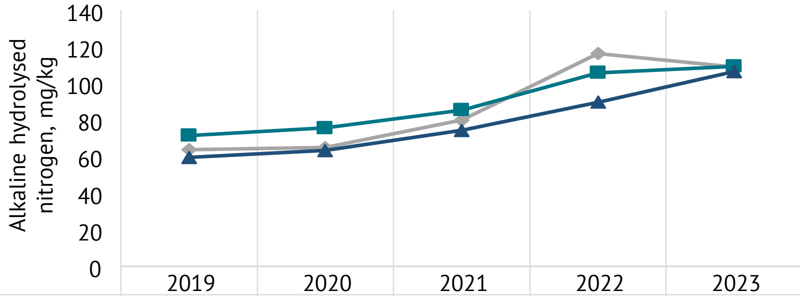
1. Biological control (root and stem residues).
2. Root and stem residues + chopped straw.
3. Root and stem residues + green manure.
4. Root and stem residues + straw + green man ure.

The experiments were replicated four times, with a sowing plot of 39.6 (3.6×11) m² and a trial plot of 25 (2.5×10) m². The experiments were conducted following the methodology and organisation of scientific research (Yevtushenko & Khizhnyak, 2019). Agrochemical analyses of the soil were conducted in the measuring laboratory of the Educational and Research Centre of Ecology and Environmental Protection of Polissia National University (certificate of conformity of the measurement system according to the requirements of DSTU ISO 10012:2005 (2007).

Humus content was determined according to Tyurin's DSTU 4289:2004 (2005); hydrolysable nitrogen content was determined according to the Kornfield method (DSTU 7863:2015, 2016); mobile phosphorus and exchangeable potassium content was determined according to Kirsanov (DSTU 4405:2005, 2006). The biological activity of the soil was determined by the application method, the degree of decomposition of linen fabric in the soil layer 0-20 cm. The application method – the specified weight of linen fabric was embedded in the soil to a depth of 20 cm, and the degree of cellulose decomposition was determined after 45 days. The species composition of micromycetes was analysed according to generally accepted methods (Azam *et al.*, 2020; Dignam *et al.*, 2022; Kara & Soylu, 2023). Experimental studies of plants (both cultivated and wild), including the collection of plant material, were following the institutional, national, or international guidelines. The study adhered to the standards of the Convention on Biological Diversity (1992) and the Convention on Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS AND DISCUSSION

It was established that in the system "cereals without the use of straw by-products", a positive humus balance can be achieved only if the structure of sown areas is at least 20-21%. In the legume-grain system, a positive humus balance is achieved only when straw and fertiliser are used on 25-30% of the sown area. The deficit-free use of humus can be overcome only if the crop rotation is saturated with cereals with the use of straw and fertilisers of at least 55-60%. A noticeable humus formation was found: the maximum increase in humus was observed in the 0-10 cm soil layer compared to the initial content (1.0-1.1%). Straw in combination with green fertilisers decomposes slowly in the soil without polluting it with high concentrations of nitrate nitrogen, promotes the development of soil fauna, increases the viability of bacteria, earthworms, and other living soil organisms, and improves the biological activity of the soil. The formation of alkaline hydrolysed nitrogen in the soil layer of 10-20 cm increased by 43.9 mg/kg over the years of research and amounted to 109.2 mg/kg, and at a depth of 20-30 cm – 106.4 mg/kg (Fig. 1).



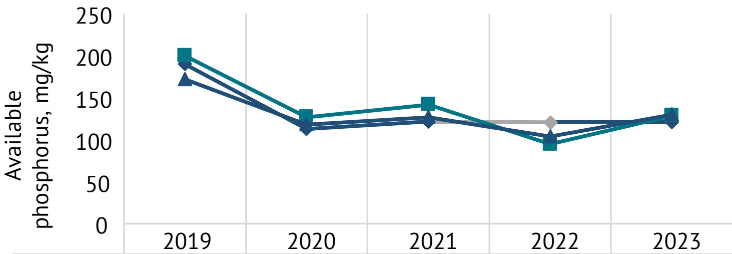
	2019	2020	2021	2022	2023
0-10	63.7	64.9	79.8	116.2	108.8
10-20	71.4	75.6	85.4	105.7	109.2
20-30	59.5	63.3	74.2	89.6	106.4

***Figure 1**. Dynamics of alkaline hydrolysed nitrogen supply in a short grain-legume crop rotation depending on the use of crop residues and green manure*

***Source:** compiled by the authors of this study*

In the soil layer of 10-20 cm, the accumulation and storage of productive moisture, improvement of soil porosity, which contributed to the processes of nitrification of nitrogen, and intensification of biological activity of decomposition of cellulose, hemicellulose, and other organic compounds were detected. With deep incorporation of organic matter (20-30 cm), anaerobic mineralisation processes were observed, accompanied by the release of low molecular weight fatty acids, which in large quantities adversely affect the vegetation of cereal crops, the process of decomposition of organic matter in the soil proceeds towards humification. Due to the mineralisation of organic residues, straw, green manure containing macro- and microelements, their amount in organic short rotation of crops increases by 45.1 mg/kg in the 0-10 cm soil layer, by 46.9 mg/kg at a depth of 10-20 cm, and by 43.1 mg/kg at 20-30 cm. Furthermore, organic residues contain a significant amount of nitrogen-free substances: glucose – 33-75%, hemicellulose – 21-22%, and lignin – 18-22%.

Phosphorus is of great importance in the decomposition of nutrients (straw, stubble, root system), as it plays an active role in the development of microorganisms and cellular metabolism. The main source of phosphorus in the soil is its content and reserves, depending on the particle size distribution of the soil and its humus content. Light grey sandy loam soils are poor in total phosphorus. The state of phosphate in the soil is influenced by organic matter, non-commodity products, soil moisture, and temperature. Insufficient availability of phosphorus reserves in the soil is conditioned by poor diffusion of phosphate ions in the soil. The relatively low utilisation rate of phosphate fertilisers in the first year of application (10-15%) is due not only to the transition of phosphates into unavailable forms, but also to their limited availability to the root system. With the introduction of organic matter and its mineralisation, available phosphorus (119.3-126.5 mg/kg) accumulates in the soil layer of 10-30 cm (Fig. 2) and is used to feed crops in crop rotation by 35-40%.

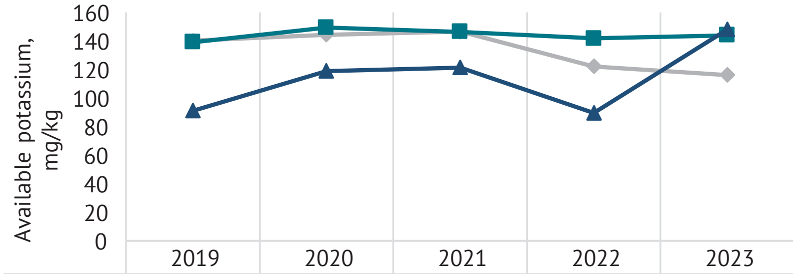


	2019	2020	2021	2022	2023
0-10	190	112.8	121.4	120.8	120.9
10-20	200	127	141.7	94.8	128.6
20-30	172	118	126.9	103.8	129.9

***Figure 2**. Dynamics of exchangeable phosphorus supply in a short grain-legume crop rotation depending on the use of crop residues and green manure*

***Source:** compiled by the authors of this study*

Potassium has a positive effect on reducing the damage to plants by fungal pathogens, thickens the wall cells. The potassium content in the 10-20 cm soil layer did not change and ranged within 144-149 mg/kg (Fig. 3).



	2019	2020	2021	2022	2023
0-10	140	144.4	146.5	122.1	116
10-20	139	149.3	146.4	141.6	144
20-30	91	118.9	121.3	89.5	148

***Figure 3**. Dynamics of potassium availability in a short grain-legume crop rotation depending on the use of crop residues and green manure*

***Source:** compiled by the authors of this study*

The mineralised potassium in organic matter is quickly transferred to the soil solution and becomes available for plant nutrition. In a short grain legume crop rotation with 10.3 t/ha of organic residues, it is not washed off and accumulates in the upper soil layers. Potassium from mineral salts in the soil solution is the best source of plant nutrition. Organic residues and green fertilisers are re-integrated into the mineral and organic plant nutrition cycle to form the next phytomass. The combination of organic residues with green manure prevents the leaching of mobile elements and their removal by groundwater, and promotes the development of soil fauna, increases the activity of bacteria and earthworms, which help improve the agrochemical and physical properties of the soil.

The findings of the study established the frequency of isolation of soil microbial communities in organic short rotation of crops (Table 1). Among them, pathogenic species (from the genera *Fusarium* spp., *Alternaria* spp., *Cladosporium* spp., *Pythium* spp.) and suppressive species (*Trichoderma* spp., *Aspergillus* spp., *Penicillum* spp., *Mucor* spp., *Rhizopus* spp.) were identified.

***Table 1**. Frequency of soil microbial isolation in organic short rotation of crops*

Micromycetes	Isolation frequency, %				
	2019	2020	2021	2022	2023
Pathogenic micromycetes					
Fusarium spp.	18.0	13.0	15.0	12.0	11.0
Alternaria spp.	12.0	9.0	10.0	6.0	4.0
Cladosporium spp.	7.0	5.0	3.0	4.0	5.0
Pythium spp.	5.0	7.0	2.0	6.0	4.0
Suppressive micromycetes					
Trichoderma spp.	12.0	16.0	14.0	19.0	20.0
Aspergillus spp.	19.0	18.0	23.0	16.0	21.0
Penicillum spp.	17.0	19.0	17.0	21.0	19.0
Mucor spp.	8.0	10.0	7.0	12.0	10.0
Rhizopus spp.	2.0	3.0	9.0	4.0	6.0

***Source:** compiled by the authors of this study*

It was found that throughout all the years, the study of 50% saturation of short rotation of crops with legumes (clover, soybean), residues of straw, and green manure contributes to the reduction of pathogenic microorganisms – the causative agents of diseases of the root system of plants in the soil and the growth of the development of suppressive micromycetes, especially *Trichoderma* spp., *Penicillum* spp., *Mucor* spp. It is the optimum selection of crops in a short organic legume crop rotation that promotes the development of trophic relationships, depriving plant pathogens of a constant source of food, which reduces their numbers. Furthermore, legumes secrete substances with natural antifungal properties, which reduces the development of phytopathogen infestation and improves soil biological activity (Table 2).

***Table 2**. Biological activity of light grey soil and crop yields of short rotation of crops, average for 2019-2023*

Organic substance	Biological activity, %	Yield, t/ha		
		winter wheat	soybean	barley
Stubble and root residues	28.2	5.6	2.2	2.9
Stubble and root residues + straw	33.4	6.1	2.4	3.2
Stubble, root residues + green manure	42.2	7.2	2.7	3.8
Stubble, root residues + straw + green manure	47.4	7.6	3.0	4.0
LSD_{05}		0.6	0.44	0.15

***Source:** compiled by the authors of this study*

The vital activity of cellulose-degrading microorganisms depends on the introduction of organic matter. The biological activity of the soil during the growing season was within 28.2-47.4%. During warming up and optimum soil moisture in the variant with the incorporation of crop residues and green manure (fodder radish), the intensity of fibre decomposition increased and reached a maximum of 42.2-47.4% in the 10-20 cm soil layer. In the surface layer at 0-10 cm, without incorporating straw and post-harvest green manure, the biological activity of the soil was only 28.2%. Thus, during the period of macrostages (BBCH 10-61, cotyledons fully opened – the beginning of flowering), the cellulosic acidity of flax fabric decomposition was 47.4%.

It was found that harvesting and chopping straw into pieces of 2.5-4.5 cm and embedding them in the soil to a depth of 10-12 cm contributed to the decomposition of straw by 5.2% faster compared to the biological control. Microorganisms that carry out mineralisation require two main elements: carbon and nitrogen, with a ratio of 10-12:1 in the soil. The low coefficient of humus formation by the ratio of N:C ratio in straw was 1:86, and the potential for humification stayed high. When straw and green mass were used, the N:C ratio was within 1:21, optimum conditions for humus formation were created, and biological activity reached 47.4%. It was found that in the short rotation of crops, on average, over the years of research, 10.5 t/ha of by-products were received, consisting of stubble and root residues (straw and green manure), which ensured a yield of winter wheat of 5.6 t/ha, soybeans of 3.0 t/ha, and spring barley of 4.0 t/ha (Table 3).

***Table 1**. Crop yields in short rotation of crops depending on the supply of organic raw materials and nitrogen of biological origin, average for 2019-2023*

Short rotation of crops	Yield, t/ha	Dry matter, t/ha				Nitrogen, kg/ha		
		stubble and root residues	straw	green manure	total	organic matter	biologically fixed	total
Clover	4.7	3.0	4.7	-	7.7	98.0	60.0	158.0
Winter wheat + by-products + green manure	5.6	6.7	8.4	4.2	19.3	16.8	-	16.8
Soybeans + by-products	3.0	2.6	4.5	-	7.1	13.6	85.0	98.6
Barley with clover undersowing	4.0	4.0	3.8	-	7.8	36.0	60.2	96.2
Total	-	16.3	21.4	4.2	41.9	164.4	205.2	369.6
Per 1 ha of crop rotation area	-	4.0	5.3	1.0	10.3	41.1	51.3	92.4

***Source:** compiled by the authors of this study*

The combination of straw and green manure created conditions for a uniform acceleration of humification and accumulation of nutrients in the topsoil, specifically nitrogen, and increased their efficiency when using green manure by 14.0-15.2%. Organic residues and green fertilisers were reintroduced into the plant nutrition cycle to form new phytomass. The combination of organic residues with green manure prevents the leaching of mobile elements and their removal by groundwater, promotes the development of the soil fraction, and improves the agrophysical properties of the soil.

The present study showed that the use of green manure and the introduction of legumes (clover, soybeans) into short rotation of crops activates soil nitrogen-fixing bacteria, increases the supply of symbiotic atmospheric nitrogen, and improves biological activity. The humified nutrients are not washed out, bind to the soil, and take part in the nutrition of crop rotation plants. V. Oliynyk (2019) argues that perennial grasses and cereals are of exceptional importance in improving the humus condition of the soil. The optimum parameters of sod-podzolic clay-sandy soils are 1.4-1.6%, and sandy loam soils – 1.6-1.8%. In fact, the humus content in ploughed soils is between 0.6-1.0%.

According to S.P. Tanchyk *et al*. (2019), S.I. Kudrya (2020), predecessors, their rotation, and the use of crop residues are important for improving soil fertility. However, the researchers do not indicate what the structure of crops should be in crop rotations on light grey soils, unlike the present study. However, in Ukraine, only 0.5 t/ha of manure is applied. To restore the fertility of light grey soils, it is necessary to return non-commodity products to the soil and introduce crop rotations with legumes saturated within 50%, which is equivalent to the application of manure. The content of alkaline-hydrolysed nitrogen in the 10-20 cm soil layer reaches 109 mg/kg, and potassium – 148 mg/kg. V.V. Gangur and V.M. Sakhatska (2019) state that the most intensive microbiological processes occur after manure application (50 t/ha), both separately and in combination with mineral fertilisers, with glucose destruction reaching 59-61%.

In studies by D. Porodzinsky (2020) and V. Kyrychenko (2020), it took 5-7 years for the soil to restore fertility functions. To accelerate the decomposition of cellulose and lignin, live strains of cellulose-degrading bacteria (destructors) should be used. According to our observations, after harvesting stubble and root residues, the complete decomposition of linen fabric took place within 3.5 years. M. Kara and E. Soylu (2023), S. Stankevich *et al*. (2020) highlight the evidence that providing the topsoil with organic residues increases the growth of fungi and bacteria that decompose cellulose, which increases cellulose-degrading activity in the 10-20 cm soil layer. M. Torbati *et al*. (2021) note that soil acts as the main nutrient medium for plant growth and development, but at the same time it can also be a carrier of harmful microorganisms that contribute to the spread of plant diseases. The main groups of phytopathogenic microorganisms in soil include bacteria (genera *Agrobacterium*, *Ervinia*), fungi (genera *Fusarium* spp., *Alternaria* spp., *Cladosporium* spp.), nematodes, etc. However, the researchers did not conduct studies in short rotation of crops on light grey soils and did not indicate the structure of pathogenic and suppressive microflora in the soil.

Thus, in this context, a short organic crop rotation is a promising solution that helps to create optimum conditions for the development of plants and beneficial microorganisms-antagonists, improves the phytosanitary condition of the soil and increases its fertility.

CONCLUSIONS

A short four-field grain and legume crop rotation with perennial legumes and legumes (50%) and cereals (50%) ensures the supply of 10.3 t/ha of organic matter to the soil in the form of stubble root residues, straw and green manure, which contributes to the cultivation of environmentally friendly agricultural products and the restoration of soil fertility. It was found that with the introduction of organic substances and their mineralisation in the soil layer of 10-30 cm, the formation of alkaline hydrolysed nitrogen increased to 109.2 mg/kg, the accumulation of available phosphorus increased to 119.3-126.5 mg/kg, and the potassium content did not change and was within 144-149 mg/kg.

Saturation of the short rotation of crops by 50% with legumes (contributes to the reduction of pathogenic microorganisms in the soil and the growth of the development of suppressive micromycetes: *Trichoderma* spp., *Penicillum* spp., *Mucor* spp. It was found that the biological activity in the soil layer of 10-20 cm of legume crop rotation after planting crop residues and green manure during the growing season reaches 47.4%. With by-products in the soil (10.5 t/ha), the short rotation of crops yielded 5.6 t/ha of winter wheat, 3.0 t/ha of soybeans and 4.0 t/ha of barley. Further research will be aimed at investigating and implementing innovative approaches to restoring and preserving soil fertility, increasing biodiversity, and using effective microorganisms to generate sustainable and high crop yields.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Відновлення родючості та покращення фітосанітарного стану ґрунту в короткоротаційній сівозміні Полісся України

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Анотація. За інтенсифікації використання сільськогосподарських земель і зміни природних умов якість ґрунтів в Україні погіршується, зменшується вміст гумусу, середньорічні втрати якого становлять близько 0,6 т/га. Тому наразі набуває актуальності його відтворення за рахунок застосування обсягів органічної сировини та введення у сівозміну зернобобових культур, що зумовлює часткову заміну азоту з мінеральних добрив на біологічний. Метою дослідження було пошук шляхів забезпечення орного шару ґрунту органічними рештками, розвитком ґрунтової мікрофлори, покращення діяльності азотфіксуючих бактерій короткоротаційної зернобобової сівозміни. Дослідження проводили впродовж 2019-2023 років, користуючись методами: візуальний – визначення етапів органогенезу; польовий – взаємодія абіотичних чинників; фізіологічний – визначення симбіотичної фіксації азоту атмосфери. Встановлено, що за рахунок мінералізації органічних решток зернобобової сівозміни збільшувалася кількість макро- і мікроелементів у шарах ґрунту 0-10, 10-20 та 20-30 см відповідно на: 45,1-46,9-43,1 мг/кг. Було досліджено, що насичення короткоротаційної сівозміни на 50 % бобовими культурами, залишками соломи та сидератами сприяло зменшенню патогенних мікроорганізмів і зростанню розвитку супресивних. Установлено, що в короткоротаційній сівозміні в ґрунт надходить 10,3 т/га органічної речовини у вигляді стерньових і кореневих решток, соломи та сидератів, що сприяло вирощуванню екологічно безпечної сільськогосподарської продукції з відновленням родючості ґрунту. Біологічна активність ґрунту за період вегетації в шарі 10-20 см досягала 47,4 %, а вміст лужногідролізованого азоту в ризосфері кореневої системи збільшувався на 43,9 мг/кг. Цінність наукових досліджень полягає в тому, що впровадження в господарствах різних форм власності короткоротаційних органічних сівозмін є інноваційним підходом у забезпеченні ясно-сірих ґрунтів органічною сировиною, відтворенні і підтриманні його родючості, покращенні фітосанітарного стану, сприянні збільшенню біологічної різноманітності, досягненні екологічної стійкості та високої врожайності

Ключові слова: сівозміна; поживні рештки; сидерати; біологічна активність; ґрунт; фітопатогени

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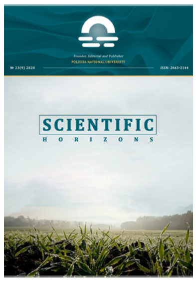

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Obtaining an important component of humate-enriched organomineral fertiliser based on oxidised lignite from Kyrgyzstan

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Abstract. The study of methods for the production and processing of humate-enriched organomineral fertilisers based on oxidised lignite represents a key aspect for modern agriculture, due to the need to improve resource efficiency, soil quality, and sustainability of production. The purpose of this study was to determine the best methods of obtaining and processing humate-enriched organomineral fertilisers based

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on oxidised lignite. To fulfil this purpose, experimental research was carried out in the laboratory of the Institute of Natural Resources of the Southern Branch of the National Academy of the Kyrgyz Republic and the educational and scientific laboratory of the Department of Ecology and Environmental Protection of the Osh Technological University named after M.M. Adyshev. The findings of the study indicate significant changes in soil characteristics after application of vermicompost and glauconite. Analyses of vermicompost revealed that it has a significant content of nutrient elements and organic compounds. The potassium content of vermicompost increased by 40%, while silicon and phosphorus content increased by 39% and 28.5%, respectively. As a result of vermicomposting, there is an improvement in soil characteristics: pH increases to 7-7.5, organic matter content increases to 35-45%, while C/N-ratio decreases to 10-12, which creates more favourable conditions for plant growth and increases nitrogen use efficiency. After addition of glauconite, an increase in potassium oxide by 0.6%, iron (III) oxide by 2.34%, and aluminium oxide by 0.41% was observed. Silicon oxide content increased by 4.9%, while the content of humic substances – by 1.1%. These changes in soil characteristics contribute to higher yields and better soil quality. The results of the study have practical significance for agriculture in improving soil characteristics, increasing yields and soil quality, and reducing dependence on chemical fertilisers

Keywords: nutrients; soil; environmental sustainability; macronutrients; glauconite; *Eisenia foetida*

INTRODUCTION

In modern agriculture, the continuous drive to increase yields and product quality necessitates the search for effective and environmentally friendly fertiliser solutions. In this context, humate-enriched organomineral fertilisers based on oxidised lignite are attracting increasing attention as a potential key component for improving soil properties and stimulating plant growth. The approach of using oxidised lignite to produce such fertilisers is an area promising considerable results in sustainable agriculture and food security (Suleymenov & Kolesnikova, 2020). The process of obtaining an essential component of humate-enriched organomineral fertilisers is based on a carefully developed technology that allows for the efficient conversion of the organic mass of oxidised lignite into biologically active and organic-rich components. This process involves not only chemical transformations but also biological processes to enrich carbon structures with nutrients and microorganisms that promote healthy plant growth (ur Rehman *et al.*, 2023; Didur *et al.*, 2023).

Z. Aslam *et al.* (2024) and T.J. Sitzmann *et al.* (2024) stress that the use of oxidised lignite in fertiliser production has considerable environmental benefits. This reduces dependence on conventional chemical fertilisers, which can have a negative impact on soil and the environment. Humate-enriched organomineral fertilisers derived from oxidised lignite contribute to the conservation of soil microflora and microfauna biodiversity, as well as to the improvement of soil structure and fertility. Moreover, according to O. Trembitska and S. Bohdan (2023), such fertilisers can have a positive effect on plant resistance to stress conditions, including drought or soil salinity, which is an important aspect in a changing climate. Thus, the development and application of humate-enriched organomineral fertilisers based on oxidised lignite represent an innovative and promising approach to agricultural production, contributing to its sustainable development and increased efficiency.

Kyrgyzstan, as a country with a high potential for agricultural development, faces challenges related to declining soil fertility and declining crop yields (Omarov *et al.*, 2024). The introduction of humate-enriched organomineral fertilisers based on oxidised lignite represents a major step towards improving soil quality and increasing crop yields in this region. Considering the limited resources and the importance of maintaining ecological balance, the use of such fertilisers could become a key component of sustainable agricultural development in Kyrgyzstan (Kurenkeev *et al.*, 2024). Furthermore, the development of local production of humate-enriched organomineral fertilisers can contribute to job creation and rural development. It can also reduce dependence on imports of chemical fertilisers, thereby increasing the country's economic sustainability (Mikos-Szymańska *et al.*, 2019; Vaschenko *et al.*, 2021).

S.B. Kenenbaev *et al.* (2023) also indicate that in the context of Kyrgyz agriculture, the introduction of humate-enriched organomineral fertilisers based on oxidised lignite contributes to the country's efficiency, sustainability, and competitiveness in the global market. Thus, despite the availability of sufficient information on the potential of humate-enriched organomineral fertilisers based on oxidised lignite, the gap that needs to be filled is related to a more detailed description of the process of obtaining humate-enriched organomineral fertilisers in agriculture, including technological aspects and production stages.

The study of the relevance of the introduction of humate-enriched organomineral fertilisers based on oxidised lignite into agriculture in Kyrgyzstan is important in the context of solving existing problems related to the sustainable development of the agricultural sector. Considering the specific climatic features and limited natural resources of the region, the search for effective methods to improve soil fertility and increase crop yields is an urgent task. The introduction of innovative technologies, such as humate-enriched lignite-based fertilisers, can significantly contribute to improving soil quality, preserving soil fertility and increasing crop productivity in the country. Thus, the study has the potential not only to improve the economic well-being of farmers, but also to reduce the negative impact on the environment by reducing the use of chemical fertilisers and maintaining the balance of ecosystems.

The purpose of this study was to determine the best methods of obtaining and processing humate-enriched organomineral fertilisers based on oxidised lignite to ensure the assimilation and effective use of important nutrient components by plants. To fulfil this purpose, the following tasks were set and implemented: study of characteristics and composition of humus produced by red wiggler worms of the *Eisenia foetida* species, evaluation of the effect of adding humus and glauconite containing natural mineral of Kyzifl-Tokoi deposit of the Kyrgyz Republic on the content of humic substances and nutrients in the soil material.

MATERIALS AND METHODS

To obtain an important component of humate-enriched organomineral fertiliser (HOMF) based on oxidised lignite, scientific experimental studies were carried out in the laboratory of the Osh Technological University of the Kyrgyz Republic. To improve the component composition of HOMF based on oxidised lignite, natural components were used: vermicompost and glauconite. Production of vermicompost (humus) was carried out using red wiggler worms (RWW) of the *Eisenia foetida* species, and production of mineral containing basic nutrients for plants in assimilable forms – by processing of natural glauconite containing mineral.

Before the vermicomposting process was organised, preparatory works were carried out:

1. Substrate preparation included the following steps: preparation of a wooden box 80 cm deep; preparation of over-digested cow dung and dry plant materials such as straw and leaves of fruit plants; preparation of necessary tools and accessories: thermometer, pH-meter F20-Standard (Mettler Toledo Switzerland), moisture meter testo 606-1 (Testo, Germany), shovel and water containers; filling the box with a substrate consisting of over-digested cow dung and dry plant materials in a 1:1 ratio; thoroughly mixing the substrate and moistening it to a moisture content of 70-80% of full capacity.

2. Substrate fermentation includes the following steps: setting the substrate for fermentation with maintaining constant humidity, moistening it with warm water and periodic stirring every 2-3 days; regular measurement of temperature and pH of the medium; at the end of the fermentation process of the components, the indicators should stay stable: pH at 7.1 and substrate temperature at 26-27°C.

3. Introduction of the worms. After the fermentation process was completed, the red wiggler worms *Eisenia foetida* were introduced. At the beginning, a small number of worms (50 individuals) were introduced to test the readiness of the environment for their activity. Then, an introduction of 1,000 specimen of red wiggler worms into the box was organised. Air oxygenation of the substrate was carried out daily by gentle stirring.

To carry out the composting process in laboratory conditions, the following technological scheme was established (Table 1).

***Table 1**. Technological scheme of organic materials composting process (vermicompost production)*

No. 1 initial substrate			
	No. 2 ready-made substrate		
		No. 3 RWW in substrate	
			Ready-made vermicompost
RWW separated from vermicompost			
	No. 2 ready-made substrate		
		No. 3 RWW in substrate	
			Ready-made vermicompost

***Source:** compiled by the authors of this study*

For enrichment of important micro- and macroelements of HOMF, glauconite containing sandstone from Kyzyl-Tokoya deposit was used, prepared to concentrated solution-suspension of glauconite, by adding 1 kg of glauconite for every 10 kg of substrate. Potassium, iron, aluminium, and silicon contents were determined using PAZH-2 device (Altavir, Ukraine), phosphorus content was determined based on spectral analysis using ULAB 101 spectrophotometer (China). The presence of minerals such as arogonite, graphite, calcite, goethite, and peaks of unknown minerals were determined by X-ray diffraction (XRD) analysis (Shimadzu XRD-7000, Japan). The mineralisation rate was determined by measuring the change in organic matter mass per month, the number of microorganisms was determined by culturing microbes from the sample on nutrient medium and then counting colonies, and air and water-air permeability was determined using a PZh 2-100-10-10/19 pycnometer (Altavir, Ukraine). The content of humic substances was determined according to the chromatography method, while X-ray fluorescence analysis was carried out on Thermo Fisher Scientific Inc. apparatus (USA) to determine the elemental composition of the samples.

The control sample was a substrate sample that was not enriched, not treated with worms, and did not contain glauconite, and represented the baseline condition. In addition, a comparative study of the effect of humate-enriched organomineral fertiliser on the growth and development of different plant species was carried out under laboratory conditions. During the study, the effects of this fertiliser on the following plant species were investigated: raw cotton variety Kyrgyz-6, rice (variety: six-line F1), Turkish tomato variety Shahti and Elpida, spice plants, particularly dill.

RESULTS

Red wiggler worms, or *Eisenia foetida*, are generally similar to common earthworms, but have several features that make them preferable for use in vermiculture. This species is characterised by rapid growth, short life cycle, and high fecundity. These worms are also more suitable for vermiculture breeding due to their ability to adapt quickly to changing conditions and reproduce rapidly. Using *Eisenia foetida* earthworms to create vermicompost and then incorporating this vermicompost into a humate-enriched organo-mineral fertiliser based on oxidised lignite will result in increased availability of micronutrients (such as iron, copper, manganese, zinc), vitamins (A and B), enzymes, antibiotics, and other beneficial factors to plants. This contributes to the activation of physiological processes in plants, improving their tolerance to diseases and pests. Furthermore, the use of such fertiliser will help in restoring soil fertility and increasing nutrient elements, which will be beneficial to plant growth and development (Al-Tawarah *et al.*, 2024).

Glauconite is used for enrichment of important trace elements as part of humate-enriched organic mineral fertilisers because of its unique properties and abilities. Glauconite is a valuable source of trace elements such as iron, manganese, potassium, phosphorus, copper, due to its chemical nature and exchange adsorption ability. Once glauconite is processed, nutrients are released and made available to plants in a digestible form. This mineral has high surface activity and a great ability to hold and release nutritional elements to plants, making it an ideal component for creating fertilisers to improve soil fertility and increase crop yields (Omarov *et al.*, 2024).

During the study, X-ray fluorescence analysis of vermicompost was carried out to determine its chemical composition and content of various elements. This allows the quality and composition of the fertiliser to be assessed and the presence of essential nutrients such as nitrogen, phosphorus, potassium, and trace elements to be verified. The results of such an analysis can be useful in optimising fertiliser application, as well as providing plants with the necessary nutrients for their health and growth. The analysis of vermicompost showed that the nutrients contained in it are presented in a form that is most favourable for assimilation by plants, thus contributing to their optimum and efficient nutrition. Vermicompost contains many organic compounds such as humus, amino acids, and trace elements that provide plants with the resources they need for healthy growth and development. This type of fertiliser can increase soil fertility and improve soil structure, contributing to crop yields and plant health.

The diffraction pattern graph represents the result of X-ray diffraction on the crystal structure of the sample. It displays the dependence of the diffracted X-ray intensity on the angle of diffraction or wavelength shift. Diffraction patterns are used to analyse the crystal structure of materials, identify crystal phases, determine the distances between atoms in the crystal lattice and other characteristics of crystalline materials. The magnitude and shape of diffraction peaks on the graph contain information about the internal structure of the sample and its crystal lattice (Fig. 1).

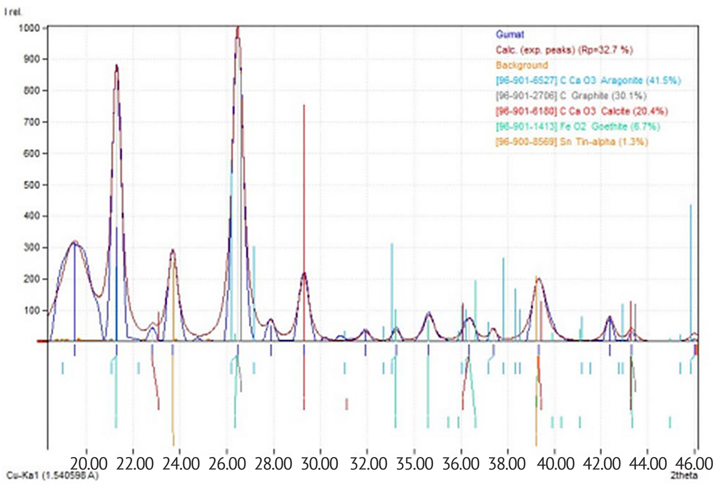

***Figure 1**. Graph of diffraction pattern of vermicompost*

***Source:** compiled by the authors of this study*

The results of the study show that HOMF containing humic substances, vermicompost, and glauconite in its composition has a positive effect on the growth and development of the plant species under study. This can manifest itself in increased plant growth rate, improved plant health, increased yields, and increased resistance to stressful conditions such as drought or disease. Such results indicate the potential effectiveness of HOMF in agricultural practices to improve the quality and quantity of agricultural products (Fig. 2).

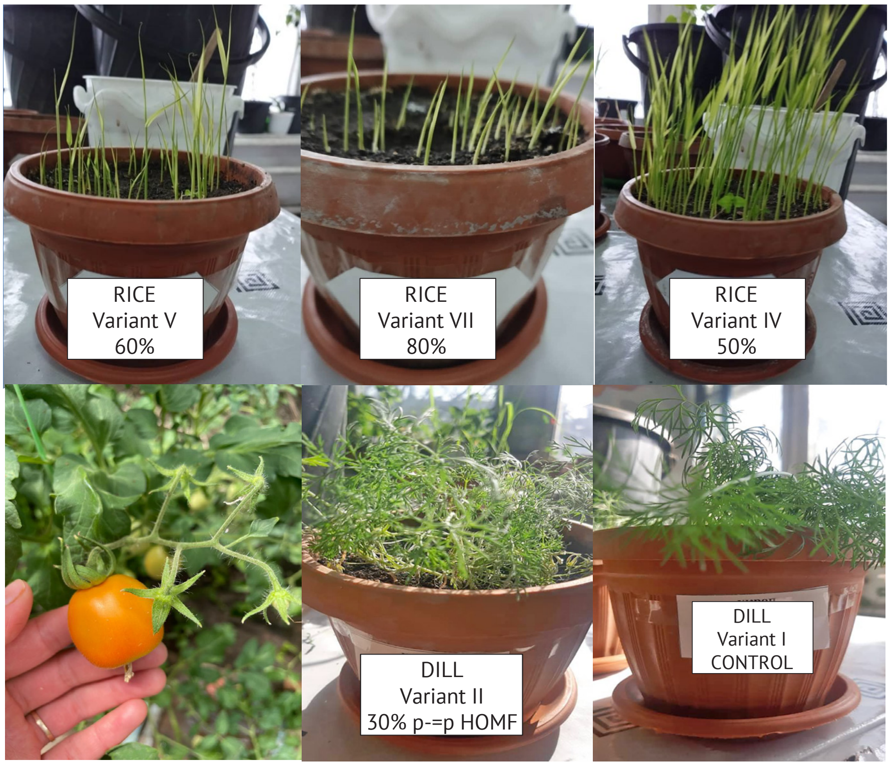

***Figure 2**. Crops under study with HOMF added to the soil*

***Source:** compiled by the authors of this study*

Glauconite available as a natural mineral was used for enrichment of HOMF, obtained by the technology of the authors of the study, considering that it contains important elements of plant nutrition (K.P.N) and micro- and macroelements necessary for physiological processes of plant organism. According to the results of analysis comparing the content of elements and minerals in control and vermicompost, it can be stated that the content of potassium in vermicompost is significantly higher (by 40%) compared to the control sample. This may indicate a higher nutrient content in vermicompost, which is favourable for plants. The iron and aluminium content in vermicompost is almost the same as in the control sample. This may indicate a retention or slight change in iron content during the vermicomposting process (Table 2).

***Table 2**. Comparison of elements and minerals content in control and vermicompost, %*

Parameter	Control	Vermicompost
Potassium	5.2	7.3
Iron	3.6	4
Aluminium	2.8	3.2
Silicon	6.1	8.5
Phosphorus	4.9	6.3
Arogonite	0.2	0.1
Graphite	0.3	0.2
Calcite	0.1	0.05
Goethit	0.15	0.08
Peaks of unknown minerals	0.05	0.03

***Source:** compiled by the authors of this study*

The silicon and phosphorus contents are also higher in vermicompost, by 39% and 28.5%, respectively, which is a positive factor for improving soil fertility. The vermicompost shows a decrease in the content of arogonite, graphite, calcite, goethite, and peaks of unknown minerals compared to the control sample. This may indicate the transformation or decomposition processes of these minerals during vermicomposting. Furthermore, the study conducted compares the characteristics of control and vermicomposted soil material. The results show that vermicomposted material has several advantages over the control. Specifically, soil pH increases after vermicomposting, which may contribute to more favourable conditions for plant growth. There is also an increase in moisture content and organic matter, which can enrich soil nutrients and improve soil fertility. Furthermore, the C/N ratio decreases, which may increase the availability of nitrogen to plants. The rate of mineralisation in vermicomposted material is also higher, indicating a more active conversion of organic matter into mineral forms. The number of microorganisms in the soil increases after vermicomposting, indicating increased biological activity of the soil. Finally, vermicomposted material has higher air and water-air permeability, which favours better air-water regime of soil and more active gas exchange. These results emphasise the benefit of vermicompost for improving soil properties and yield (Table 3).

***Table 3**. Characteristics of control and vermicomposted soil material*

Parameter	Control	Vermicompost
pH	6.5-7	7-7.5
Humidity (%)	30-40	40-50
Organic matter content (%)	20-30	35-45
C/N ratio	12-15	10-12
Mineralisation rate (g/kg/month)	15-25	30-40
Number of microorganisms (colonies/g)	4×10^6-6×10^6	7×10^6-9×10^6
Air permeability (%)	50-70	70-80
Water-air permeability (%)	40-60	60-70
Structure and texture	Loose	Crumb

***Source:** compiled by the authors of this study*

Overall, the study found that more intensive organic waste composting processes occur when red wiggler worms *Eisenia foetida* are used. Because of this process, an active mineralisation of organic matter occurs, which leads to the release of biologically active components into the soil. The resulting product, or humus, which is added to the soil, contributes to the formation of a special soil structure. Furthermore, the results of laboratory experimental studies indicate that vermicomposting using red wiggler worms *Eisenia foetida*, results in effective decomposition of organic material, resulting in a quality vermicompost. This process accelerates composting and converts organic waste into a more digestible form of plant nutrients. The resulting vermicompost contains nutrients in the form most available to plants, which improves soil fertility and stimulates plant growth.

Glauconites, whose structure is usually colloidal, are formed by complex syntheses involving many intermediate structures. Potassium plays a significant role in strengthening the structure of glauconite by facilitating the transformation of its intermediate forms into more stable ones. Humatised organomineral fertiliser enriched with glauconite and humus is superior to industrial mineral fertilisers in its effectiveness. Humic substances in HOMF improve the assimilability of basic and micronutrients for both plants and soil. Properties of the natural mineral glauconite, including its adsorption properties and structure features, contribute to the conversion of basic nutrition elements and trace elements necessary for physiological processes of plants into assimilable forms. This allows plants to utilise nutrients efficiently, which improves their growth and development. A humate-enriched organomineral fertiliser produced based on oxidised lignite has a full range of essential nutritional elements, including important micro- and macroelements. Due to enrichment with glauconite extracted from the natural mineral of Kyzyl-Tokoi deposit and humus substances of vermicompost, such fertiliser has an advantage over industrially produced mineral fertilisers.

Based on the data obtained during the study, it can be stated that the content of potassium oxide increased by 0.6%, from 7.4% in the control sample to 8% after the addition of glauconite, also increased the content of iron (III) oxide from 24.51% in the control sample to 26.85%, the content of aluminium oxide – by 0.41%, and the content of silicon oxide – from 41.57% in the control sample to 46.47% after the addition of glauconite. Notably, the humic content also increased by 1.1% after the addition of glauconite. Increasing humic content has many positive implications for soils, ecosystems, and agriculture. This includes improving soil structure and fertility, reducing erosion, improving fertiliser use efficiency, and maintaining biodiversity. Such changes contribute to sustainable agriculture, environmental protection, and healthy ecosystems (Table 4).

Table 4. Element content in control sample and with added glauconite, wt. %

Parameter	Control	Glauconite
K_2O	7.4	8
Fe_2O_3	24.51	26.85
Al_2O_3	2.75	3.16
SiO_2	41.57	46.47
Humic substances content, wt. %	2.5	3.6

Source: *compiled by the authors of this study*

The findings obtained, which show an increase in the content of all measured parameters after the addition of glauconite, emphasise its importance as an effective means of improving soil quality and stimulating plant growth. This is important not only for agriculture but also for environmental conservation. Improved soil quality provides more favourable conditions for plant development, which can lead to higher yields and reduced need for crop protection products. Plant growth stimulation, especially using natural and environmentally friendly materials such as glauconite, contributes to sustainable and ecologically balanced farming systems. This can reduce the negative environmental impacts of agricultural production, such as soil and water pollution, soil erosion and reduced biodiversity, and reduce the need for chemical fertilisers and pesticides by improving natural plant defences. Thus, the addition of glauconite to soil represents a promising solution to improve its quality and increase yields, considering the principles of sustainable agriculture and environmental sustainability.

Based on the presented data, the use of vermicompost (humus) produced by red wiggler worms can be recommended for enrichment of oxidised lignite to increase their humic content. This enhances the transformation of element compounds into forms available for plant uptake. It is also recommended to use glauconite isolated from a natural mineral, e.g., glauconite from Kyzyl-Tokoya deposit, for soil enrichment in combination with vermicompost (humus). This allows the fertiliser to contain the main nutritional elements and the necessary micro- and macronutrients for plants. The developed humate-enriched organic-mineral fertiliser containing all necessary nutrients, as well as micro- and macroelements for plants, can become a full-fledged alternative to chemical fertilisers, unlike which, it contributes to the preservation of soil fertility during the following years without depleting its structural content. Furthermore, the use of vermicompost and the enrichment of glauconite make it possible to create a fertiliser that provides plants with essential nutrients in an assimilable form, which contributes to their healthy growth and physiological development.

The findings of the study demonstrate that both vermicompost and the addition of glauconite to soil have considerably positive effects on soil quality and fertility. Vermicompost enriches the soil with organic compounds and nutrients, which contributes to effective plant nutrition and improved plant growth. The addition of glauconite increases the content of basic nutritional elements and humic substances, which also has a favourable effect on soil characteristics, highlighting the importance of using such techniques for sustainable agriculture and maintaining ecological balance.

DISCUSSION

Humified organomineral fertilisers based on oxidised lignite represent a vital component in modern agriculture, ensuring efficient and sustainable crop production. The use of such fertilisers is based on the use of lignite, which is subjected to an oxidation process that promotes the development of organic components with a high content of humic acids. Humate-enriched oxidised lignite contains a range of bioactive substances such as humus substances, humates, amino acids, and trace elements essential for healthy plant growth and development (Koković *et al.*, 2021).

The studies carried out by scientists and their findings also reveal significant aspects concerning the production of a vital component of humate-enriched organomineral fertiliser based on oxidised lignite. Specifically, Y. Bouhia *et al.* (2022) and C. Amoah-Antwi *et al.* (2021) found that vermicompost has a significant content of organic compounds, amino acids, and trace elements, which contributes to optimum plant nutrition. The conducted study agrees with the findings of other scientists, particularly M. Berca *et al.* (2017) and M.N. Ashraf *et al.* (2020), which confirm the increase of potassium, silicon, and phosphorus content in vermicompost. These elements play a key role in improving the nutrient composition of the soil and promote healthy plant growth. Moreover, H.S. Jat *et al.* (2019) also observed an increase in nutrient elements in vermicompost, which confirms the similarity of results.

The study on the use of red wiggle worms in organic waste composting is consistent with previous research including T. Keller *et al.* (2021) and A. Mustafa *et al.* (2020), which confirm the activity of organic matter mineralisation involving worms of *Eisenia foetida* species, as well as the positive effect of vermicompost on soil structure and plant growth. L. Joos *et al.* (2020)

aimed at investigating the effect of worms and minerals on improving soil carbon retention proved that the addition of worms changed the concentrations of organic carbon and nitrogen in certain density fractions and increased the thermal stability of organic matter in heavy fractions. These fractions contained higher proportions of aromatic and proteinaceous materials. Consequently, worms under composting conditions can effectively stabilise organic matter by forming aggregates and/or organo-mineral compounds containing more material originating from microorganisms (Domaratskiy *et al.*, 2023).

B. Symanowicz and R. Toczko (2023) confirm the improvement of soil characteristics under the influence of vermicompost, including increase in pH, moisture content, and organic matter. These changes create favourable conditions for plant growth and promote soil fertility, which is also reflected in the findings of the present study. Furthermore, confirmation of the obtained results can be found in the studies of S. Li *et al.* (2023) and G. Wang *et al.* (2020), which indicate the positive effect of vermicompost on soil biological activity. This indicates that vermicompost helps to improve microbiological processes in the soil, which can improve soil fertility. Comparable results were obtained by B. Al-Tawarah *et al.* (2024), who noted that the use of vermicompost improves soil structure by activating biological processes and enriching the soil with organic matter. This results in increased water-holding capacity of the soil and increased availability of nutrients to plants. The researchers also note that vermicompost is an effective tool in controlling soil erosion and preserving soil fertility for the long term.

S. Pot *et al.* (2022), in their studies on the microbiological characteristics of composts and management wastes compared to peat substrates, highlight that the composition of microbial communities in composts based on different raw materials can be more variable compared to peat substrates. In addition, I. Oyege and M.S. Balaji Bhaskar (2023) confirm that classification of management wastes based on parameters such as pH and hemicellulose content can be important for characterisation of bacterial and fungal communities, respectively.

Findings from many studies indicate the potential of various types of composts to improve plant growth or suppress pathogens, depending on their composition (Vinyukov *et al.*, 2022). Thus, green composts and vegetable composts may be most effective for non-acidophilic plants, while grass clippings and woody compost fractions may be beneficial for calcifuging plants (Lutz *et al.*, 2020; Le Mer *et al.*, 2020). Thus, the findings of the present study are in agreement with previous studies which also found the positive effect of vermicompost on soil properties and plant growth. Confirmation of these results under different conditions and using different techniques demonstrates the wide potential of vermicompost in improving agricultural sustainability and environmental conservation (Chimdessa & Sori, 2020; Ibrahim *et al.*, 2021).

The study confirms the positive effect of glauconite on soil characteristics, which agrees with the findings of I. Asghar *et al.* (2023), which shows an increase in the content of major nutritional elements and humic substances in the soil. This indicates that the addition of glauconite does improve soil fertility and stimulate plant growth. L. Liang *et al.* (2021) observed that the application of lignite-based humified organomineral fertiliser affects soil structure, making it more friable and improving water-holding capacity. This promotes more efficient penetration of moisture and nutrients to the plant roots. Humified fertilisers also help improve soil resistance to drought and erosion. Improved soil structure enriched with organic components helps to conserve moisture and prevent soil layers from being washed away by intense rainfall or irrigation (Tandy *et al.*, 2021; Ros *et al.*, 2020). These results demonstrate the potential of using local resources, such as lignite, to develop sustainable and effective organomineral fertilisers that can contribute to improved agriculture and environmental conservation in Kyrgyzstan (Sun *et al.*, 2020).

M. Berca *et al.* (2017) note the potential of using humate-enriched fertilisers as a tool to improve the economic efficiency of agriculture in Kyrgyzstan. Increasing yields and reducing fertiliser and tillage costs can lead to improved profitability and sustainability of agricultural enterprises. Consequently, the development of humate-enriched fertilisers based on lignite from Kyrgyzstan opens new prospects for sustainable agricultural development and environmental conservation in this region. It is also important to emphasise that the findings of Q. Wu *et al.* (2020) suggest that the use of lignite-based humate-enriched fertiliser can reduce the dependence of agriculture on chemical fertilisers. This can lead to reduced negative environmental impacts and lower costs of agricultural production. The use of humate-enriched fertiliser enhances plant immunity and resistance to stressful conditions such as drought, severe temperature fluctuations and diseases, which can improve yield and quality of produce (Vandecasteele *et al.*, 2021).

Considering the cited studies, as well as the findings of the present study, it can be stated that the use of humate-enriched fertilisers based on oxidised lignite is an effective way to improve soil fertility and stimulate plant growth. Thus, the agreement with the results of other studies confirms the reliability and importance of the obtained findings and emphasises the significance of obtaining a vital component of the humate-enriched organomineral fertiliser based on oxidised lignite of Kyrgyzstan as a fertiliser to improve soil properties and ensure healthy plant growth. The similarity of the results of various studies conducted by different groups of scientists in different regions confirms the consensus on the positive effects of humate-enriched fertilisers on soil properties and crop yields. These fertilisers help to improve soil structure, increase nutrient content, and activate biological processes necessary for healthy plant growth and development (Smetanska *et al.*, 2023).

Thus, based on the agreed results of various studies, it can be concluded that the use of humate-enriched fertilisers based on oxidised lignite in agriculture is promising to achieve a more sustainable and productive agriculture. Consequently, the findings of this study reflect the results of other researchers, confirming the positive effect of vermicompost and glauconite on soil properties and plant growth. This emphasises the importance of using these materials in agriculture to improve crop yields and soil quality. The combination of such studies allows for a full assessment of the efficiency and potential of the component of the humate-enriched fertiliser based on oxidised lignite.

CONCLUSIONS

The study provided significant data on vermicompost and its effect on soil characteristics. The analysis of vermicompost showed its richness in organic compounds and nutrient elements, which are presented in the form most assimilated by plants. The study found that the potassium content of vermicompost increases by 40% and silicon and phosphorus content is higher by 39% and 28.5%, respectively. Improvement in soil characteristics after vermicomposting was also observed: soil pH increased to 7-7.5, organic matter content increased to 35-45% and C/N ratio decreased to 10-12. This indicates more favourable conditions for plant growth and more efficient use of nitrogen.

The study also found that the addition of glauconite increased potassium oxide content by 0.6%, iron (III) oxide content increased by 2.34%, and aluminium oxide content increased by 0.41%. In addition, silicon oxide content increased by 4.9% and humic substances content increased by 1.1% after the addition of glauconite. Increasing humic substances brings many positive changes for the soil, including improving soil structure and fertility, reducing erosion, increasing fertiliser efficiency and maintaining biodiversity. Thus, the study found that the use of red wiggler worms of the species *Eisenia foetida* in the composting of organic waste promotes intensive composting processes and active mineralisation of organic matter. The resulting product, vermicompost enriched with glauconite and humus, helps improve soil structure and fertility, which stimulates plant growth. Hence, the addition of glauconite can be an effective way to improve soil quality, which is important for sustainable agriculture and environmental conservation.

A limitation of the study is that laboratory conditions may not fully reflect the complex interactions that can occur in a real soil environment. For instance, factors such as climatic conditions, biological activity of soil microorganisms, and soil chemical composition can substantially influence the effectiveness of vermicompost and glauconite as fertilisers. The prospect for further research is to evaluate the synergistic effects of vermicompost and glauconite with other fertilisers, soil adjustments, and agronomic practices to determine the best combinations of their application.

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None.

CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Отримання важливого компонента гуматизованого органомінерального добрива на основі окисленого бурого вугілля Киргизстану

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Анотація. Дослідження методів отримання та обробки гуматизованих органомінеральних добрив на основі окисленого бурого вугілля є ключовим аспектом для сучасного сільського господарства, з огляду на необхідність підвищення ефективності використання ресурсів, поліпшення якості ґрунту та забезпечення стійкості виробництва. Мета роботи – визначити оптимальні способи отримання та обробки гуматизованих органомінеральних добрив на основі окисленого бурого вугілля. Для досягнення поставленої мети проведено експериментальні дослідження в лабораторії інституту Природних ресурсів південного відділення Національної академії Киргизької республіки та навчально-науковій лабораторії кафедри Екологія та охорона довкілля Ошського технологічного університету імені М. М. Адишева в Киргизстані. Результати дослідження свідчать про значні зміни в ґрунтових характеристиках після застосування вермикомпосту та глауконіту. Аналіз вермикомпосту виявив, що він має значний вміст поживних елементів і органічних сполук. Вміст калію у вермикомпості збільшився на 40 %, а вміст кремнію і фосфору відповідно зріс на 39 % і 28,5 %. У результаті вермикомпостування спостерігається поліпшення характеристик ґрунту: pH підвищується до 7-7,5, вміст органічної речовини збільшується до 35-45 %, а C/N-відношення знижується до 10-12, що створює сприятливіші умови для росту рослин і підвищує ефективність використання азоту. Після додавання глауконіту відмічено збільшення вмісту оксиду калію на 0,6%, оксиду заліза (III) на 2,34%, та оксиду алюмінію на 0,41 %. Вміст оксиду кремнію зріс на 4,9 %, а гумінових речовин – на 1,1 %. Ці зміни в ґрунтових характеристиках сприяють підвищенню врожайності та поліпшенню якості ґрунту. Результати дослідження мають практичну значущість для сільського господарства в рамках поліпшення ґрунтових характеристик, підвищення врожайності та якості ґрунту, а також у зниженні залежності від хімічних добрив

Ключові слова: поживні речовини; ґрунт; екологічна стійкість; макроелементи; глауконіт; *Eisenia foetida*

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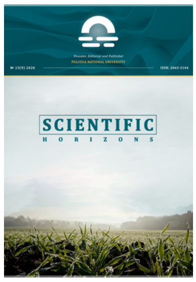

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Growth performance, yield, and proline content of two local cultivars of black rice irradiated by gamma rays for drought resistance

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Abstract. One of the critical effects of climate change on the agricultural environment is drought stress. This study aimed to determine the growth, yield performance, and proline content of black rice local cultivars Bantul, Boyolali mutant M2 at various doses of gamma rays and the proline content of mutant M3 during drought stress. The experiment used a Completely Randomized Design (CRD) with the factor dose of gamma-ray irradiation (0 Gy, 100 Gy, 200 Gy, and 300 Gy), applied in both paddy fields and polybags to observe and compare the growth, yield, and proline content of black rice plants with the control treatment (0 Gy). Laboratory test results showed that Boyolali and Bantul cultivars irradiated at 100 Gy and 200 Gy, had the highest proline content, which increased under drought stress conditions in both cultivars. At 100 Gy,

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the average plant height and the number of productive tillers of black rice of Boyolali and Bantul cultivars were the highest, and the diversity of the two local cultivars at this dose was higher than at other irradiation doses. Applying 100 Gy and 200 Gy gamma-ray irradiation doses on the Boyolali cultivar significantly affected the panicle length character and the number of seeds per panicle. For the Bantul cultivar, panicle length and the number of seeds per panicle at an irradiation dose of 200 Gy showed significantly different results. The conclusion is that a reduced amount of gamma-ray irradiation can enhance the growth, yield, and proline content of M2 black rice

Keywords: functional food; rice plant; water stress; agriculture; climate change

INTRODUCTION

Black rice (*Oryza sativa* L.) is a viable alternative to white rice and holds significant economic importance. It has gained widespread recognition due to the growing health consciousness among the public. Considered a functional food, black rice is rich in anthocyanin, fibre, and iron, making it beneficial for health (Rahim *et al.,* 2022). According to F. Agustia *et al.* (2019), black rice contains 7.5% dietary fibre and 5.8% hemicellulose. The fibre and hemicellulose in white rice are only 5.4% and 2.2%, respectively. Black rice has flavonoid content that is five times higher than white rice, and the calories of black rice are 362 kcal/100 grams lower than other rice (Aini *et al.,* 2023). However, local black rice still has the characteristics of a tall plant posture (>150 cm), a long life (>145 days), and low productivity, so farmers rarely cultivate black rice. Plants that are too tall will fall quickly. Boosting black rice productivity will become more profitable, if these weaknesses are resolved. Black rice M2 local cultivars Boyolali and Bantul have good characteristics from the M1 parents, namely low plant height and high yields seen from the weight of grain per clump. These good traits will be passed down and tested on M2 generation black rice, which is expected to be more stable than the elders. M2 hopes for higher genetic stability and will enter the advanced test stage to serve as a superior cultivar in the future. The genetic diversity of growth and yield characters displayed by M2 black rice is still high. According to R. Darmawan and D. Damanhuri (2019), plant breeding activities require a wide genetic diversity for the selection process to be more effective.

Today, global warming and the increasing human population pose problems in increasing water availability for agriculture. One of the effects of global warming is drought. It is a challenge in agriculture in maintaining plant growth and productivity in drought conditions. Over 4 million hectares of rainfed lowland and highland rice areas spread throughout the regions in Indonesia including Java, Sumatra, Kalimantan, Sulawesi, Bali, Nusa Tenggara, Maluku, and Papua. The area of rainfed rice fields, 2.07 million hectares, is divided between 33.8% of Java and other regions (Erythrina *et al.,* 2021). Drought tolerant rice cultivars that are tolerant to drought are needed to deal with water shortages in the future. It can use mutation breeding and selection activities to improve plant traits and maintain good plant traits. In addition, it is also expected to obtain new mutant lines that have the potential to be drought-tolerant and have high yields. According to Nandariyah *et al.* (2021), mutation breeding using gamma-ray irradiation can enhance the characteristics of black rice for the better. Mutations with gamma-ray irradiation have been widely applied to various types of plants to increase plant resistance to drought stress, such as soybeans (Oktavianus *et al.,* 2019), rice (Dama *et al.,* 2020), peanuts (Hemon *et al.,* 2020) and soybean cultivar namely kipas putih.

Plants undergo various metabolic adjustments to regulate morphological adaptations and physiology to environmental changes (Zaib *et al.,* 2023). Proline accumulation is an indicator of a plant's physiological response to drought. Proline acts as an osmolyte and maintains osmotic regulation in plant organs so plants remain protected when water potential is low (Ozturk *et al.,* 2020). M. Meena *et al.* (2019) added that proline contributes to counteracting ROS and sustaining the structure of membranes and proteins. The response of the cempo ireng rice line to drought stress by increasing the leaf proline content (Patmi *et al.,* 2020). Drought tolerant plants will accumulate more proline than sensitive plants. According to A. Furlan *et al.* (2020), the elevated levels of proline content in plants indicate a relationship with drought tolerance. The growth, yield, and proline content information of Boyolali and Bantul M2 black rice cultivars are limited. It must know the research on the tolerance level of local cultivars of black rice Boyolali and Bantul M2 before selecting individual drought-tolerant plants. The proline content in each black rice cultivar indicates the tolerance level to drought stress. Therefore, it is necessary to research to examine more deeply the growth performance, yield, and proline content of local cultivars of black rice Boyolali and Bantul M2 that are irradiated at various doses of gamma-ray irradiation. This study aimed to determine the growth and yield performance and the proline content of black rice local cultivars of Boyolali and Bantul M2 due to gamma irradiation. Furthermore, observation of M3 black rice proline content at various doses of gamma rays when subjected to drought stress treatment.

MATERIALS AND METHODS

The research was conducted in 2023 and carried out in two places, namely the planting of M2 black rice in the rice fields of Jati Village, Jaten District, Karanganyar

Regency, and M3 black rice planting in Kaloran Village, Wonogiri District, Wonogiri Regency in polybags. The proline content test was carried out at the Biochemistry Laboratory, Universitas Gadjah Mada. Implementation in the rice fields of Jaten Karanganyar used a single factor completely randomized design (CRD), namely the dose of gamma-ray irradiation (0 Gy, 100 Gy, 200 Gy, and 300 Gy) without replication because the experimental unit is homogeneous. The materials used were black rice seeds selected by M1 from Boyolali and Bantul irradiated with 100 Gy, 200 Gy, and 300 Gy gamma rays and control (0 Gy). Other materials used were urea fertilizer, ponskha, SP-36, pesticides, and chemicals to test the leaf proline content. In this study, the growth, yield, and proline content of individual black rice irradiated by gamma rays were observed and compared with the control treatment (0 Gy).

The second experiment was carried out in polybags with drought stress treatment. The material used was black rice produced by local cultivars M3 Boyolali and Bantul irradiated by gamma rays. The study used a completely randomized design (CRD) with gamma-ray irradiation factors (irradiation levels of 100 Gy, 200 Gy, and 300 Gy) subjected to drought stress and without drought. This second experiment tests the proline content of local cultivars of black rice Boyolali and Bantul due to gamma-ray irradiation. According to T. Handayani and H. Kurniawan (2018), the procedure for analyzing leaf proline content is to smooth black rice leaf samples of each black rice cultivar irradiated with gamma rays and without irradiation with 3% sulfosalicylic acid. The smooth leaves were filtered with Whatman #2 filter paper, then the filtrate was added with ninhydrin acid and glacial acetic acid at 100°C. One hour later, it was extracted with toluene to form two phases. It took the upper phase (chromophore) and measured it at an absorbance of 520 nm – calculation of proline concentration with a standard curve.

Observation variables included number of productive tillers, plant height, panicle length, number of seeds per panicle, the weight of 100 seeds, and proline content of black rice planted in paddy fields and polybags. The growth and yield components of local cultivars of black rice from Boyolali and Bantul that were exposed to gamma-ray irradiation at levels of 100 Gy, 200 Gy, and 300 Gy were compared descriptively with those of black rice that was not irradiated. The growth and yield data were further analyzed using a T-test to determine the significance of the differences between the two cultivars of gamma-irradiated with black rice (0 Gy). The proline content data were analyzed descriptively by comparing the proline content of each cultivar under different doses of gamma-ray irradiation and a control group (not exposed to gamma-ray irradiation). Data were presented in tabular form. The authors adhered to the standards of the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS AND DISCUSSION

The T-test indicated that the mean height of rice plants (Table 1) and the number of productive tillers (Table 2) were significantly different between black rice cultivars from Boyolali and Bantul irradiated with 100 Gy gamma rays compared to non-irradiated control black rice (0 Gy). The average plant height of Boyolali and Bantul cultivars has the highest dose of 100 Gy, with the highest standard deviation in each black rice cultivar. It showed that the black rice's two local cultivars exhibited greater diversity in plant height and number of productive tillers at a irradiation dose of 100 Gy compared to other doses.

***Table 1**. Plant height of M2 mutant black rice cultivars Boyolali and Bantul irradiated by gamma rays with various doses of irradiation*

Cultivars	Doses (Gy)	Lowest		Highest		Range	Average (cm)
		Plant Code	Height (cm)	Plant Code	Height (cm)		
Boyolali	0	Control	104.5	Control	139	104.5-139	122.5±8.47
	100	M2-BY1-1-40	112	M2-BY1-1-12	155.5	112-155.5	132.6±13.29*
	200	M2-BY2-2-35	109	M2-BY2-2-03	149	109-149	125.6±11.40
	300	M2-BY3-7-07	96.5	M2-BY3-7-17	121.5	96.5-121.5	117.7±10.66
Bantul	0	Control	104	Control	144	104-144	117.6±10.76
	100	M2-BT1-4-10	112.2	M2-BT1-4-17	160.3	112.2-160.3	135.5±12.8*
	200	M2-BT2-5-21	106	M2-BT2-5-06	153.5	106.5-153.5	123.3±11.37
	300	M2-BT3-8-22	101	M2-BT3-8-20	168	101-168	119.7±11.96

***Note:** the numbers are followed by sign (*) were significantly different from the control based on the T-test $\alpha = 0.05$*
***Source:** laboratory test results*

Table 2. *Number of productive tillers of M2 mutant black rice Boyolali and Bantul cultivars irradiated with gamma rays with various doses of irradiation*

Cultivars	Doses (Gy)	The Lowest		The Most		Range	Average (Stem)
		Plant Code	Total (Stem)	Plant Code	Total (Stem)		
Boyolali	0	Control	8	Control	21	8-21	12.2±3.28
	100	M2-BY1-1-35	9	M2-BY1-1-1	23	9-23	15.3±3.79*
	200	M2-BY2-2-36	7	M2-BY2-2-30	21	7-21	11.9±3.79
	300	M2-BY3-7-09	9	M2-BY3-7-13	20	9-20	13.5±3.15
Bantul	0	Control	6	Control	21	6-21	13.8±4.15
	100	M2-BT1-4-06	10	M2-BT1-4-08	28	10-28	16.6±4.66*
	200	M2-BT2-5-06	5	M2-BT2-5-17	26	5-26	13.9±4.32
	300	M2-BT3-8-14	8	M2-BT3-8-08	21	8-21	15.0±3.40

Note: *the numbers are followed by sign (*) were significantly different from the control based on the T-test* $\alpha = 0.05$
Source: *laboratory test results*

The panicle length and the number of seeds per panicle were significantly affected by the application of gamma-ray irradiation doses of 100 Gy and 200 Gy on the Boyolali cultivar, as shown in Table 3 and Table 4. Meanwhile, black rice of the Bantul cultivar shown significantly different in panicle length (Table 3) and the number of seeds per panicle (Table 4) when subjected to an irradiation dose of 200 Gy. The mean value and standard deviation indicated the highest diversity in panicle length for the Boyolali cultivar at a dose of 200 Gy (Table 3). The Bantul cultivar, plant code M2-BT1-4-17 (Table 4), produced the greatest number of seeds per panicle whe exposed to 100 Gy gamma-ray irradiation. The Boyolali cultivar treated with 100 Gy irradiation showed significant differences from the control plants on the 100-grain weight, while the Bantul cultivar treated with 200 Gy irradiation (Table 4).

Table 3. *Panicle length of mutant black rice M2 Boyolali and Bantul cultivars irradiated by gamma rays with various doses of irradiation*

Cultivars	Doses (Gy)	The Shortest		The Length		Range	Average
		Plant Code	Panicle Lenght	Plant Code	Panicle Lenght		
Boyolali	0	Control	20.96	Control	26.48	20.96-26.48	23.4±1.61
	100	M2-BY1-2-29	22.52	M2-BY1-1-43	26.78	22.52-26.78	24.7±1.17*
	200	M2-BY2-2-28	20.6	M2-BY2-2-02	27.68	20.6-27.68	24.7±1.67*
	300	M2-BY3-7-03	19.7	M2-BY3-7-05	26	19.7-26	22.8±1.39
Bantul	0	Control	20.3	Control	26.42	20.3-26.42	22.9±1.38
	100	M2-BT1-4-07	20.8	M2-BT1-4-28	27.4	20.8-27.4	23.8±1.82
	200	M2-BT2-5-12	17.85	M2-BT2-5-06	24.56	17.85-24.56	22.2±1.63*
	300	M2-BT3-8-11	18.56	M2-BT3-8-20	26.68	18.56-26.68	23.4±1.77

Note: *the numbers are followed by sign (*) were significantly different from the control based on the T-test* $\alpha = 0.05$
Source: *laboratory test results*

Table 4. *Weight of 100 grains of mutant black rice M2 Boyolali and Bantul cultivars irradiated with gamma rays with various doses of irradiation*

Cultivars	Doses (Gy)	The Lowest		The Highest		Range	Average (g)
		Plant Code	Weight (g)	Plant Code	Weight (g)		
Boyolali	0	Control	2.17	Control	3.04	2.17-3.04	2.6±0.20
	100	M2-BY1-2-07	2.45	M2-BY1-1-35	3.18	2.45-3.18	2.8±0.16*
	200	M2-BY2-2-13	2.08	M2-BY2-2-30	3.2	2.08-3.2	2.8±0.25
	300	M2-BY3-7-19	2.1	M2-BY3-7-15	2.71	2.1-2.71	2.7±0.19
Bantul	0	Control	1.9	Control	3.29	1.9-3.29	2.6±0.22
	100	M2-BT1-4-06	2	M2-BT143-02	3.35	2-3.35	2.6±0.37
	200	M2-BT2-5-05	2.25	M2-BT2-5-19	3.08	2.25-3.08	2.7±0.23*
	300	M2-BT3-8-16	2.43	M2-BT3-8-12	3.08	2.43-3.08	2.7±0.12

Note: *the numbers are followed by sign (*) were significantly different from the control based on the T-test* $\alpha = 0.05$
Source: *laboratory test results*

Laboratory test results showed that Boyolali 100 Gy and Bantul 200 Gy cultivars had the highest proline content (Table 5). The proline content increased under drought stress conditions in both Boyolali and Bantul cultivars (Table 6). The highest proline accumulation was in Boyolali and Bantul cultivars when exposed to 100 Gy gamma rays in dry conditions (Table 6).

***Table 5**. Proline content of mutant black rice M2 in Boyolali and Bantul cultivars irradiated with gamma rays with various doses of irradiation in an experiment in paddy fields*

Dose (Gy)	Repetition	Proline Content	
		Boyolali	Bantul
0	I	9.57	6.14
100		20.14	8.70
200		17.64	17.24
300		1.33	2.36
0	II	15.63	8.703
100		19.42	10.11
200		16.61	10.16
300		1.62	2.03

***Source:** laboratory test results*

***Table 6**. Proline content of mutant black rice M3 in Boyolali and Bantul cultivars irradiated by gamma rays with various doses of irradiation under drought stress*

Dose (Gy)	Treatment	Proline Content	
		Boyolali	Bantul
0	Control	4.15	3.99
100		9.49	7.74
200		5.91	6.14
300		3.81	3.70
0	Drought Stress	5.13	4.33
100		11.72	9.06
200		6.36	6.83
300		4.21	4.33

***Source:** laboratory test results*

The shortest plant heights in Boyolali and Bantul cultivars are 112 cm and 112.2 cm, respectively. Plant codes M2-BY1-1-40 and M2-BT-1-4-10 (Table 1). The mean value and standard deviation of black rice plant height of Boyolali cultivar irradiated with 100 Gy gamma rays were the highest, namely 132.6 cm and 13.29, respectively, as well as the Bantul cultivar with an average plant height of 135.5 cm and a standard deviation of 12.18 (Table 3). It is in line with the statement A. Gaur *et al.* (2018), that exposure to higher doses of gamma rays is inhibitory, while lower doses of radiation are sometimes stimulants. Free radicals produced by gamma rays can be destructive to affect the morphophysiology, anatomy, and biochemistry of plants depending on the level of radiation. Co-60 gamma-ray irradiation changes and increases genetic diversity so that the success rate of plant breeding is greater (Riviello-Flores *et al.*, 2022).

The number of productive tillers directly affects grain yield. Productive tillers produce panicles that produce grain so that which is associated with increased rice productivity. Gamma-ray radiation of 100 Gy led greater number of productive tillers in Boyolali and Bantul black rice cultivars with wider genetic diversity compared to other treatments (Table 2). The value of high genetic diversity in the number of productive tillers during the M2 generation allows selection for that generation. M. Shabani *et al.* (2022), also reported gamma-ray irradiation led to an increase in genetic diversity, resulting in higher number of productive tillers and yields per wheat plant. The greatest amount of productive tillers was in Bantul 100 Gy is 28 with code M2-BT1-4-8, while Boyolali 100 Gy had the greatest amount of productive tillers 23 at M2-BY1-1-11 (Table 2). These findings align with research W. Hanifah *et al.* (2020), that exposure to 100 Gy gamma-ray irradiation resulted in an increase in productive tillers. However, it depends on the individual plant because the results of irradiation in plants occur randomly.

Panicle length of M2 mutant black rice Boyolali cultivar at irradiation doses of 100 Gy and 200 Gy, respectively 22.52-26.78 cm and 20.6-27.68 cm longer than the control (20.96-26.48 cm) (Table 3). The plant M2-BY2-2-02 had the longest panicle among individuals measuring an average of 24.7 cm, compared to the control plants, which had an average panicle length of 23.4 cm. According to M. Huang *et al.* (2020), long panicles allow higher yield per unit area because more grain is produced. Even though the dose of gamma-ray irradiation was consistent, the observed growth and yield of each plant varied. Each plant is affected uniquely by its environmental adaptability as it grows. The genetic potential of each plant is different. T. Begna (2021), added that different genetic potentials cause growth diversity and individual yields to be different.

Longer panicles tend to increase the number of seeds per panicle, indicating a positive correlation between panicle length and seed yield (Mai *et al.,* 2021). The Boyolali 200 Gy cultivar, specifically plant code M2-BY2-2-09, had the greatest seeds number per panicle, reaching 166.2 seeds (Table 4), although it was still lower than the control. The number of productive tillers also affects the number of seeds per panicle. The greater the productivity of tillers, the higher the number of panicles per unit area, leading to the formation of grains in the panicles (Asis *et al.,* 2021) also added that the grain yield was high in long panicles if accompanied by many secondary panicle branches. The weight of 100 grains determines the yield potential of a rice cultivar. The weight of 100 grain in the Boyolali cultivar was highest at gamma-ray irradiation of 200 Gy, which was 3.2 g, while in the Bantul cultivar at a dose of 100 Gy, the highest weight of 100 grain reached 3.35 g (Table 4). According to M. Syafi'ie and D. Damanhuri (2018), the weight of grain can be influenced by the shape and size of the grain so that the weight value of each plant will be different. The process of panicle formation and environmental conditions related to nutrient uptake significantly affect grain weight. D. Novitasari *et al.* (2021) also added that the value of seed mass is determined by the dry matter content of the assimilate used in filling rice seeds.

Exposing Boyolali cultivar black rice to gamma-ray irradiation at a dose of 100 Gy resulted in an increase in proline content, reaching an average of 19.78. In Bantul cultivar, the highest was in gamma-ray irradiation of 200 Gy with an average proline content of 13.70 (Table 5). A. Aly *et al.* food sterilization and medicinal healing. In the present study irradiation techniques were applied to investigate the effect of gamma irradiation on germination and physiological aspects of pigeon pea seedlings. Pigeon pea (Var. BSMR 736(2018) also reported that exposure to gamma radiation alone, significantly increased proline accumulation. Gamma-rays, a form of ionizing radiation, create free radicals within cells, which impact the structure and metabolic processes of plant cells. This includes expanding thylakoid membranes, influencing photosynthesis, regulating antioxidant systems, and increasing the accumulation of phenolic compounds (Hong *et al.,* 2022). Gamma irradiation causes an increase in the proline content of black rice under drought stress. The highest increase in proline content in Boyolali and Bantul cultivars at an irradiation dose of 100 Gy was 10.5% and 7.85%, respectively (Table 6). An increase in significant proline also occurred in seedlings *Arabidopsis* irradiated at a dose of 50 Gy under heat stress conditions, in contrast to those without irradiation (Wang *et al.,* 2022).

This study investigated the effects of gamma-ray irradiation on various traits of black rice cultivars from Boyolali and Bantul. Results indicate significant differences in plant height, number of productive tillers, panicle length, seed yield, and proline content in response to different doses of irradiation, with implications for genetic diversity and plant productivity. These findings underscore the complex interplay between gamma-ray exposure, genetic variability, and physiological responses in black rice, contributing to a nuanced understanding of its potential for crop improvement and stress tolerance.

CONCLUSIONS

Gamma-ray irradiation increased the average number of productive tillers, plant height, panicle length, number of seeds per panicle, the weight of 100 M2 black rice grains of both Boyolali and Bantul cultivars. It showed that the genetic diversity of M2 black rice exposed to gamma rays is broader compared to those not exposed. Gamma-ray irradiation at 100 Gy resulted in a greater number of productive tillers in both cultivars. The M2 mutant black rice Boyolali cultivar showed longer panicle length and higher number of seeds per panicle at irradiation doses of 100 Gy and 200 Gy. The weight of 100 grains was highest in Boyolali at 200 Gy and in Bantul at 100 Gy. The highest proline content on black rice was planted in paddy fields, namely Boyolali cultivars produced by gamma-ray irradiation of 100 Gy and Bantul cultivars of 200 Gy during drought stress, the proline content in Boyolali and Bantul black rice cultivars increased following irradiation with 100 Gy gamma rays. A reduced dose of gamma-ray irradiation can enhance the growth, yield, and proline content of M2 black rice.

These findings highlight the potential of gamma-ray irradiation to induce genetic variability and enhance traits associated with crop productivity and stress tolerance. Additionally, the study underscores the importance of understanding the complex interactions between radiation, genetic diversity, and physiological responses in plants. Further research in this area could explore the specific genetic changes responsible for these observed effects, contribute to the development of novel breeding strategies for improving crop resilience and yield in the face of environmental challenges.

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CONFLICT OF INTEREST

None.

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Ростові показники, врожайність та вміст проліну двох місцевих сортів чорного рису, опромінених гамма-променями для підвищення посухостійкості

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Анотація. Одним із критичних наслідків зміни клімату для сільськогосподарського середовища є стрес від посухи. Метою дослідження було визначити ріст, урожайність та вміст проліну у чорного рису місцевого сорту Бантул, мутанта Бойолалі М2 за різних доз гамма-опромінення та вміст проліну у мутанта М3 під час посушливого стресу. В експерименті використовувався повністю рандомізований дизайн (CRD) з факторною дозою гамма-опромінення (0 Гр, 100 Гр, 200 Гр і 300 Гр), що застосовувався як на рисових полях, так і в поліетиленових мішках для спостереження і порівняння росту, врожайності та вмісту проліну в рослинах чорного рису з контрольною обробкою (0 Гр). Результати лабораторних досліджень показали, що сорти Бойолалі і Бантул, опромінені в дозах 100 Гр і 200 Гр, мали найвищий вміст проліну, який збільшився в умовах стресового впливу посухи в обох сортах. При дозі 100 Гр середня висота рослин і кількість продуктивних стебел чорного рису сортів Бойолалі і Бантул були найвищими, а різноманітність двох місцевих сортів при цій дозі була вищою, ніж при інших дозах опромінення. Застосування доз гамма-опромінення 100 Гр і 200 Гр на сорті Бойолалі суттєво вплинуло на характер довжини волоті та кількість насінин у волоті. Для сорту Бантул довжина волоті та кількість насінин у волоті при дозі опромінення 200 Гр суттєво відрізнялися. Зроблено висновок, що зменшена кількість гамма-опромінення може покращити ріст, врожайність та вміст проліну в чорному рисі сорту М2

Ключові слова: функціональні продукти харчування; рослина рис; водний стрес; сільське господарство; зміна клімату

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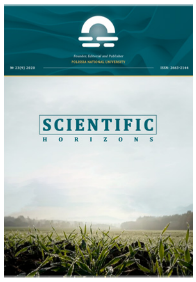

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Assessment of the impact of COP decisions on biodiversity and ecosystems

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Abstract. The relevance of the study is conditioned by the fact that any anthropogenic load or economic activity has an impact on the functioning of natural ecosystems, as demonstrated by the widespread practice of oil palm expansion. The purpose of this study was to develop methods for assessing strategies that play an important role in strengthening measures to protect biodiversity and ecosystems in Africa and Latin America, considering the impact of the decisions of the United Nations Climate Change Conference. The study considers the materials of various international organisations on the problems of biodiversity related to climate crises, inefficient territorial planning,

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expansion of land for growing crops that deplete the soil or reduce the area of land needed to accommodate biota. The paper presents a sequence of steps to assess the impact of the decisions of the United Nations Climate Change Conference on biodiversity and ecosystems. The aspects of conservation of biodiversity and ecosystems in the context of the expansion of palm plantations are substantiated. The indicators of the ratio between the available land area for palm oil cultivation and the area threatening to reduce the biodiversity of Africa are analysed. Environmental solutions are substantiated, which correspond to the provisions of the United Nations Climate Change Conference in the context of biodiversity and ecosystem conservation, taking into account the expansion of large areas for palm plantations in Colombia. The practical significance of this study lies in the development of a methodology for the economic and mathematical assessment of the decisions of the United Nations Climate Change Conference, which affect the conservation of biodiversity and ecosystems, and are the driving mechanism in creating an effective control system for tracking oil palm plantations, territorial development with the least environmental losses and high economic efficiency

Keywords: United Nations; climate transformations; territorial planning; environmental management; resource potential; oil palm plantation

INTRODUCTION

The United Nations Climate Change Conference 29 (COP) will be held from November 11 to 22, 2024 in Azerbaijan. This is an extremely important event to address various aspects of climate change, biodiversity conservation and protection, and to decide on the next steps in combating global environmental challenges. Recent meetings at the UN Climate Change Conference, in particular COP 27 and COP 28, were devoted to the issues of changing the temperature regime, which has a serious impact on the global ecosystem, including the structure of forests, oceans, mixed and polar ecosystem landscapes (United Nations Development Programme, 2023). For example, rising temperatures and changes in precipitation can lead to landslides, flooding, and other disasters that negatively affect biodiversity.

Assessing the impact of COP decisions provides insight into how effective the measures taken have been in conserving biodiversity and ecosystems. This is important for assessing the effectiveness of policies and identifying possible ways to improve, identifying possible conflicts between different initiatives and priorities, and understanding which solutions require greater coordination and cooperation between countries (United Nations Development Programme, 2023).

In recent years, the world community for the protection of natural resources has been paying more and more attention to the problem of palm plantations expanding significant areas of land. First of all, this situation is caused by the fact that the demand for palm oil is constantly growing due to its widespread use in the food and light industries, and in the production of alternative fuels. The expansion of palm plantations leads to the destruction of natural ecosystems and loss of biodiversity. The destruction of forests and other natural environments leads to the extinction of various plant and animal species, including those that are unique or already endangered (Kross *et al*., 2022).

The forests capable of absorbing carbon dioxide from the atmosphere are destroyed. This leads to the release of large amounts of CO_2, which contributes to climate change. In addition, reducing the number of trees in forests can lead to the loss of other ecosystem services, such as water conservation and maintaining high levels of soil fertility. In particular, the cultivation of palm plantations is associated with the use of pesticides and other chemicals, which has a detrimental effect on the health of the local population (de Mendonça *et al*., 2022).

The impact of the expansion of palm plantations on agriculture in Latin America was investigated by A. Castellanos-Navarrete *et al.* (2021). The results of the study show that industrial plantations entail risks of disturbance and degradation of local landscapes, especially on forest borders. This leads to loss of biodiversity, conflicts between small-scale palm plantation owners and the local population, which depends on the level of ecosystem services of forests.

The features of the transformation of natural savannahs into a plantation of oil palms in the Llanos of Colombia and their impact on the diversity of birds are analysed by L. López-Ricaurte *et al.* (2020). Significant changes in biodiversity and the number of bird species were detected as a result of this expansion. The researchers give suggestions on the formation of a detailed monitoring system for the conservation of natural savannahs.

The main technical and technological parameters of palm oil certification, considering the standards of the Roundtable on Sustainable Palm Oil (RSPO) (n.d.) and Malaysian Sustainable Palm Oil, are described in the paper by M.A. Hassan *et al.* (2024). Technological progress in this area corresponds to the zero emissions system and contributes to cyclical bioeconomics and reduction of negative impact on the environment. The expansion of oil palm plantations in Amazonian forests leads to a significant reduction in the area of perennial forests and degradation of unique natural landscapes, as noted by K.C.L. da Silva *et al.* (2023). There are almost irreversible changes in land use with accelerated loss of forests and deterioration of the ecological situation. The management of this sector requires strategies aimed at the conservation or restoration of biodiversity landscapes.

Despite a number of studies, the problems of individual bioregions that are most affected by the large-scale expansion of oil palms remain rather neglected. Another important issue is the characterisation of specific biota species, especially vulnerable to this process. The problems of restoring degraded soils after deforestation are also worthy of attention. As a result, methods for assessing the decisions of the UN Conference on Climate Change affecting the conservation of biodiversity and ecosystems as a result of the expansion of palm plantations should be improved (Tashiro *et al.*, 2019).

The aim of this study is to analyse the effectiveness of various measures taken by COP to conserve biodiversity in unique natural ecosystems using the example of the impact of palm plantation expansion in Africa and Latin America, followed by the development of recommendations for economic and mathematical assessment of strategies to enhance the protection of flora, fauna. The main problems that need to be addressed are based on the use of economic instruments that are necessary within the framework of COP for the protection of flora and fauna, but at the same time may be ineffective in the context of the expansion of palm plantations in Africa and Latin America. The use of mathematical models in the process of conducting economic assessments can improve the effectiveness of strategies for protecting biodiversity in regions affected by palm plantations.

It is also necessary to adapt existing COP strategies for the protection of flora and fauna to the specific conditions of a particular region, considering the features of the expansion of palm plantations. And increased investments in innovative technologies and methods for the protection of biodiversity can lead to a reduction in the loss of natural ecosystems in different regions of the world, which are subject to the expansion of palm plantations.

MATERIALS AND METHODS

The research information base has compiled data from the report by United Nations Development Programme (2023), report and the COP conference's work streams on climate change, biodiversity conservation and protection, and decision-making on the way forward in addressing global environmental challenges. Data from the Intergovernmental Panel on Climate Change (2022) synthesis report were used, related to climate crises, inefficient spatial planning, expansion of land for crops and tree stands that deplete the soil or reduce the amount of land needed to accommodate biota. For a more detailed justification of COP's decisions in the field of conservation and protection of biodiversity within unique ecosystems, the provisions of the International Union for Conservation of Nature (2023), National Adaptation Plans (2023) and long-term low-emission development strategies (United Nations, 2023) were considered. The data of the Joint Research Centre (2023) and the French Agricultural Research Centre for International Development (2023) regarding the main indicators of the potentially serious impact of the expansion of the palm oil industry in Africa were analysed. The results of reports by RSPO (n.d.) on market transformation in Latin America, the Dutch non-governmental organisation AidEnvironment, National Oil Palm Federation (2020), the non-governmental organisation Solidaridad (2022) and Rainforest Alliance (2022) were studied.

The scientific research was based on economic (the law of self-interest, the law of supply and demand), environmental laws (laws of biodiversity, climate change, toxic and dangerous substances, land and water), mathematical patterns of their concepts, categories and modern geographical theories. The paper summarises the decisions taken within the framework of COP 28 on biodiversity conservation, considering the position of the International Union for Conservation of Nature in achieving the goals of the Paris Agreement in the context of the areas of protection, restoration and sustainable management of global ecosystems. Environmental solutions are considered, which are evaluated based on the indicators of economic effect with the conservation of biodiversity and the implementation of sustainable development goals.

A systematic analysis has been applied to substantiate the main aspects of biodiversity and ecosystem conservation in the context of palm plantation expansion, which are influenced by COP decisions. The relationship between the available land area for palm oil cultivation and the area threatening the reduction of primate biodiversity in Africa is characterised. The influence of the expansion of oil palm plantations on the differentiation of biodiversity and the state of natural ecosystems within the territory of Africa and Latin America is compared. In particular, environmental solutions regulated by the COP in the context of the conservation of biodiversity and ecosystems, considering the expansion of large areas of land for palm plantations in Colombia, are indicated. The study provides a rationale for mathematical and economic methods that are used to assess decisions and strategies related to the conservation of biodiversity and ecosystems in the context of the expansion of oil palm plantations.

RESULTS

The decisions taken within the framework of COP 28 on biodiversity conservation, taking into account the position of the International Union for Conservation of Nature, provided a critical opportunity to assess collective progress towards achieving the goals of the Paris Agreement (López-Ricaurte *et al.*, 2020). During the discussions, proposals were made to eliminate the existing problems of conservation and protection of biota, considering the specific features of natural ecosystems. The provision of the International Union for Conservation of Nature during COP 28 regulates the clear definition, support, and substantiation of the areas of restoration and sustainable management of global ecosystems (United Nations Development Programme, 2023). It is noted that any conservation decisions are evaluated against indicators of economic benefits with biodiversity conservation and the realisation of sustainable development goals.

The representative office of the International Union for Conservation of Nature emphasises the importance of ensuring greater operational synergy in all key international policy processes governing the functioning of terrestrial, freshwater, coastal and marine ecosystems of the world. In particular, it is important to establish and strengthen appropriate functional links between key environmental management tools and their policy mechanisms. In particular, this refers to the Order of the President of the Republic of Azerbaijan "On Approval of the 'National Strategy of the Republic of Azerbaijan on Conservation and Sustainable Use of Biodiversity for 2017-2020" (2016), Convention on Biological Diversity (1993) and Paris Agreement (2015).

In particular, the provisions of the International Union for Conservation of Nature contain regulatory issues for the conservation of biodiversity and natural ecosystems and regulate their inclusion in National Adaptation Plans (2023) and long-term low-emission development strategies (United Nations, 2023). One of the important decisions taken at COP 27, which will further affect the situation with biodiversity, was based on consideration of the feasibility of environmental decisions or approaches related to ecosystem changes in general, taking into account UN Assembly Resolution 5/5 on the environment. In particular, it is necessary to implement measures to mitigate the effects of reducing biological diversity and adapting to changes in the territorial climate regime, while ensuring appropriate social and environmental guarantees (Decision 1/CP.27, paragraph 52 and Decision 1/CMA.4, paragraph 81) (United Nations Development Programme, 2023).

The International Union for Conservation of Nature encourages all actors implementing environmental solutions through the market or non-market mechanisms to follow a high-integrity approach when developing, verifying, and expanding such initiatives. To do this, it is necessary to use nature-based solutions (NBS) that contribute to the conservation of biodiversity, and can also have additional benefits for society, such as improving air and water quality, improving people's physical and psychological health, reducing the risk from natural disasters, increasing resilience to climate change.

The problem of conservation and protection of biodiversity and ecosystems should be resolved through reliable accounting systems, which will contribute to the observance of appropriate social and environmental guarantees. Assessing the impact of the decisions of the UN Climate Change Conference on biodiversity and ecosystems is critically important, since actions taken to combat climate change have a significant impact on the natural environment. For example, the deployment of renewable energy sources such as solar and wind energy can have both positive and negative impacts on biodiversity and ecosystems (Fig. 1).

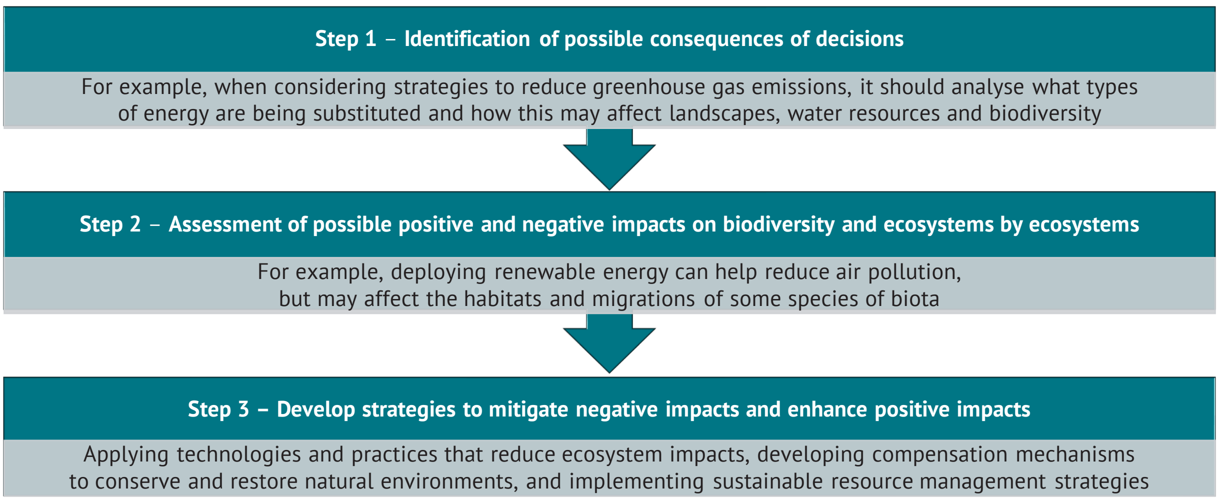

***Figure 1**. Step-by-step rational for assessing the impact of the UN Climate Change Conference decisions on biodiversity and ecosystems*
***Source:** compiled by the authors based on United Nations Development Programme (2023)*

The expansion of palm oil plantations poses a serious challenge to biodiversity, especially in the tropical regions where they are usually located. In this case, tropical forests are often used to create palm oil plantations, which leads to their deforestation and destruction of natural habitats for numerous species of plants and animals. This problem is especially acute for those animal species that are on the verge of extinction or are already in danger of extinction.

In recent years, Africa and South America have been key regions for the expansion of palm plantations due to their favourable climatic conditions and potential for palm cultivation. In the region of Southeast Asia, which used to be the main centre of palm oil production, the limits of acceptable spatial spread and land-loading have almost been reached. Therefore, many companies producing this product cooperate with Nigeria and Cameroon. Latin America is becoming increasingly attractive for the expansion of palm plantations. Colombia, Ecuador, Peru, and Brazil are countries with the potential to expand palm plantations. In particular, decisions taken during the COP and strategies to address environmental challenges also have a significant impact on biodiversity in Africa and Latin America, especially in the context of the expansion of oil palm plantations (Table 1).

***Table 1**. Substantiation of the main aspects of biodiversity and ecosystem conservation in the context of palm plantation expansion, which are influenced by COP decisions*

Aspect	Substantiation
Regulation of land use	COP's decisions include international agreements and regulations related to the use of land and forests. These decisions may affect the location and development of oil palm plantations, including limiting the conversion of forests into agricultural land.
Support for alternative energy sources	Reducing the use of petroleum products and switching to renewable energy sources may reduce the demand for palm oil as a biofuel. This can reduce the need for palm oil production and pressure on the ecosystems involved in their cultivation.
Support for local communities	Strategies to address environmental challenges may include measures to support local communities to promote their participation in natural resource management. This can help in the implementation of sustainable forest management practices and reduce the pressure on forest ecosystems.

***Source:** compiled by the authors based on Intergovernmental Panel on Climate Change (2022)*

In the context of the expansion of oil palm plantations, it is important that decisions and strategies ensure a balance between economic and social needs and the conservation of biodiversity and ecosystems. Only in this way can sustainability of development be achieved, contributing to both economic prosperity and environmental conservation. The results of studies conducted by the Joint Research Centre (2023) and the French Agricultural Research Centre for International Development (2023) confirm the potential serious impact of the expansion of the palm oil industry on African primates. Only a few small areas in Africa are considered highly suitable for oil palm cultivation and have a low potential impact on the species of primates living there (Fig. 2).

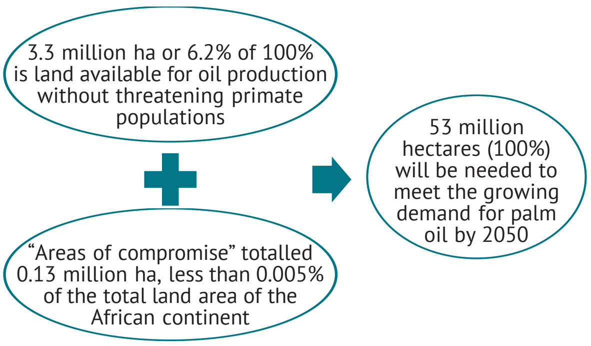

***Figure 2**. The ratio between the available land area for palm oil cultivation and the area threatening to reduce primate biodiversity in Africa*
***Source:** compiled by the authors based on R. Sourokou et al. (2023)*

Figure 2 shows a diagram of the proportions between the "compromise zones" suitable for palm oil cultivation and the general demand for such land until 2050. It also demonstrates the limited availability of areas for palm oil cultivation without harming the primate population. In light of the catastrophic impact of industrial oil palm plantations on wildlife in Southeast Asia, a large-scale assessment of the expected effects of oil palm expansion on the diversity of African primates should be carried out, with particular emphasis on challenges that may arise in the future (Convention on Biological Diversity (1993). Economic assessment is quite difficult due to the heterogeneity of the economic value of biodiversity and difficulties in determining economic parameters. However, several approaches can be used to assess the expected effects of oil palm expansion on the diversity of African primates.

The development of ecotourism areas in the overall biodiversity conservation strategy contributes to increasing public awareness and support for basic nature conservation measures. Ecotourism provides an opportunity for monitoring and research on biodiversity, which can help identify vulnerabilities and ineffective conservation strategies. In the case of one region of Africa, if primates attract tourists, their loss may lead to a decrease in income from ecotourism. The equation is recommended to be formulated as follows (1):

$$E = N \times V, \quad (1)$$

where E – total economic impact, N – number of tourists who want to observe primates, V – average cost of the tour.

If the loss of primates leads to a loss of genetic diversity, which may affect the biopharmaceutical industry and other industries. The equation is recommended to be formulated as follows (2):

$$G = Q \times P, \quad (2)$$

where G – cost of loss of genetic resources, Q – number of useful genetic properties, P – average cost of a genetic resource.

Changes in primate diversity can affect ecosystem services such as plant pollination or seed distribution. The equation is recommended to be formulated as follows (3):

$$F = \sum_{i-1}^{n} (Ai \times Vi), \quad (3)$$

where F – total cost of ecosystem functioning change, Ai – number of ecosystem services changed, Vi – cost of each service. Equations can be modified depending on the available data.

Thus, decisions regarding the problems of conservation and protection of biodiversity in the context of the uniqueness of ecosystems affect the solution of issues of palm oil plantation expansion and primate vulnerability by developing environmental criteria for the transformation of land. This approach can be an effective key to minimise the species-specific impact on the expansion of oil palm plantations. In particular, such environmental criteria may include:

1. Prohibition of the placement of plantations in particularly vulnerable areas – legislative restriction on the placement of oil palm plantations in areas where vulnerable primate species live or where their biodiversity is especially significant.

2. Creation of natural corridors – preservation and creation of natural corridors that provide a link between the tracts where primates live, to facilitate migration and the spread of populations.

3. Primary forest conservation is the prevention of deforestation of primary forests and the preservation of these environments as irreplaceable habitats for many primate species.

4. Setting boundaries for plantation expansion – definition of boundaries for the expansion of oil palm plantations to avoid habitat loss and destruction of ecologically important territories for primates.

These conservation criteria can be effective tools to ensure the conservation of primates and other species in the wild in the face of increasing pressure on land resources to expand oil palm plantations. Although the palm oil-producing countries in Latin America do not lead the global palm oil market, they successfully maintain relatively stable indicators of consistency. This situation is developing because this region has the highest percentage of certified palm oil in the world, which is about 35% according to RSPO (n.d.) experts in Latin America. It is important to note that in Latin America, palm oil plantations are mostly located in forested areas that are unsuitable for other crops, and tree stands and are degraded or unproductive. This afforestation concept is in line with COP directions in the context of preserving forest ecosystems and implementing effective conservation measures.

Despite the high level of environmentally safe palm oil production and, in general, effective territorial planning of plantations of this type of stand, nevertheless, experts of the National Oil Palm Federation (2020) note the threat of deforestation for the cultivation of oil palm. Thus, in the period from 2021 and 2022, 23,311 hectares of forest were burned around these plantations to make room for their further expansion. Currently, the territory of Colombia is involved in solving issues of maintaining the stability of palm oil production. National Oil Palm Federation (2020), the NGO Solidaridad (2022) and Rainforest Alliance (2022), and the initiators of the review, correction and improvement of methodologies of the International Union for Conservation of Nature and the Dutch NGO AidEnvironment.

The RSPO uses various procedures, including the Remediation and Compensation Procedure (RaCP), to prevent deforestation and ensure regeneration. RaCP covers companies that control lands of high conservation value) and require restoration or compensation for lost value. This may include direct restoration of sites or support for conservation projects funded over 25 years. This mechanism is considered as a form of "green washout", emphasising the need to improve the quality of medicinal products through cooperation with other product certification standards. Environmental decisions regulated by the COP in the context of biodiversity and ecosystem conservation, considering the expansion of large areas of land for palm plantations in Colombia, may include the following aspects:

1. Zoning strategies – identify and protect areas of high biodiversity, such as nature reserves, national parks, and other unique landscapes. It is also important to consider the potential of migration corridors to ensure the conservation of species diversity.

2. Use of sustainable forest management – continuation and development of sustainable forest management programmes that contribute to the conservation of natural forests and their ecosystems instead of cutting them down in favour of palm plantations.

3. Certification and standardisation – the application of certification standards such as the RSPO, which establish requirements for sustainable palm oil production, including the conservation of forests and biodiversity.

4. Ecosystem restoration – support programmes for the restoration and rehabilitation of ecosystems damaged as a result of the exploitation of palm plantations to restore biodiversity and environmental sustainability.

5. Risk management – identification and reduction of potential negative impacts of palm plantation expansion on biodiversity and ecosystems.

Assessment of COP decisions affecting the conservation of biodiversity and ecosystems as a result of the expansion of palm plantations can be carried out using mathematical and economic methods. Modelling of biodiversity loss occurs through the use of mathematical models to assess the impact of palm plantation expansion on the diversity of species and ecosystems in a particular area. Models can include factors such as changes in land use, loss of natural habitats of species.

The assessment of economic losses is determined due to the economic value of the loss of biodiversity and ecosystem due to the expansion of palm plantations. This may include the loss of environmental services such as air and water purification, soil fertility maintenance. The analysis of the cost of losses and income from palm oil production is carried out by comparing the economic cost of losses of biodiversity and ecosystems with the profit from palm oil cultivation. This will help to understand the economic acceptability of COP decisions related to palm plantations. The development of mathematical models or optimisation algorithms to find strategies takes place to minimise the loss of biodiversity and the ecosystem, ensuring economic sustainability (Fig. 3).

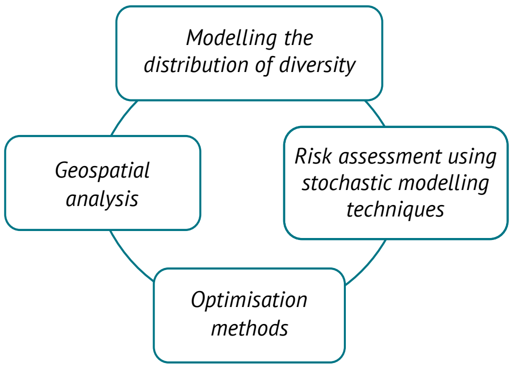

***Figure 3**. Mathematical methods used to assess decisions and strategies related to the conservation of biodiversity and ecosystems in the context of the expansion of oil palm plantations*
***Source:** compiled by the authors*

Considering the information from Figure 3, it is possible to create mathematical models that include factors affecting the distribution of diversity, such as loss of the natural environment and fragmentation of local ecosystems. Stochastic modelling methods can be used to assess the risks associated with the expansion of oil palm plantations for biodiversity. This allows considering the uncertainty and randomness of the impact of these actions on ecosystems. Mathematical optimisation methods can be used to find optimal strategies for resource management and land use for biodiversity conservation. This may include maximising biodiversity conservation while limiting losses from the expansion of oil palm plantations.

Mathematical methods of geospatial analysis can be used to identify areas with high levels of biodiversity that are most at risk due to the expansion of oil palms. This method simplifies the adoption of important environmental management decisions to preserve ecosystems. These mathematical methods can help in assessing the impact of decisions and strategies adopted at the Conferences of the Parties (COP) on biodiversity in Africa and Latin America, in particular in the context of the expansion of oil palm plantations. The economic assessment of COP decisions affecting the conservation of biodiversity and ecosystems as a result of the expansion of palm plantations can be made up of several key elements.

The cost of loss of biodiversity and ecosystem includes losses of ecosystem services, economic losses, threats to human health and well-being, and a decrease in the potential of environmental management, which cannot be estimated in monetary terms. Therefore, the protection and sustainable use of natural resources, including the management of palm plantations, should include careful sustainability and measures to minimise negative impacts on biodiversity and ecosystems (4):

$$C = \sum_{i-1}^{n}(Pi \times Qi), \tag{4}$$

where Pi – economic value of the lost biodiversity or ecosystem for a species or ecosystem, Qi – amount of lost biodiversity or ecosystem.

One of the main reasons for growing an oil palm is the high productivity of this plant in relation to the oil it produces. Palm oil is widely used in various industries, including the food industry, cosmetics industry, household chemicals and fuel industry (5):

$$B = R - C, \tag{5}$$

where R – total profit from the sale of palm oil.

The cost of alternatives (A) may include the loss of other ecosystem services (water and climate management, biodiversity maintenance) that can be provided by natural landscapes occupied by palm plantations (6):

$$A = \sum_{j-1}^{m}(Cj), \tag{6}$$

where Cj – cost of loss of biodiversity or ecosystem for alternative j.

The cost estimate of conservation measures (E) includes the costs of establishing and maintaining nature reserves, national parks and other areas where economic activities are prohibited or restricted, including palm plantations. In addition, the costs of monitoring the implementation of legislation in the field of environmental protection and compliance with sustainable management standards should be considered (7):

$$E = \sum_{k-1}^{l}(Ck), \tag{7}$$

where Ck c– cost of conservation measures for a species or ecosystem k.

The Economic Sustainability Index (ESI) allows a balanced assessment of the economic aspects of the expansion of palm plantations and their impact on the sustainable development of the region. This indicator helps to identify potential risks and problems, and opportunities to improve business practices to achieve more sustainable results (8):

$$ESI = \frac{B+E}{B-E} \times 100\%, \tag{8}$$

where B – economic benefits of palm plantations, E – cost of conservation measures.

The use of equations 1-8 helps to identify new opportunities for the creation of innovative economic instruments, models, development strategies and financing mechanisms that contribute to the conservation of biodiversity and ecosystems. However, indicators of the potential for the development of ecosystem services markets will increase. There will also be the creation of compensation funds and programmes to support sustainable land management.

In general, the economic assessment of COP decisions on biodiversity and ecosystem conservation in the context of palm plantation expansion plays a key role in making informed decisions that contribute to achieving sustainable development. It provides a framework for the development of effective strategies and activities that consider both economic and environmental aspects and contribute to the achievement of global goals to combat climate change and preserve biodiversity.

DISCUSSION

The expansion of palm oil plantations has a serious impact on biodiversity, as it leads to the loss of forests, the destruction of habitats for numerous species of flora and fauna, and a decrease in their diversity. Such dynamics are becoming a threat to unique natural ecosystems, which can have negative consequences for the entire planetary environment and human well-being. This study helps to understand the complex relationships between the expansion of palm oil plantations and changes in natural ecosystems. This helps to understand the scale of the problem, determine the main factors causing the destruction of biodiversity, and identify vulnerable areas. The results obtained help to identify key ecosystems and species that require special attention and protection, as demonstrated by the example of Africa and Latin America. These substantiations allow developing a strategy for the conservation and restoration of natural resources and increasing the resilience of ecosystems to the effects of anthropogenic activities. It is important for the public to understand the problem associated with the expansion of palm oil plantations and their impact on biodiversity and natural ecosystems. Overall, this study is a critical step towards the sustainable use of natural resources and ensuring the ecological stability of the planet.

During the COP, various approaches to reducing the impact of the expansion of oil palm plantations on biodiversity are discussed. The decisions taken at the COP are important for the development and implementation of policies, laws and strategies aimed at protecting biodiversity and countering forest loss due to the expansion of oil palm plantations. The same conclusion in their research was reached by Z. Jia *et al.* (2024) and E. Meijaard *et al.* (2023). This is important because biodiversity is a key component of sustainable development and ensuring the ecological sustainability of the planet, according to M. Cordella *et al.* (2022). Conservation of biodiversity is essential for ensuring food security, supporting ecosystem services and adapting to climate change.

The assessment of the impact of the expansion of oil palm plantations in Thailand on biodiversity has a significant impact on the conservation of the diversity of bird and insect species in five regions of the country, which is substantiated in the paper by U. Jaroenkietkajorn *et al.* (2021). Scientific research contains data on the availability of biota species, and on available land use areas to determine the relationship between these two elements of the ecosystem. Special attention is focused on the deforestation of oil palm forests, which is considered the greatest threat to wildlife according to N. Boke Olén *et al.* (2021). The results of the study show that the possibility of survival of animals in former mangrove forests is higher compared to other types of forests after occupation. It was also found that deforestation in the northern region leads to fewer species losses in the area. It is impossible to disagree with the fact that the impact of expansion of palm oil plantation areas on faunal species diversity depends on the particular taxon and the combination of region and forest type. This study provides a substantiation for the case in Africa, where there is a problem of primate conservation, caused precisely by the need to expand the area of land for the cultivation of oil palms. At the same time, the characteristics of a particular region and type of forest are considered in order to preserve biodiversity.

The European Commission expresses strong arguments on palm oil production's potential negative impacts on land use, in particular, due to changes in the scale of production that could lead to deforestation of tropical forests according to B. Azhar *et al.* (2021) and M. Romero *et al.* (2019). Representatives of the main producing countries, such as Indonesia and Malaysia, argue that the measures proposed by the EU may create excessive barriers to environmental initiatives in the industry and violate the principles of free trade. These issues were considered by Y. Xin *et al.* (2022). However, the solution to this problem, which correlates with COP initiatives, is to reform land use policies for the main producing countries due to the mandatory division of land with farmers who have lost land for some reason or are forced to change their location. These mechanisms will help reduce deforestation and encourage the use of existing palm plantations for agricultural purposes (Naime *et al*., 2020; Bai *et al*., 2023), and the strategy is consistent with the requirements of palm oil certification for social and environmental sustainability (Vincenza *et al*., 2024).

Mitigation of the risk of negative land use change in palm oil production is a high priority. Indeed, indicators of qualitative and quantitative parameters of land resources also affect effective territorial planning, which, in turn, depends on the uniqueness of natural ecosystems and the location of diverse biota (Liu *et al*., 2022). Thus, R. Sourokou *et al*. (2023) examined the relationship between the available land area for palm oil cultivation and the area threatening to reduce primate biodiversity in Africa. It has been possible to establish that there is limited availability of areas in Africa for palm oil cultivation without affecting the primate population.

C. Basnou *et al*. (2020) investigated the features of the development of a spatial distribution plan that considers the expansion of oil palm plantations and the impact of this expansion on biodiversity. A detailed study of the situation in Colombia revealed that there are territories where the expansion of the oil palm is fraught with a variety of vertebrates that are under threat of extermination. Especially low biodiversity indicators and risks are observed in the territories of Serrania de la Macarena, the transition between the Andes and the Amazon, Darien and the Tumaco forests. So these territories should be avoided when planning the expansion of oil palm plantations. That is why the application of an approach to effective territorial development may be acceptable for other countries facing the expansion of oil palms to ensure a balance between production and conservation of biodiversity.

The situation in Colombia regarding the placement of palm oil plantations and their expansion has also been investigated in this study. Indeed, the issue of effective territorial planning is extremely important in solving problems of conservation and protection of biodiversity. Thus, within the framework of the COP for the conservation of biodiversity and ecosystems in Colombia, possible conservation measures include zoning strategies. This means the identification and protection of areas with high biodiversity, such as protected areas and national parks, as indicated by K. Xu *et al*. (2020) and J. Yuan *et al*. (2023). In addition, according to G. Guzmán *et al*. (2022), it is important to consider possible migration routes to ensure the conservation of species diversity.

The main areas of conservation of important territories to improve communication in support of biodiversity conservation, adaptation to climate change and the rights of indigenous peoples, which is a key area of environmental policy, which, in turn, is regulated by the Convention On Biological Diversity (1993). These issues were analysed by S. Yeasmin *et al*. (2021). The researchers considered the main provisions of the Kunming-Montreal Global Biodiversity Framework, recently adopted and aimed at the need to preserve up to 30% of terrestrial and marine territories by 2030, using interconnected and fairly managed conservation and conservation systems. Through various methods, including a literature review and consultations with stakeholders, six favourable conditions have been identified for the successful implementation of the programme (Serrano-Ramírez *et al*., 2021). These conditions include the interaction and involvement of indigenous peoples, the establishment of clear commitments, stable financing and compliance with international legal norms, and the development of appropriate large-scale criteria for the development of monitoring reports (Cao *et al*., 2020).

The decisions made at COP on the Convention on Biological Diversity (1993) are important to the global community for several reasons. First of all, cooperation between countries is being stimulated to jointly solve biodiversity problems. The decisions taken at the COP create international obligations that force countries to work together to preserve the natural ecosystems of the entire planet. Participating countries are obliged to implement these solutions in their national legislation, which contributes to solving biodiversity problems at the local level. The COP establishes monitoring and reporting mechanisms to monitor progress towards achieving set goals and respond to changes in biodiversity in a timely manner.

The above aspects are measures for the occurrence of environmental disasters, as in Malaysia or Indonesia. It is important to consider experience and take control and regulatory measures to avoid repeating mistakes in these regions. Only through a coordinated approach and by all stakeholders can the sustainable development and conservation of natural resources in Africa and Latin America be ensured during the massive transformation of forests into oil palm plantations.

CONCLUSIONS

Thus, the expansion of palm plantations leads to a significant decrease in biodiversity, as these large-scale monocultures mainly displace natural ecosystems such as tropical forests. That is, there is a loss of the natural environment to accommodate the numerous species of flora and fauna living in these ecosystems. This pattern leads to a decrease in the level of populations of certain species or increases the level of their extinction. Notably, the ecological balance in the relevant region is disrupted as a result of the increase in the area of palm plantations, as has already begun to happen in South America or Africa, due to the destruction of natural ecosystems that perform important functions such as maintaining a high level of soil fertility, the qualitative properties of aquifers and combating countless degradation actions.

The results of this study confirm once again that the issue of conservation of biodiversity and ecosystems should be addressed and correlated with environmental policy and sustainable climate goals of the COP. That is why these solutions should stimulate cooperation between diverse management and executive sectors, and improve public-private partnerships in order to implement comprehensive measures to protect biodiversity. This cooperation will facilitate the development and implementation of regional strategies and national development programmes for the conservation of biodiversity, since the effectiveness of measures may vary significantly depending on the socio-economic, political, and environmental characteristics of the region.

In the context of further promising areas of research in the field of protection and conservation of biodiversity and ecosystems, in the context of the impact of COP decisions, it is possible to highlight the strengthening of cooperation between countries and international organisations to exchange experience, technologies, and investment projects. It is important to develop and implement alternative strategies for preserving the uniqueness of natural ecosystems in the context of sustainable forestry, the latest agroecological approaches to agriculture. It is necessary to involve the public in the decision-making process at the COP and ensure broad support for the conservation of biodiversity and ecosystems. This may include conducting public consultations, educational campaigns, and involving local initiatives in conservation work.

It should be noted that Africa and Latin America are extremely promising territories in agriculture, economic growth and the development of the energy industry. However, one of the main problems in the study of the expansion of oil palm trees in significant areas of land in the above regions is the insufficient amount of data on environmental indicators of land use, nature management, and differentiation of flora and fauna. An equally defining limitation in studying the problem of biodiversity, which is associated with the expansion of palm oil production, was the development of methods for economic and mathematical assessment of this impact, as there is no single acceptable standard for these indicators in international practice.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Оцінка впливу рішень COP на біорізноманіття та екосистеми

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Анотація. Актуальність теми обґрунтована тим, що будь-яке антропогенне навантаження, господарська діяльність впливають на функціонування природних екосистем, що демонструє поширена практика експансії олійних пальм. Метою даного дослідження було формування методів оцінки стратегій, що відіграють важливу роль у посиленні заходів з охорони біорізноманіття та екосистеми на території Африки, Латинської Америки з урахуванням впливу рішень Конференції Організації Об'єднаних Націй зі зміни клімату. У статті проаналізовано матеріали різних міжнародних організацій щодо проблем біорізноманіття, пов'язаних із кліматичними кризами, неефективним територіальним плануванням, експансією угідь для вирощування сільськогосподарських культур, що виснажують ґрунти або зменшують площі земель, необхідних для розміщення біоти. У роботі представлено послідовність кроків щодо проведення оцінки впливу рішень Конференції Організації Об'єднаних Націй зі зміни клімату на біорізноманіття та екосистеми. Обґрунтовано аспекти збереження біорізноманіття та екосистем у розрізі експансії пальмових плантацій. Проаналізовано показники співвідношення між доступною площею земельних угідь для вирощування пальмової олії та площею, що загрожує зменшенню біорізноманіття Африки. Обґрунтовано природоохоронні рішення, які співвідносяться з положеннями Конференції Організації Об'єднаних Націй зі зміни клімату в контексті збереження біорізноманіття та екосистем з урахуванням експансії великих площ для пальмових плантацій на території Колумбії. Практичне значення даного дослідження полягає у формуванні методики економіко-математичної оцінки рішень Конференції Організації Об'єднаних Націй зі зміни клімату, що впливають на збереження біорізноманіття, екосистеми та є рушійним механізмом у створенні дієвої системи контролю щодо відстеження плантацій олійних пальм, територіального розвитку з найменшими екологічними втратами і високими показниками економічної ефективності

Ключові слова: Організація Об'єднаних Націй; кліматичні трансформації; територіальне планування; природокористування; ресурсний потенціал; плантації олійної пальми

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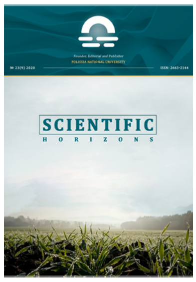

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The economic impact of agroforestry practice in production forest areas, Central Java province, Indonesia

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Abstract. The concept of Joint Community Forest Management in Indonesia is an effort to involve communities in forest management through agroforestry to support forest sustainability. Agroforestry (or persilan in local language) carried out by pesanggem in the KPH Kebonharjo and KPH Mantingan Working Areas significantly contributes to the pesanggem household income. This research wants to measure the provision of agroforestry on pesanggem household income and examine agroforestry factors that influence pesanggem household income. Data collection techniques through surveys and observations of 216 pesanggem households were carried out in the KPH Kebonharjo and

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KPH Mantingan areas, divided into eight villages respectively. The data was then analyzed descriptively and by multiple linear regression analysis. The research results found that the agroforestry pattern through land plots refers to agricultural activities by pesanggem in forest areas by utilizing forest land belonging to Perum Perhutani as agricultural/persilan for planting seasonal crops. Persilan contributions provide a moderate contribution to pesanggem households in KPH Kebonharjo and KPH Mantingan with a total contribution percentage of 38% or IDR 11,000,000 per household per year with details for each KPH Kebonharjo 44% (IDR 10,900,000 per household per year) and KPH Mantingan 33% (Rp. 11,200,000 per household per year). Based on the results of linear regression analysis, some variables significantly influence measurements with degrees of confidence of 85%, 95%, and 99%. The variables agricultural equipment (sig. 0.133), distance to agroforestry land (sig. -0.051), labor utilization (sig. 0.038), and land area of land persil (sig. 0.001) show an influence on the agroforestry income of pesanggem households in KPH Kebonharjo. In addition, the variables farming income (sig. -0.143), other income (sig. 0.048), area of land plots (sig. 0.037), and distance to agroforestry land (sig. 0.001) show an influence on the agroforestry income of pesanggem households, at KPH Mantingan. This research provides valuable insights into the complex relationships between agroforestry, production forests, and economic dynamics, providing a foundation for sound policy-making and encouraging environmentally conscious economic growth

Keywords: Agroforestry Practice; Household; Perhutani Areas; Pesanggem; Production Forest

INTRODUCTION

Forests play an essential role in maintaining ecosystem balance, supporting human life, and being a home for various plant and animal species. Forests are natural resources that can provide organic infrastructure, which plays a vital role in human life, maintains ecosystem balance (Liepa *et al.,* 2023), and reduces the risk of natural disasters (Mihardja *et al.,* 2023), including floods (Viezzer *et al.,* 2022), drought (Portela *et al.,* 2023), landslides and other extreme events. Forests are also able to mitigate climate change through carbon sequestration, contribute to oxygen balance, and protect river watersheds because they can supply 75% of freshwater worldwide (Bremer *et al.*, 2019). As a home for various species of flora and fauna, forests are considered capable of providing organic infrastructure that is important for human life. Forests can offer multiple natural resources that are important for humans, including wood, bamboo, fruit, spices, and other non-wood products, which are sources of building materials, food, medicine, and other materials (Kusters & Belcher, 2004).

Forest areas in Indonesia have long been the nation's identity. Around 48.8 million Indonesians live in forest areas. Many people depend on forests for their livelihoods to provide food sources (Jendresen & Rasmussen, 2022), livelihoods (Akter *et al.,* 2022), and other activities. However, the facts show that according to the largest poor group in Indonesia has been identified as living in forest areas. Moreover, the forest has become an area of conflict between the community and the state, which has resulted in many cases of wood theft and at its peak in 1998, known as the "looting" period, which saw 300,000 hectares of forest become denuded. These problems are caused because forest areas are considered separate areas from communities and village areas. The community and forest officers seem to be chasing cats and dogs, so many conflicts often occur. Therefore, in 2001, the government initiated the Joint Community Forest Management (PHBM) program to overcome this problem (Sopaheluwakan *et al.,* 2023).

Joint Community Forest Management is a solution to bring communities closer to forests. The PHBM concept is an elaboration of social forestry, which is an effort to involve the community in forest management to support forest sustainability. Social forestry practices in Indonesia refer to government initiatives to involve local communities in the management and utilization of forest resources (Budi *et al.,* 2021). This approach allows communities to make decisions about forest use, such as harvesting non-timber forest products and agroforestry practices (Wong *et al.,* 2020). The implementation of PHBM is carried out by 57 Forest Management Units (KPH) throughout Java. One of the KPHs that implement CBFM practices in the Central Java Province Region is KPH Kebonharjo and KPH Mantingan, which have working areas in 113 villages divided into three districts, namely Rembang Regency (24,083.48 Ha), Blora Regency (7,345.60 Ha) in Java Province Central and Tuban Regency (3,057.44 Ha) in East Java Province. Forest utilization through agroforestry in KPH Kebonharjo and KPH Mantingan is known as "Persilan". Persilan is an agricultural activity carried out by pesantren farmers, where pesantren farmers use forests as cultivated land to meet their subsistence needs. Planting with corn, cassava, or nuts commodities is carried out in empty spaces between trees to maintain tree sustainability because Pesanggem farmers do not carry out haphazard logging (Owsianiak *et al.,* 2021).

Agroforestry practiced by pesanggem (forest farmers) in the KPH Kebonharjo Working Area makes a significant contribution to farmers' household income

(Public Summary of KPH Kebonharjo, 2022). Through diverse income streams, agroforestry allows farmers to enter profitable markets for timber and non-timber forest products (Luswaga, 2023). In addition, agroforestry-based businesses provide opportunities for added value and increased resilience, which further improves the financial welfare of farming households (Admasu & Jenberu, 2022). By embracing agroforestry, farmers can experience improved livelihoods and sustainable economic growth. Research related to forest communities, which is studied through socio-economic aspects, is exciting to discuss. Several studies have been carried out by Hardiyanti *et al.* (2021) to determine agroforestry management patterns and calculate the contribution to farmers' income. In this article, authors present a calculation of the gift of agroforestry to the household income of pesanggem farmers and assess the agroforestry factors that influence the agroforestry income of farmer households.

MATERIALS AND METHODS

Selected of Study Sites. This study was conducted to measure the economic impact of the Joint Community Forest Management (PHBM) program, which has been implemented in the Perum Perhutani area (the entire Java-Bali region) since 2000. Two working areas of the Perum Perhutani Forest Management Unit (KPH) in Central Java Province The focus of this research is KPH Kebonharjo and KPH Mantingan. Kebonharjo KPH has a geographical location between 111°20′00"-111°30 00" E 06°30′00"-06°60′00" South Latitude with an area of 17,734.6 Ha covering forest areas in Blora Regency and Rembang Regency, Province Central Java, Tuban Regency, East Java Province. Apart from that, Mantingan KPH has a geographical location between 111°10′00"-111°28′00" E 06°45′00"-06°56′00" South Latitude with an area of 16,751.92 Ha covering forest areas in Blora and Rembang Regency, Central Java Province, Pati Regency, Central Java Province.

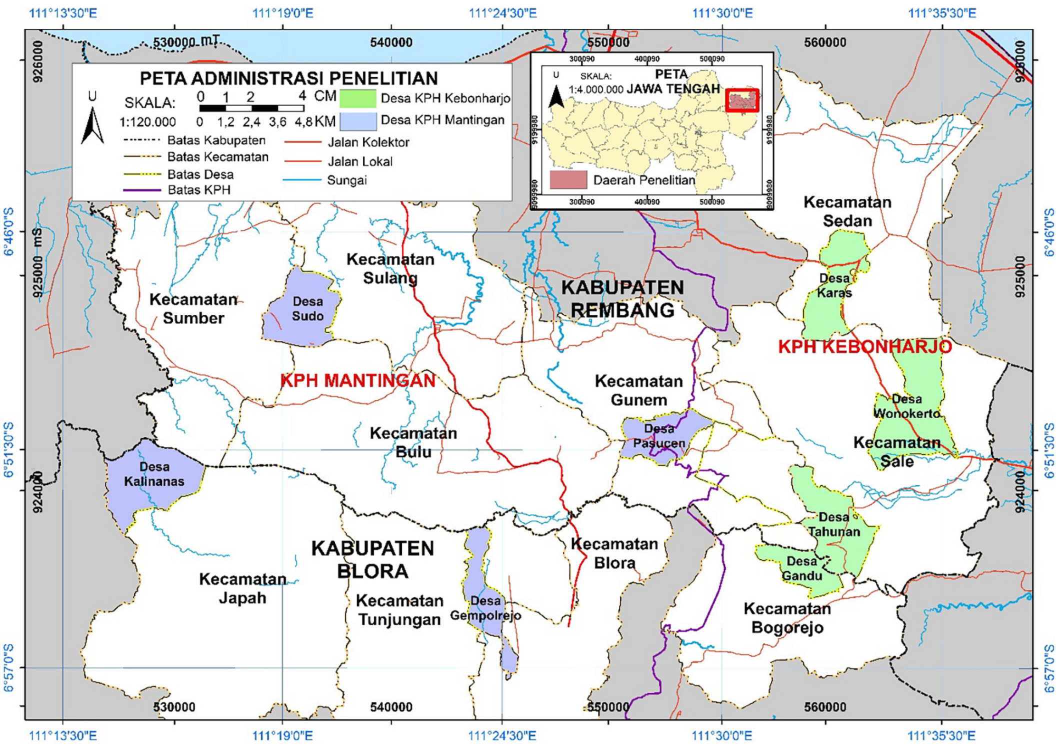

Figure 1*. Location of field study*
Note: *the Kebonharjo KPH area includes the villages of Karas, Wonokerto, Tahunan, and Gandu which are shown in green, Mantingan KPH includes the villages of Sudo, Pasucen, Gempolrejo, and Kalinanas which are shown in green*
Source: *formed by authors*

Based on studies conducted at KPH Kebonharjo and KPH Mantingan, eight villages were selected for the survey. The selection of villages as research objects was carried out by looking at geographical conditions, forest existence, community characteristics, and population so that they were able to represent the entire KPH Kebonharjo and KPH Mantingan areas. The villages selected in the KPH Kebonharjo area include Karas, Wonokerto, Tahunan, and Gandu. Apart from that, the chosen villages in the KPH Kebonharjo area are Pasucen, Sudo, Gempolrejo, and Kalinanas.

Source of Data and Sampling. Data has been collected by collecting household data through survey techniques. The household survey was aimed at pesanggem households with a total of 213 respondents consisting of KPH Kebonharjo (N=116) and KPH

Mantingan (N=97), as in Table 1. The selection of respondents was carried out by calculating the survey's wealth ranking based on field data while still considering the area — forest land owned by pesanggem households. Questionnaires were also used to explore the demographic characteristics of the respondents' villages and families in more depth. Meanwhile, information related to agroforestry patterns, types of plants cultivated, information associated with the seasonal calendar, and income can be obtained through in-depth interviews and participant observation to know the household activities of pesanggem farmers directly.

***Table 1**. Selected Village and Respondents*

Forest Management Units	Regencies	Districts	Villages	Total Pesanggem Household	Samples	Percentage (%)
KPH Kebonharjo	Rembang	Sedan	Karas	25	14	12.07
		Sale	Wonokerto	97	28	24.14
		Sale	Tahunan	51	25	21.55
	Blora	Bagareja	Gandu	286	49	42.24
KPH Mantingan	Rembang	Gunem	Pasuscen	25	18	18.56
		Sulang	Sudo	28	21	21.65
	Blora	Tunjungan	Gempolrejo	41	19	19.59
		Japah	Kalinanas	78	39	40.20
Total	**2**	**7**	**8**		**213**	

***Source:** authors' development*

In addition, data collection through focus group discussions (FGD) was carried out twice in the KPH Kebonharjo and KPH Mantingan areas. Several stakeholders who participated in the FGD included the head of the KPH, the Head of the Forest Management Unit (BKPH), "Mantri", the Head of the Partnership Division, the Field Facilitator, the Village Head and Head of the Forest Village Community Institution (LMDH). Apart from that, critical secondary data was also collected from several sources used in this research, including KPH's annual socio-economic documents, forest product production data, village monographs, journals, and newspapers. In this study, agricultural and non-agricultural income are considered dependent variables. In contrast, age, household size, education, agricultural land area, non-agricultural income, agricultural training access to credit facilities, and participation in institutions, and etc are considered explanatory variables. The survey was conducted from February to March 2023.

Data Analysis: Calculation of Contributions and Influencing Factors. Based on the framework carried out by D. Desmiwati *et al.* (2021) in calculating total household income, this study decided to use a formula for adding up income from agroforestry products with other income, which includes on-farm and off-farm income., non-farm, to remittances. The total revenue of each pesanggem household surveyed is obtained from these calculations. This calculation is more clearly shown in the following formula:

$$Total\ Income = \sum(Agroforestry\ Income + Other\ Income). \quad (1)$$

Next, to calculate the contribution of agroforestry (% I_{gf}) in two regions (KPH Kebonharjo and KPH Mantingan), the data was analyzed by calculating the total income of pesanggem households for a year. In calculating the contribution of agroforestry (% I_{gf}), the following formula is used:

$$\%\, I_{gf} = \left(\frac{I_{gf}}{I_{tot}}\right) \times 100\%, \quad (2)$$

where % I_{gf}: Contribution of agroforestry income to total household income of pesanggem farmers (per year); I_{gf}: Total income from agroforestry activities (IDR/year); I_{tot}: Total income of pesanggem farmers (IDR/ year)

In measuring the significance of the influence of agroforestry factors on the income of pesanggem farmers, measurements are carried out through multiple linear regression analysis with the following formula:

$$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n + e. \quad (3)$$

To clarify the variables being measured, they can be explained in Table 2 as follows:

***Table 2**. Description and measurement of variables*

Variables	Description and Measurement	Expected Signs
Gender	Dummy: 1 if the household head is male and 0 other answers	±
Age	Actual number	+
Married	Dummy: 1 if the household head is married and 0 other answers	
Education	Education of household head is measured by level of study	+
Household Size	The total number of members in a household	+
Number of Livelihoods per Household	The number of types of livelihoods owned by one household	+

Table 2. Continued

Variables	Description and Measurement	Expected Signs
Land Area	The total area of agroforestry land owned by one household (m^2)	+
Number of Plants	The number of types of plants planted in one growing season	+
Agricultural tools	Dummy: 1 if complete agricultural equipment and 0 other answers	
Labor Utilization	The number of workers used for agroforestry production in one planting season	+
Non-Agroforestry Income	Non-agroforestry income includes non-agroforestry, non-farm on-farm and off-farm income, up to remittances (Rupiah)	-
Savings	Dummy: 1 if the household has savings and 0 other answers	-
Credit Access	Dummy: 1 if the household has access to credit and 0 other answers	+
Access training/ extension	Dummy: 1 if the household has access to training/ extension and 0 other answers	±
Participation in Institutions	Dummy: 1 if the household has participated in institutions and 0 other answers	±
Distance to Agroforestry Land	The total distance required by farmers to get to agroforestry land	±

Source: *authors' development*

The research was conducted in accordance with the rules of the Helsinki Declaration (1975).

RESULTS

Agroforestry practice in KPH Kebonharjo and KPH Mantingan Areas. Agroforestry is an agricultural system that combines tree crops with agricultural or livestock crops on the same land. The main goal of agroforestry is to create ecological, economic, and social sustainability through positive interactions between trees, plants, and livestock. This model offers a diverse and sustainable approach to land use, which can provide benefits such as increased productivity, biodiversity, and resilience to climate change. One form of agroforestry in Central Java province, especially in the KPH Kebonharjo and KPH Mantingan areas, is "Persilan".

The intercropping system implemented refers to the Joint Community Forest Management Program (Sahide *et al.,* 2020). Perlan refers to agricultural activities carried out by communities in forest areas who have access to utilize forest land owned by Perum Perhutani as agricultural/arable land for growing seasonal crops. Planting is carried out on forest land previously cut down and processed into land ready for planting. Planting is carried out by pesanggem in empty fields among replanted tree seedlings to preserve the forest simultaneously. The management community is responsible for maintaining and caring for the tree seedlings planted on the plot of land as compensation for cultivating the land. The plants planted on the plots of land, including corn, cassava, and nuts, do not require much water. Even though farming communities have large land areas, they still carry out land plots. This reason is that persilan has become a tradition deeply rooted in the lives of communities around the forest (Table 3).

Table 3*. The agroforestry pattern adopted by pesanggem in KPH Kebonharjo and KPH Mantingan*

Agroforestry	Intercropped Plants KPH Kebonharjo			Intercropped Plants KPH Mantingan		
	Primer	Secondary	Additional	Primer	Secondary	Additional
Type 1	*Zea mays*			*Zea mays*		
Type 2	*Zea mays*	*Arachis hypogaea*		*Zea mays*	*Manihot esculenta*	
Type 3	*Zea mays*	*Manihot esculenta*	*Allium cepa*	*Zea mays*	*Solamun melongena*	
Type 4	*Zea mays*	*Manihot esculenta*	*Musa paradisiaca*	*Zea mays*	*Oryza sativa*	
Type 5	*Zea mays*	*Manihot esculenta*	*Arachis hypogaea*	*Zea mays*	*Manihot esculenta*	*Solamun melongena*
Type 6	*Manihot esculenta*	*Arachis hypogaea*	*Capsicum annum*	*Manihot esculenta*	*Oryza sativa*	*Glycine max L / Arachis hypogaea*

Source: *authors' development*

The land managed by farmers cultivating the "pesanggem" forest is owned by Perum Perhutani or what people usually call "persilan" with an average area managed of 0.25 Ha per farmer. The main crops planted on agroforestry land are forestry plants (Jati Plus Perhutani (JPP), lamtoro (*Leucaena leucocephala*) intercrops, kosambi (*Schleichera oleosa*) filler plants, mahogany edge plants, Multiple Purposes Trees Species (MPTS), and secang (*Biancaea sappan*) hedge plants) with an age of 3-5 years. After the forest plants grow tall, the farmers must move to new land, according to the land where logging has been completed. Before working on logged-over land, farmers must clear the land and clean it independently. There are several types of crops planted between forest plants, including corn (*Zea mays*), chili (*Capsicum annuum*), cassava (Manihot esculenta), eggplant (*Solanum melongena*), rice (*Oryza sativa*), shallots (*Allium cepa*), and peanuts (*Arachis hypogaea*) with different planting patterns based on suitability and location of arable land (Table 3).

The Contribution of agroforestry practice to pesanggem household income. Agroforestry income refers to financial resources generated through various activities related to agroforestry practices. Agroforestry systems are designed to integrate trees with crops and/or livestock, creating a diverse and sustainable land use system (Brandt & Staiss, 2019). This integration allows farmers to obtain income from various sources, thereby increasing the economic resilience and sustainability of the livelihoods of "pesanggem" farming households (Table 4).

***Table 4**. The Contribution of agroforestry practice to pesanggem household income*

Income per Household	KPH Kebonharjo		KPH Mantingan		Kebonharjo + Mantingan	
	Income	Contribution	Income	Contribution	Income	Contribution
Forest	10.9	0.44	11.2	0.33	11.0	0.38
Non-Forest	13.9	0.56	22.4	0.67	17.8	0.62
Total	24.8	1	33.6	1	28.8	1

***Note:** Income (million)*
***Source:** authors' development*

Table 4 shows the contribution of agroforestry to pesanggem household income in KPH Kebonharjo and KPH Mantingan. The contribution of agroforestry in KPH Kebonharjo reaches an average of 44% or an average of IDR 10,900,000 per household per year. Apart from that, the contribution of agroforestry to KPH Mantingan is 33% or an average of IDR 11,200,000 per household year. These results show that agroforestry contributes moderately to pesantren households in KPH Kebonharjo and KPH Mantingan, with a total contribution percentage of 38% or IDR 11,000,000 per household annually.

Factors of agroforestry practice that influence on the farmers' household income. Descriptive statistics of the explanatory variables in this study are explained by calculating the minimum value, maximum value, mean and standard deviation of eighteen factors that are thought to influence the agroforestry income of pesanggem households in two regions, namely KPH Kebonharjo and KPH Mantingan (as in Table 5). Based on the results of measurements on the age variable, in the KPH Kebonharjo area the average age of pesanggem is 52.71 years with a standard deviation value of 12.47. In comparison, in the KPH Kebonharjo area the average age of pesanggem is 50.07 years with a standard deviation of 11.94. In the married variable, in the KPH Kebonharjo and KPH Mantingan areas, the average number of people who have been married shows a mean value of 0.98 and 1, respectively. Hence, the standard deviation value is not visible, showing 0.13 and 0. Furthermore, the variable level of education possessed by pesanggem in the KPH Kebonharjo and KPH Mantingan areas respectively show mean figures of 1.04 and 1.12; this value indicates that the average pesanggem education in the KPH Kebonharjo and KPH Mantingan areas is still at elementary school level.

***Table 5**. Descriptive statistics on selected variables*

Variables	KPH Kebonharjo				KPH Mantingan			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Gender	0	1	0.88	0.88	0	1	0.78	0.41
Age (years)	25	81	52.71	12.47	26	75	50.07	11.94
Married	0	1	0.98	0.13	1	1	1	0
Education	0	4	1.04	0.76	0	3	1.12	0.86
Household Size	1	8	3.45	1.37	1	7	3.45	1.30
Number of Livelihoods	1	5	2.33	0.86	1	6	2.93	0.99
Land Area (Persilan)	0.3	4	0.57	0.60	0.13	2	0.68	0.51
Land Area Productive	0	1.5	0.09	0.21	0	5	0.25	0.58
Number of Plants	0	4	1.27	0.59	0	4	1.42	0.69
Agricultural tools	0	1	0.54	0.50	0	1	0.48	0.50
Labor Utilization	0	72	4.21	8.08	0	67	7.47	9.58
Farm Income (Rupiah-million)	0	48	1.23	5.64	0	40	1.79	5.62
Other Income (Rupiah-million)	0	67	12.65	14.92	0	144	20.65	27.56
Savings	0	3	0.22	0.48	0	1	0.16	0.37
Credit Access	0	1	0.43	0.50	0	1	0.44	0.50

Table 5. Continued

Variables	KPH Kebonharjo				KPH Mantingan			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Access training/ extension	0	1	0.40	0.49	0	1	0.24	0.43
Participation in Institutions	0	1	0.37	0.49	0	1	0.29	0.46
Distance to Agroforestry Land (km)	0	70	4.59	7.38	1	28	4.39	4.57

Source: *authors' development*

The pesanggem household income variable was other than agroforestry and is divided into income from paddy field farming and income other than farming in the KPH Kebonharjo and KPH Mantingan areas. Pesanggem household income from rice field/moor farming shows mean figures of 1.23 million and 1.79 million in Rupiah, respectively, with standard deviation values of 5.64 and 5.62. Besides that, other income of pesanggem households apart from agroforestry and paddy field farming are 12.65 million and 20.65 million in Rupiah, with standard deviations of 14.92 and 27.56 respectively. Based on Table 5 above, seven variables have standard deviation values less than the mean, including gender, age, marriage, education, household size, number of livelihoods, and number of plants, which shows that these variables have poor data distribution. Varies or the mean value can be used as a representation of the entire data. Meanwhile, eleven variables, including land area and income, have standard deviation values that are more than the mean, meaning that the distribution of the data varies or the mean value is a poor representation of the overall data. This result shows some outlier data (too extreme data) in the income and follow institutions variables.

The relationship between agroforestry factors and pesanggem household income from forest management in the KPH Kebonharjo and KPH Mantingan areas was analyzed using a multiple linear regression model, which is presented in Table 6, and the results of the multicollinearity test are shown in Table 7. Some of the variables analyzed include gender, age, marriage, education, household size, number of jobs in one household, land area, ownership of agricultural equipment, amount of labor utilization, household income, savings, access to loans, access to training/counseling, participation in institutions, to the distance variable towards agroforestry land. Confidence degrees of 85%, 95%, and 99% are used to measure the significance level between the variables analysed.

Table 6*. The linier regression analysis on selected independent variables*

Variables	KPH Kebonharjo				KPH Mantingan			
	Coef.	Std.Err.	t-value	Sig.	Coef.	Std.Err.	t-value	Sig.
Constanta.		8.445	-0.634	0.528		9.295	0.099	0.921
Gender	0.040	2.912	0.483	0.630	-0.007	2.709	-0.067	0.946
Age	-0.026	0.082	-0.292	0.771	-0.073	0.111	-0.626	0.533
Married	0.091	6.282	1.270	0.207	-	-	-	-
Education	0.117	1.345	1.320	0.190	0.053	1.427	0.489	0.626
Household Size	0.046	0.612	0.620	0.537	-0.108	0.890	-1.065	0.290
Number of Livelihoods	-0.037	1.054	-0.465	0.643	0.067	1.279	0.604	0.547
Land Area (Persilan)	0.691	1.878	7.020	0.001**	0.262	2.747	2.123	0.037*
Land Area Productive	-0.039	4.326	-0.506	0.614	0.081	2.438	0.645	0.521
Number of Plants	-0.010	1.556	-0.128	0.898	0.120	1.643	1.202	0.233
Agricultural tools	0.120	1.826	1.515	0.133+	0.122	2.257	1.229	0.223
Labor Utilization	0.176	0.119	2.102	0.038*	-0.109	0.144	-0.893	0.374
Farm Income	0.009	0.146	0.131	0.896	-0.157	0.215	-1.478	0.143+
Other Income	-0.038	0.061	-0.496	0.621	0.211	0.044	2.005	0.048*
Savings	-0.003	1.820	-0.040	0.968	-0.038	2.987	-0.385	0.701
Credit Access	-0.066	1.814	-0.846	0.400	0.143	2.247	1.451	0.151
Access training/ extension	-0.056	1.800	-0.731	0.466	0.069	2.754	0.665	0.508
Participation in Institutions	-0.058	1.735	-0.789	0.432	-0.063	2.451	-0.648	0.519
Distance to Agroforestry Land	-0.172	0.135	-1.973	0.051+	0.371	0.277	3.339	0.001**

Note: *+, *, ** indicates significant diference $p < 0.15$, $p < 0.05$, and $p < 0.001$ respectively*
Source: *authors' development*

Based on the results of the analysis, the variables that influence the agroforestry income of pesanggem households in KPH Kebonharjo, namely Land Area (Persilan) with $p < 0.001$, Labor Utilization with $p < 0.05$, as well as ownership of agricultural equipment and distance of agroforestry land from residence show significance at $p < 0.15$. On the other hand, some variables influence the agroforestry income of pesanggem households in KPH Mantingan, including the distance from the house to the agroforestry land, which shows significance with $p < 0.001$, Land Area (Persilan) and other income, which shows importance at $p < 0.05$, as well as the variable income from paddy fields offers an effect at $p < 0.15$.

The land area of persil owned by pesanggem households in KPH Kebonharjo shows a significance value (Sig. 0.001) at p-value < 0.001, which means that the variable land area of land plots has a very significant influence on the agroforestry income of pesanggem households in KPH Kebonharjo. The same results were also shown in the variable land area of persil by pesanggem households in KPH Mantingan, which showed a significance value (Sig. 0.037) at p-value < 0.05, which means that the variable land area of persil had a very significant influence on household agroforestry income of pesanggem at KPH Mantingan. Pesanggem households with larger areas of forest land have more tremendous opportunities to increase their household income. Land productivity is also closely related to farmer capacity, access to resources, and access to markets, so a more expansive land tenure policy will provide benefits to forest farming communities, increasing farmers' income and welfare.

The variable for completeness of agricultural tools owned by pesanggem at KPH Kebonharjo shows a significance value (0.133) at p-value < 0.15. This value means that the completeness of agricultural equipment is essential in increasing the income of pesanggem households from the agroforestry sector. However, the variable for completeness of agricultural equipment owned by pesanggem in KPH Mantingan did not show any influence even though the p-value was < 0.15, so it can be said that the variable for completeness of agricultural equipment did not influence increasing the income of pesanggem households from the agroforestry sector. The variable utilization of labor by pesanggem in agroforestry in KPH Kebonharjo significantly influences pesanggem household income with a significance value (Sig. 0.038) at p-value < 0.05. These results show that the more workers are utilized in the Kebonharjo KPH area agroforestry, the higher the income pesanggem households will receive. This labor utilization variable is directly proportional to the more significant the average area of land managed by pesanggem households, that the greater the land area owned by pesanggem eating households, the greater the utilization of labor will be. It will influence the increase in income of pesanggem households from the sector agroforestry.

The variable farm income and other income of pesanggem households in KPH Mantingan shows the influence on the income of pesanggem households from the agroforestry sector. The results of the analysis show that there is an influence of the farm income variable with a value (Sig. 0.143) at p-value < 0.15 and other income variables with a value (Sig. 0.048) at p-value < 0.05. These results conclude that pesanggem household income from agroforestry will increase if income from other agricultural industries (rice fields/moorland) is decreased. On the other hand, pesanggem household income from the agroforestry sector will increase if they get income from other income (i.e. non-agroforestry income and not from rice fields/moors) (Table 7).

***Table 7**. Multicolliniearity test for independent variables*

Variables	Collinearity Statistics			
	KPH Kebonharjo		KPH Mantingan	
	Tolerance	VIF	Tolerance	VIF
Gender	0.638	1.568	0.737	1.358
Age	0.552	1.812	0.532	1.881
Married	0.857	1.166	-	-
Education	0.559	1.788	0.619	1.615
Household Size	0.812	1.232	0.693	1.443
Number of Livelihoods	0.704	1.421	0.575	1.738
Land Area (Persilan)	0.454	2.200	0.468	2.136
Land Area Productive	0.727	1.375	0.456	2.193
Number of Plants	0.684	1.463	0.722	1.386
Agricultural tools	0.700	1.428	0.721	1.387
Labor Utilization	0.626	1.598	0.484	2.068
Farm Income (Non-Agroforestry)	0.847	1.181	0.636	1.572
Other Income	0.736	1.360	0.643	1.554
Savings	0.768	1.301	0.746	1.340
Credit Access	0.715	1.398	0.736	1.359
Access training/ extension	0.744	1.344	0.668	1.496
Participation in Institutions	0.821	1.218	0.743	1.345
Distance to Agroforestry Land	0.583	1.715	0.579	1.726

***Source:** authors' development*

The distance of residence to agroforestry land is a variable that influences the income of pesanggem households in both KPH Kebonharjo and KPH Mantingan. The variable distance from residence to agroforestry land in the KPH Kebonharjo area shows (Sig. 0.051) at p-value < 0.15, meaning that there is an influence between the distance from residence to agroforestry land on increasing the income of pesanggem households from the agroforestry sector. The negative coefficient value on this variable means that the income of pesanggem households from the agroforestry sector in KPH Kebonharjo will increase if the distance between residence and agroforestry land is closer, thus providing effectiveness in agroforestry management. The average distance to agroforestry land in KPH Kebonharjo is 4.59 kilometers. On the other hand, the distance from residence to agroforestry land in the Mantingan KPH area shows (Sig. 0.001) at p-value < 0.001, meaning that there is an influence between the distance from residence to agroforestry land on increasing the income of pesanggem households from the agroforestry sector. The coefficient on this variable gives a positive value, which means that the income of pesanggem households from the agroforestry sector in KPH Kebonharjo will increase if the distance between the residence and the agroforestry land is further. The average distance to agroforestry land in KPH Mantingan is 4.39 kilometers. Table 7 shows the multicollinearity test on the variables, showing each variable's variance inflation factor (VIF). There is a high level of tolerance between variables, indicating no severe multicollinearity between the variables used in the analysis.

DISCUSSION

Agroforestry reflects awareness of preserving the environment while meeting food and other resource needs. Studies on the economic impacts of agroforestry practices in production forest areas are critical to explore the potential benefits obtained in economic, social, and environmental ecosystems. First of all, from a financial perspective, agroforestry can significantly contribute to the income of farmers and local communities (Desmiwati *et al.,* 2021; Kauppi *et al.,* 2022). The integration of tree crops with agricultural or livestock crops can create additional sources of income through the sale of forest products, timber, or agricultural products. Agroforestry also has the potential to create new jobs. Planting, maintaining, and harvesting trees and related activities can provide employment opportunities for local communities. This potential can improve living standards and reduce poverty, especially in areas dependent on agricultural and forestry activities. The economic impact of agroforestry also involves the development of local markets. Agroforestry products, such as fruit, wood, and other agricultural products, can be an attraction for the local economy. Increased access to markets and promotion of local products can significantly boost local and regional economic development. It is also important to note that agroforestry can benefit the ecosystem in the long term (Zada *et al.,* 2022; Kilonzo, 2022). Farmers can maintain their production and income by creating a sustainable agricultural system without damaging the surrounding environment. This attempt can help prevent land degradation and sustain long-term productivity, ultimately supporting economic sustainability.

Agroforestry is essential in increasing community employment opportunities because it creates a sustainable and multifunctional agricultural system (Sulistiyowati *et al.,* 2023). First, agroforestry can increase food production by combining crops, forestry plants, and livestock, creating jobs in various agricultural sectors. Second, sustainable agroforestry requires sustainable land care, creating job opportunities in forest and environmental maintenance. In addition, planting multiple types of crops provides diverse results, which can increase the community's economic sustainability. Agroforestry also creates employment opportunities for planting, maintaining, and harvesting crops. Apart from that, the industrial sector related to forest product processing can also provide additional employment opportunities. By implementing agroforestry, communities can develop new skills in natural resource management and sustainable agriculture, opening up training and education opportunities to increase their capacity. Overall, agroforestry is not only about increasing agricultural productivity but also positively impacts the environment and creates sustainable employment opportunities for people.

Agroforestry plays an essential role in diversifying farmers' income sources (Jagger *et al.,* 2022; Jamison *et al.,* 2023). By combining crops, plantations, and trees on one land, farmers can expand their portfolio. First, agroforestry produces various products that can be sold, such as fruit, wood, and non-timber forest products. This method provides more stable economic opportunities because it does not depend on just one type of plant. In addition, agroforestry can increase land productivity by utilizing positive interactions between plants. Trees planted with crops can provide shelter, provide nutrients through fallen leaves, and reduce soil erosion. This effort increases agricultural yields and reduces the risk of crop failure. In addition to direct income from agroforestry products, farmers can also access incentive programs and government assistance that support these sustainable practices. For example, reforestation or environmentally friendly programs can provide financial incentives for farmers to implement agroforestry. Overall, agroforestry is an agricultural method and a long-term investment strategy for farmers. By harnessing the diversity of natural resources on their land, farmers can create systems more resilient to climate and economic change while improving their well-being.

Agroforestry plays a vital role in climate change mitigation and adaptation efforts. In terms of mitigation, this system functions as an effective carbon store, helping to reduce greenhouse gas emissions by sequestering and storing carbon in trees and soil (Viezzer *et al.,* 2022; Moreda, 2023). In addition, integrating livestock in agroforestry can minimize methane emissions from livestock. Meanwhile, in a conservation context, agroforestry helps overcome climate challenges by increasing plant resilience through production diversification. The trees in this system can protect from high temperatures and winds while improving air and soil conservation. To care for degraded land, agroforestry contributes to ecosystem restoration and increases resilience to natural disasters. Thus, agroforestry is a sustainable agricultural model and an integral solution to reduce climate change's impact and increase community agriculture's adaptability.

However, the mindset of forest farmers in Central Java Province, dependent on seasonal farming in the forest, can negatively impact environmental sustainability, food security, and community welfare. Farmers who rely too heavily on seasonal farming in forests (such as corn, cassava, and rice) may face risks from weather and winter climates (Robinne, 2021; Ruba & Talucder, 2023). Annual cropping patterns tend to be vulnerable to seasonal changes or natural disasters, increasing the risk of production and income losses. Dependence on agriculture for one season can also create a cycle of poverty that is difficult to end. Farmers who rely on one growing season for their primary income may face high financial risks if crops fail. These practices can hinder economic development and the welfare of the agricultural community (Akpan & Zikos, 2023). Farmers accustomed to seasonal farming may need more knowledge and skills to switch to sustainable farming practices. It is essential to change the mindset of farmers by educating them about the diversification of agricultural benefits, sustainable resource management, and the use of enabling technology. Encouraging passion and resilience must be a focus so farmers can better face future challenges.

Specific case studies or the presentation of success stories of agroforestry initiatives in Central Java are important to deepen the understanding of the implementation and impact of sustainable agricultural practices at the local level. Through this research, authors can gain in-depth insight into the factors that influence the success of agroforestry from an economic, ecological, and social perspective. These success stories inspire and can guide farmers and policymakers in selecting and implementing sustainable farming models. By understanding the local context, this research can identify specific solutions to the challenges facing agricultural communities in Central Java. In addition, research results can form the basis for development policies that support the implementation of agroforestry and sustainable agriculture at regional and national levels. In other words, this case study research not only provides a comprehensive picture of the potential of agroforestry in Central Java but also plays a crucial role in outlining practical steps to achieve more sustainable agriculture in the future.

Increasing the economic impact of agroforestry in Central Java requires active involvement from policymakers, practitioners, and stakeholders (Octavia *et al.,* 2022). First, developing training and education programs is key to increasing farmers' understanding and skills in implementing agroforestry effectively. Community empowerment should also be a focus by encouraging active participation in the planning and implementation of agroforestry practices. Strong partnerships between government, the private sector, and NGOs can provide support in terms of capital, technology, and market access, which is a strong foundation for the success of agroforestry. The importance of recognition and incentives cannot be understated; Rewards for farmers who implement agroforestry, such as sustainable certification and tax incentives, can be a strong incentive to implement this model. In addition, business diversification in the agroforestry sector and local market development can help create added economic value. A good monitoring and evaluation system must also be implemented to provide positive impacts and identify improvement areas. Effective outreach and education campaigns can play a key role in increasing public awareness of the economic benefits of agroforestry. By accepting this recommendation, it is hoped that agroforestry in Central Java can develop as a pillar of a sustainable economy and improve the welfare of local communities.

CONCLUSIONS

Studies on the economic impacts of agroforestry practices in production forest areas are critical to explore the potential benefits obtained in economic, social, and environmental ecosystems. The results of research conducted through a survey of 216 pesanggem households in the KPH Kebonharjo and KPH Mantingan areas show the contribution of agroforestry to the income of pesanggem households in KPH Kebonharjo and KPH Mantingan. The average contribution of agroforestry in KPH Kebonharjo reaches 44% or an average of IDR 10,900,000 per household per year. Apart from that, the contribution of agroforestry to the Mantingan KPH is 33% or an average of IDR 11,200,000 per household per year. These results show that agroforestry significantly contributes to Islamic boarding school households in KPH Kebonharjo and KPH Mantingan, with a total contribution percentage of 38% or IDR 11,000,000 per household annually.

Based on the analysis results, several variables significantly affect measurements with confidence levels of 85%, 95% and 99%. The variables agricultural tools (sig. 0.133), distance to agroforestry land (sig. -0.051), labor utilization (sig. 0.038), and land area (sig. 0.001) show an influence on the agroforestry income of pesanggem households, at KPH Kebonharjo. In addition, the variables farming income (sig. -0.143), other income (sig. 0.048), land area (sig. 0.037), and distance to agroforestry land (sig. 0.001) show an influence on agroforestry income. pesanggem household, at KPH Mantingan. These results indicate the need to increase the area of agroforestry land management in the two KPH areas (KPH Kebonharjo and KPH Mantingan) so that it is likely to increase agroforestry income significantly. However, this research also suggests that there needs to be an understanding effort for pesanggem households to only sometimes depend on seasonal crop farming so that they do not cause losses due to uncertain climate changes, which will result in financial losses.

This research provides valuable insights into the complex relationships between agroforestry, production forests, and economic dynamics, providing a basis for sound policy-making and encouraging environmentally conscious economic growth. However, this research still has limitations, so there is a need for further research that presents success stories of agroforestry initiatives that play an essential role in deepening the understanding of the implementation and impact of sustainable agricultural practices at the local level. Through this research, authors can gain in-depth insight into the factors that influence the success of agroforestry from an economic, ecological, and social perspective. These success stories not only provide inspiration but can also serve as practical guidance for farmers and policymakers in selecting and implementing sustainable farming models and can form the basis for development policies that support the implementation of agroforestry and sustainable agriculture at regional and national levels.

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CONFLICT OF INTEREST

This article contains no elements of conflict of interest.

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Економічний вплив практики агролісомеліорації на виробничих лісових територіях, провінція Центральна Ява, Індонезія

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Анотація. Концепція спільного ведення лісового господарства громадами в Індонезії – це спроба залучити громади до управління лісами через агролісомеліорацію для підтримки стійкості лісів. Агролісомеліорація (або персілан місцевою мовою), яку здійснюють песанггем у робочих зонах КРН Кебонхарджо та КРН Мантінган, робить значний внесок у дохід домогосподарств песанггем. Це дослідження має на меті виміряти вплив агролісомеліорації на доходи домогосподарств песангемів та вивчити фактори агролісомеліорації, які впливають на доходи домогосподарств песангемів. Збір даних здійснювався шляхом опитування та спостереження за 216 домогосподарствами песангемів у районах КРН Кебонхарджо та КРН Мантінган, розділених на вісім сіл відповідно. Потім дані були проаналізовані описово та за допомогою множинного лінійного регресійного аналізу. Результати дослідження показали, що модель агролісомеліорації через земельні ділянки відноситься до сільськогосподарської діяльності песанггема в лісових районах шляхом використання лісових земель, що належать Perum Perhutani, як сільськогосподарських/персілан для посадки сезонних сільськогосподарських культур. Внески персілан забезпечують помірний внесок домогосподарств песангем у КРН Кебонхарджо та КРН Мантінган із загальним відсотком внеску 38 % або 11,000,000 індонезійських рупій на домогосподарство на рік, з деталізацією для кожного КРН Кебонхарджо 44 % (10,900,000 індонезійських рупій на домогосподарство на рік) та КРН Мантінган 33 % (11,200,000 індонезійських рупій на домогосподарство на рік). За результатами лінійного регресійного аналізу,деякі змінні суттєво впливають на вимірювання зі ступенем достовірності 85%, 95% та 99%. Змінні сільськогосподарське обладнання (sig. 0,133), відстань до агролісомеліоративних земель (sig. -0,051), використання робочої сили (sig. 0,038) та площа земельної ділянки персіл (sig. 0,001) демонструють вплив на дохід від агролісомеліорації домогосподарств песанггем в КРН Кебонхарджо. Крім того, змінні дохід від фермерства (sig. -0,143), інші доходи (sig. 0,048), площа земельних ділянок (sig. 0,037) та відстань до агролісомеліоративних земель (sig. 0,001) демонструють вплив на дохід від агролісомеліорації домогосподарств песангемів у КРН Мантінган. Це дослідження надає цінну інформацію про складні взаємозв'язки між агролісомеліорацією, виробничими лісами та економічною динамікою, забезпечуючи основу для розробки обґрунтованої політики та сприяння екологічно свідомому економічному зростанню

Ключові слова: практика агролісомеліорації; домогосподарство; райони Перхутані; Песанггем; виробничі ліси

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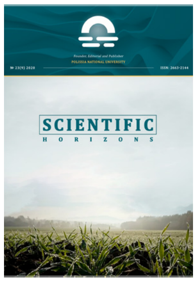

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Financial modelling of cash flow management for agribusiness security: Accounting and analytical aspect

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Abstract. The study related to cash flows of agricultural enterprises is relevant, since the agricultural sector is one of the key sectors of the national economy, and food security of Ukraine is becoming a strategic priority in the context of post-war reconstruction and requires effective management approaches to increase investment attractiveness and sustainable development in the field of agricultural activities. The purpose of this study was to analyse, develop, and improve the existing priority approaches to cash flow management for the financial security of agribusiness, considering the accounting and analytical aspect. Among the methods used were the statistical method, forecasting, and financial modelling methods, analytical method, and discounting method. During the study, an analytical assessment and modelling of the prospects for changes in the net cash flow of an agricultural enterprise in the medium term was carried out, the need to use actuarial accounting and reporting to attract the necessary amount of foreign investment in the development of the industry in the context of financial security of farmers was determined. The role of actuarial accounting support in managing the value of agribusiness for its financial security

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was investigated. The study modelled the process of cash flow management at the agricultural enterprise PJSC "Agrofirma "Provesin". The study investigated actuarial information support from the actuarial accounting system for assessing the prospective changes in the property potential of agribusiness. It was noted that cash flow management in agriculture involves the use of innovative accounting and analytical management support, which would help to improve the image of investment attractiveness in foreign markets. The practical significance of this study lies in the development of concrete practical recommendations for the top management of agricultural enterprises to effectively attract the necessary amount of financial resources for sustainable development and post-war recovery of agribusiness

Keywords: monetary assets; investment attractiveness; agriculture; actuarial reporting; food security

INTRODUCTION

Ukraine's agricultural sector has found itself in a rather demanding situation under martial law and needs comprehensive support from both the government and real and potential foreign investors. Ensuring the sustainable development of Ukrainian agricultural enterprises is not only a guarantee of food security, but also the basis for further post-war economic recovery and the restoration of agribusiness. However, effective management of cash flows of agricultural enterprises is an urgent task, as it allows optimising the structure of assets, increasing solvency and liquidity, providing a cost estimate of economic potential, and promoting the investment attractiveness of agriculture. On the other hand, Ukraine's integration into the international community opens new opportunities and requires the country to ensure that the accounting and reporting system of agricultural enterprises is understandable to real and potential foreign investors and other capital providers, i.e., meets European standards. This makes the problem of effective cash flow management in agriculture even more important and justified in terms of the post-war recovery of agribusiness and the iteration of agrarians into the international arena.

According to O.Z. Mykytin (2023), the need for effective management of enterprise cash flows, in the context of martial law in Ukraine, is a crucial and necessary process. One of the main conclusions of the researcher was the considerable impact of the rationality of cash flow management on the efficiency of enterprises, both in the internal and external markets. The researcher emphasises that a comprehensive model of cash flow planning of a business entity should be based on concrete set of methodological tools. At the same time, Mykytin argues that a significant place among the methods of cash flow planning is occupied by financial modelling; the method of optimising financial decisions; calculation-analytical and regulatory methods. According to the researcher, the final managerial decision on the use of certain methods should be made only in the context of the financial strategy. Admittedly, only in consultation with the company's top management and considering the economic value of the business and the present value of net cash flow in the planned and factual periods of evaluation can the capital structure be effectively optimised. However, unlike a few years ago, the current conditions of martial law in Ukraine and the situation in the agricultural sector require additional financial resources in the form of both external and internal investments.

T.E. Kucherenko and G.Yu. Anishchenko (2022) assessed the modern accounting and analytical support for enterprise cash flow management and suggest that it is formed based on a combination of financial and internal accounting systems. The interaction of these systems results in accounting and reporting information that serves as the basis for analytical assessment of cash flows based on express analysis in the context of business development prospects. The researchers' conclusions show that effective cash flow management contributes to financial balance and improves the level of solvency of an enterprise. Reliable accounting and reporting support from the actuarial accounting system forms the quality content for attracting investments.

Yu.O. Kostenko *et al*. (2023) emphasise the significance of effective management of financial resources of an enterprise in the context of implementing a financial management strategy, analysis, and forecasting of cash flows. The increase in the value of an enterprise's assets is determined by investment and financial decisions. The cash flow management strategy aims to achieve the following objectives: maximising net cash flow; optimising cash flow distribution; and reducing the risk of insolvency. The researchers emphasise that investment decisions determine the size and structure of tangible assets required for business activities. Financial decisions relate to obtaining sources of funding for these assets in terms of their structure. Notably, an objective analytical assessment of the prospects for increasing the company's value, based on the information content of actuarial reporting, can help to increase the level of investment attractiveness of an enterprise.

Yu. Nikolchuk and O. Lopatovska (2022) analysed the problems of developing a financial strategy in the financial management system of an enterprise. They identified several key aspects of the financial strategy, considering the prospects for the financial development of the enterprise, which form the financial policy. Important components of the financial policy are the management of cash flows; investments; assets; capital and financial risks. It is proposed to assess the trends in the financial condition of the enterprise and its own capabilities in the future. Attracting the necessary amount of additional financial resources and their efficient use stays a critical issue under martial law that requires a comprehensive approach. Such a comprehensive approach is important to increase the investment attractiveness of Ukrainian agricultural enterprises and ensure the recovery of the national economy.

Generally, there is a need to improve the efficiency of cash flow management based on actuarial accounting and analytical support to increase the investment attractiveness of agribusiness. Rebuilding the post-war economy requires effective cash flow management and ensuring the recovery of the agricultural sector. The purpose of this study was to develop an algorithm for managing cash flows in agriculture in Ukraine, considering the accounting and analytical aspect and ensuring the recovery of the agricultural sector. To fulfil this purpose, the following tasks were set: to calculate the forecast cash flow of the enterprise; to reflect the discounted value of assets in the actuary's financial statements; to assess the change in the value of an individual agribusiness entity in the context of its investment attractiveness.

MATERIALS AND METHODS

The study was based on official documents and reports of the National Bank of Ukraine (n.d.), Committee on Agrarian and Land Policy (n.d.), as well as materials from the official websites of the European Bank for Reconstruction and Development (2022), Stock market infrastructure development agency of Ukraine (2023) and Thomson Reuters (2023), Decree of the President of Ukraine No. 722/2019 (2019).

The study of the projected cash flow of the Private Joint Stock Company (PJSC) "Agrofirma "Provesin" until 2027 was conducted according to the financial statements for 2018-2023. PrJSC "Agrofirma "Provesin" is a modern agricultural enterprise engaged in the cultivation of various organic vegetables in greenhouse conditions, melons, roots and tubers, and is located in Western Ukraine (registered address: 152 Hlynianskyi Trakt Str., Lviv, 79067). PrJSC "Agrofirma Provesin" provides food to the population through local and all-Ukrainian retail chains, which is strategically important in the context of martial law and food security in Ukraine.

The statistical method was used to investigate the data on the average annual growth rate of income and expenses of an agricultural enterprise. Ex-ante economic analysis made it possible to carry out financial modelling of the management of cash assets of agricultural enterprises based on the calculation of the forecast net cash flow for the last 5 years, by applying the following formula:

$$\overline{G}_{rate} = \sqrt[n-1]{\frac{y_1}{y_0}}, \tag{1}$$

where y_1 – is the value of the indicator in the reporting period; y_0 – is the value of the indicator in the base period; n is the number of periods.

The use of the discounting method made it possible to bring the future value of an agricultural enterprise's cash flows to their value in the current period through the lens of actuarial management reporting. Accordingly, the economic value of an agricultural enterprise is formed from the present value of net cash flow in the planning period and the present value of net cash flow in the final period using the following formulas:

$$NCF_{pl} = \sum_{t=1}^{T} \frac{NCF_t}{(1+cc)^t}, \tag{2}$$

$$NCF_f = \frac{NCF_{T+1}}{cc-g} x \frac{1}{(1+cc)^T}, \tag{3}$$

where NCF_{pl} – is the value of net cash flow in the planning period; NCF_f – is the value of net cash flow in the final period; NCF_t –is the net cash flow in the planning period; cc – is the cost of capital; T – is the duration of the strategic plan for the development of the agricultural enterprise; g – is the percentage change in the volume of activities (sales).

$$PV = \frac{FV}{(1+i)^n}, \tag{4}$$

where FV – is the future value of the enterprise; PV – is the present value of the enterprise; i – is the discount rate (the average annual refinancing rate of the National Bank of Ukraine (NBU)).

The discount rate is an important indicator in the calculation, as its increase will reduce the present value of the agricultural enterprise. Based on the results obtained, concrete recommendations will be developed to improve the image of the investment attractiveness of Ukrainian agricultural enterprises through the practical application of accounting and reporting content of actuarial statements, which will contribute to the post-war recovery of the agricultural sector of Ukraine.

RESULTS

The full-scale losses of Ukraine's agricultural sector as a result of the war are significant. Thus, according to the Ministry of Agrarian Policy and Food of Ukraine, the total amount of losses incurred by the agricultural sector was USD 6.6 billion. Furthermore, the total amount of indirect losses of farmers as a result of the reduction in agricultural production in Ukraine is USD 34.25 billion (Rusan, 2022). The accounting and analytical support of cash flow management of an agricultural enterprise should be considered as a set of information data from the accounting system to model the level of reasonable assessment of the financial security of the business entity and its sustainable development. That is, in the context of rebuilding the post-war economy of Ukraine, accounting should move to a new stage of development – actuarial. Actuarial accounting is aimed at real and potential investors or other capital providers and can increase the investment attractiveness of Ukrainian agricultural business in the context of not only food but also financial security. To ensure the financial security of Ukrainian farmers under martial law, the actuarial accounting system should be developed in such a way that it creates an image of protection of their activities from the negative effects of military operations and can quickly mitigate various external threats. Financial security is determined by the financial position of the enterprise, the efficiency of *cash flow (asset)* management, the level of security and the prospective (forecast) property potential of business development (Oriekhova *et al.*, 2022). Given the volatile market environment and the overall threats at the macro level, it is worth abandoning short-term forecasts. The optimum forecast period should be no more than 5 years. The calculation of forecast indicators for agricultural enterprises is based on past trends, considering the specifics of trends in the agricultural sector as a whole. Furthermore, the forecast excludes significant large-scale impacts, such as pandemics, wars, etc.

Net cash flow is reflected in the accounting system, specifically in the public financial statements such as the Cash Flow Statement (Form 3), which is modified in the actuarial accounting system into the Actuarial Balance Sheet (Form 3-a). In terms of content, net cash then represents the amount of cash available to investors, which is calculated by deducting investments in working capital and non-current assets from cash flows from operating activities. The total amount of free cash flow should be an identical amount both from the standpoint of the enterprise and from the standpoint of the capital provider (real or potential investor).

Therefore, it is advisable to use the forecasted values of cash inflows and outflows to an agricultural enterprise for a range of periods. The difference between these projections is the net cash flow of the agricultural business entity. Financial modelling of cash flow management is a key stage in the preparation of actuarial reports, specifically, Form 1-a (Actuarial Balance Sheet (Actuarial Statement of Financial Position) and Form 3-a (Actuarial Cash Flow Statement). This factors in the dynamics of the company's cash flows over a range of periods and recalculates their value considering the forecast growth rates of income and expenses. The task of improving the efficiency of cash flow management is addressed in the actuarial accounting and reporting system. To calculate the net cash flow, i.e., the amount of cash available to investors of PJSC "Agrofirma "Provesin", the study used information on the dynamics of expenses and income of the agricultural company for 2018-2023 (Table 1).

***Table 1**. Dynamics of income and expenses of PrJSC "Agrofirma "Provesin" for 2018-2023*

Period	Revenues, (UAH thsd)	Expenses, (UAH thsd)
2018	8,128	7,369
2019	10,318	8,128
2020	10,414	10,261
2021	6,012	8,305
2023	7,246	9,950
$\overline{G}_{rate}$	0.944	1.077

***Source:** compiled by the authors of this study*

An analytical assessment of the dynamics of income and expenses of the agricultural firm over the past 5 years according to the financial statements indicates negative financial results of its operation in the agricultural market and the need to attract additional financial resources. However, conventional financial statements are of no interest to a potential investor, as they do not reflect the present value of future cash flows in the long term, but only state the carrying value of the objects of accounting. Therefore, to create an image of investment attractiveness for Ukrainian agricultural enterprises, it is necessary to have accounting and reporting content that would allow a potential investor to immediately see the additional benefit of investing temporarily free financial resources, i.e., the return on investment.

The average annual rate of decrease (increase) in income and expenses of PJSC "Agrofirma Provesin" for 2018-2023 is -5.6% and +7.7%, respectively, which is a reasonable trend both in the context of the pandemic and martial law in Ukraine. It is worth using the forecasted values of cash inflows and outflows to the enterprise for 2024-2027. The difference between these projections is the net cash flow (Table 2).

***Table 2**. Projected net cash flow of PrJSC "Agrofirma "Provesin" for 2024-2027*

Period	Revenues, (UAH thsd)	Expenses, (UAH thsd)	*Cash flow* (UAH thsd)
$2024_{forecast}$	6,811	10,746	-3,935
$2024_{forecast}$	6,403	11,606	-5,203
$2025_{forecast}$	6,018	12,534	-6,516
$2026_{forecast}$	5,657	13,537	-7,880
$2027_{forecast}$	5,318	14,620	-9,302
Total	**37,453**	**72,992**	**-35,539**

***Source:** compiled by the authors of this study*

Accordingly, the amount of free cash flow should represent the same amount from the standpoint of the agricultural enterprise as well as from the standpoint of a real and potential investor or other capital provider. Determining the discount rate is a rather controversial stage in the preparation of actuarial reports, specifically, Form 1-a "Actuarial Balance Sheet (Actuarial Statement of Financial Position)" and Form 3-a "Actuarial Balance Sheet of Cash Flows". In the current environment, it can be stated that there is no single methodology for calculating the discount rate. Undoubtedly, discounting is an important mechanism that allows providing reliable information about the financial position of an agricultural enterprise, since the present value of future financial flows may differ substantially from the nominal value.

The procedure for selecting the discount rate should be recorded in the accounting department's notes and also prescribed in the accounting policy. Furthermore, it is advisable to use open and widely known sources (the NBU, or information from global rating agencies, such as Thomson Reuters). In addition, the accounting certificate should substantiate the increase in the discount rate if the debt has risks of non-repayment and assess them.

For PrJSC "Agrofirma "Provesin", the discount rate was chosen at the level of the NBU's average annual rate, which as of 01.01.2022 was 9.92% (however, from 16.06.2023 it reached 25.00%). The calculation of the discounted value of assets will be presented on the example of calculating the discounted value of fixed assets, the decoding of which for PJSC Agrofirma Provesin is presented in Table 3.

***Table 3**. Property, plant and equipment of PrJSC "Agrofirm "Provesin" (Lviv) as of 1 January 2022*

Name of the item of property, plant and equipment	Residual cost, UAH thsd	Residual service life, years
Buildings and structures	5,926	12
Machinery and equipment	371	3
Vehicles	7	4
Land plots	0	(no minimum allowable useful lives are set)
Other property, plant and equipment	77	9
Total:	6,381	x

***Source:** compiled by the authors of this study*

The next step is to allocate the cash flow to the company's assets using the discounting method to reflect in the Actuarial Balance Sheet (Actuarial Statement of Financial Position), Form No. 1-a, assets and liabilities with a maturity of more than 12 months. Thus, the distribution of cash flow from operating activities will be carried out only in terms of non-current assets (Table 4). Ukrainian researchers propose to use the residual value of fixed assets as the basis for distribution, which is given in the analytical report on fixed assets. Assets that are expected to be disposed of during the forecast period are excluded from the calculation of the distribution base as they are disposed of (Sergienko *et al.*, 2022; Kyrychenko, 2023).

When calculating the discounted value, information on the residual value of non-current assets at the end of the forecast period is also important (Table 5).

The discounted value of intangible assets for PrJSC "Agrofirma "Provesin" is calculated as follows (Table 6).

Table 4. Distribution of cash flows by items of non-current assets PJSC "Agrofirma Provesin" (Lviv)

Period	2024		2025		2026		2027	
Name of the item of property, plant and equipment	Cash flow allocation basis, %	Cash flow, UAH thsd	Cash flow allocation basis, %	Cash flow, UAH thsd	Cash flow allocation basis, %	Cash flow, UAH thsd	Cash flow allocation basis, %	Cash flow, UAH thsd
Buildings and structures	92.87	2,497.26	92.87	3,211.43	92.87	3,750.07	92.87	5,236.91
Machinery and equipment	5.81	156.34	5.81	201.05	5.81	234.77	5.81	327.86
Vehicles	0.11	2.95	0.11	3.79	0.11	4.43	0.11	6.19
Land plots	-	-	-	-	-	-	-	-
Other property, plant and equipment	1.21	32.45	1.21	41.73	1.21	48.73	1.21	68.05
Total:	**100.0**	**2,689**	**100.0**	**3,458**	**100.0**	**4,038**	**100.0**	**5639**

Source: *compiled by the authors of this study*

Table 5. Estimated residual value of non-current assets of PJSC "Agrofirma "Provesin" (Lviv) as of 31 December 2027

Indicator	Discount period				Discounted value non-current asset
	31 December 2024	31 December 2025	31 December 2026	Residual value as of 31 December 2027	
1	2	3	4	5	
Rate calculation					
Discount factor	0.7530	0.6850	0.6232	x	
Non-current assets:	Discounted value				
Buildings and structures	2,418.20	2,568.80	3,263.64	1,973.27	13,774.33
Buildings and structures	2,418.20	2,568.80	3,263.64	1,973.27	13,774.33
Machinery and equipment	151.39	160.82	204.32	123.54	862.35
Vehicles	2.86	3.03	3.86	2.33	16.27
Other property, plant and equipment	31.42	33.38	42.41	25.64	178.98
Total:	**2,603.87**	**2,766.03**	**3,514.22**	**2,124.78**	**14,831.93**

Source: *compiled by the authors of this study*

Table 6. Intangible assets of PJSC "Agrofirm "Provesin" (Lviv) as of 1 January 2022

Operation	Initial cost, UAH thsd	Discount rate	Useful life, years	Note
1	2	3	4	5
Intangible assets	10.0	9.92	2	-
Total:	***10.0***	***X***	***X***	***X***

Source: *compiled by the authors of this study*

$$PV = 10 \div (1 + 0.0992)^2 = 10 \div \div 1.20824064 = 8.28 \textit{ UAH thsd.} \quad (5)$$

According to the data presennted in Tables 3-6, in the Actuarial Balance Sheet (Actuarial Statement of Financial Position), Form No. 1-a, the carrying value of fixed assets of "Agrofirma "Provesin" PrJSC is UAH 6,381 thsd, and the discounted value is UAH 14,832 thsd, while intangible assets are UAH 10 thsd and UAH 8 thsd, respectively. Thus, the difference in valuation amounts to UAH 4,899 thsd for fixed assets and UAH 2 thsd for intangible assets, and should be reflected in the equity of the agricultural enterprise in the eponymous Section IV, Form 1-a. Due to this information content of actuarial reports, the investor will immediately see the prospects for changes in the property potential of agribusiness in the future, which will encourage them to invest temporarily free financial resources in its restoration and further progressive development.

Generally, the differences between the book and discounted valuation of not only assets but also liabilities of an enterprise should be recorded in the actuarial accounting system in 3D actuarial accounts, which should be opened as a separate class 10 in the Chart of Accounts. Thus, to account 10 "Fixed assets", it is worth opening an actuarial 3D subaccount of the corresponding procedure 1010 "Fixed assets at discounted value", which will facilitate the procedure for reflecting such an assessment of these objects in the actuary's reports.

DISCUSSION

The diagnostics of the situation of Ukrainian agricultural enterprises under martial law indicates an urgent need not only to preserve but also to strengthen their position in the international agricultural arena. For this, agricultural enterprises need additional funding and full and comprehensive support from both the state and foreign partners. Due to these circumstances, it is quite reasonable to model the cash flow management of agribusiness in the context of its financial security. Effective financial modelling of cash flow management of an agricultural enterprise requires appropriate information support to assess the prospective change in the property potential of agribusiness, from the actuarial accounting system, which will help to improve the image of its investment attractiveness in foreign markets. Agribusiness value management based on the information content of actuarial accounting and reporting includes modelling net cash flows for the future and assessing the value of its property potential aimed at actual and potential external investors or other capital providers in the financial markets.

The post-war recovery of the national economy requires improvement of the conventional accounting methodology based on the actuarial aspect. That is why actuarial accounting and reporting should become structural elements of the accounting system and the information basis for an objective analytical assessment of the prospects for changes in the economic potential of agribusiness. In other words, the level of investment attractiveness of an agricultural enterprise should be reflected in its actuarial statements. PJSC "Agrofirma "Provesin" does not prepare actuarial reports, as it is not yet regulated by law in Ukraine. The purpose of actuarial reporting is to create an image of investment attractiveness of the business for a potential foreign investor or other capital provider to attract the necessary financial resources, with further improvement of liquidity and solvency in the context of asset security, security of interaction with counterparties and financial security of the enterprise as a whole. In addition, when preparing actuarial reports, under the conditions of martial law in Ukraine, it is necessary to consider such external financial risks as inflation and currency risks. Actuarial accounting support in the n-dimensional format is the information basis for analytical assessment of the dynamics of monetary assets and prospects for changes in the value of an agricultural enterprise. As a result, conventional accounting accounts are transformed into 3D actuarial force accounts that form a spatial interpretation of the prospects for changes in the property and financial potential of a business in a multidimensional format.

S.I. Vasylyshyn *et al*. (2023) demonstrated the need to develop a system of accounting and information support for the potential of agricultural enterprises. Scientists emphasise that Ukraine is a leading exporter of agricultural products under martial law. Agricultural enterprises play a strategic role in the context of global food security. The researchers describe the importance of applying innovative approaches to managing the economic potential of agricultural enterprises based on high-quality accounting and information support to assess it reliably. The meaningful projection of economic potential has a direct impact on the accounting classification of non-current and current assets. This essential and substantive unity determines the need for the accounting service to consider the institutional influence of the external and internal environment on the activities of an agricultural enterprise. It is important to distinguish between accounting, analytical, and control subsystems, which transform incoming financial and non-financial information flows into integrated reporting. Such reporting comprehensively describes the main components of economic potential, such as financial, production, human resources, innovation, science and technology, environmental, information, marketing and management. An objective assessment of the value and economic potential of an agricultural enterprise can be quickly made based on actuarial management accounts prepared within the Conceptual Framework for Financial Reporting and understandable to a foreign investor or other capital provider, as they meet European requirements.

M.V. Leifura (2023) investigated the valuation of companies based on the Firm DCF (Discounted Cash Flow) model and covered the main advantages and disadvantages of applying this model in practice. The researcher found that to estimate the value of a company using modelling, it is necessary to apply a reasonable approach based on a comprehensive analytical assessment of financial and management reports and forecasting trends in income and expenses. Leifura suggests that the most effective method for valuing a company in the context of the income approach is the discounted cash flow (DCF) method. This position is fully supported by the scientist, since it is the discounting procedure that allows for an objective assessment of the present value of future cash flows and provides reliable information about the financial condition of an agricultural entity through the lens of actuarial reporting.

M.I. Diachenko and V.O. Zhmudenko (2023) assessed the current state of the agricultural sector of Ukraine and the areas of restoring economic potential in the context of transformational changes. The researchers stressed that despite the significant potential of Ukrainian agriculture, the war has a significant impact not only on global but also on internal food security. The researchers describe the current model of agribusiness development under martial law. The war against Ukraine threatens food security around the world, as Ukraine is a significant food producer and exporter. Financial support from partners for Ukrainian agriculture is helping to overcome the global food crisis, according to the Committee on Agrarian and Land Policy. The European Bank for Reconstruction and Development (EBRD) and the Netherlands, as a founding member of the EBRD and an important donor to the Bank's activities in Ukraine, will provide a EUR 25 mln financial guarantee to support private companies, specifically those operating in critical sectors related to agriculture and food processing. The support will focus on food security and will help to leverage further funding from the EBRD in Ukraine.

According to L.O. Boiko and V.O. Boiko (2023), an important priority for the development of the Ukrainian agricultural sector is free access to financial flows of farmers, as well as the availability of credit lines. Authors fully agree with this approach, since the specifics of the functioning of agricultural enterprises are characterised by the seasonality of agricultural production, and, accordingly, certain periods of time require significant amounts of funding to ensure the continuity of production processes. The author strongly emphasises that under martial law, agricultural producers should use all available resources to preserve agribusiness and ensure food security of the state. At the same time, the scientist argues that the key area in the Agricultural Sector Recovery Plan is the Green Deal, i.e., sustainable, clean, and safe environmental management by European countries. According to the researcher, Ukrainian farmers are making significant efforts to ensure the country's food security and hope for financial assistance from the international community. Admittedly, attracting additional funding in the form of foreign direct investment will help modernise all sectors of Ukrainian agriculture, including processing. The actuarial concept of value-based financial management of companies aimed at a foreign capital provider in the medium term can improve the image of the investment attractiveness of domestic farmers.

V. Ilchuk *et al*. (2020) emphasise the importance of the mechanism of value-based financial management of enterprises, which in the context of investment activity involves increasing the investment rating and investment attractiveness. The information subsystem of such a mechanism includes accounting and analytical, regulatory and methodological support. Accordingly, accounting and analytical support is formed by financial and management reports; accounting registers; primary accounting information on business transactions, etc. That is, according to scientists, the initial data for analytical research is formed based on accounting information from the following systems: accounting (which is divided into financial and management), tax, and statistical accounting. Notably, accounting has moved to a new stage of development – the actuarial stage, and therefore the modern accounting and analytical support of the mechanism of value-based financial management of agricultural enterprises should be supplemented by actuarial accounting, which is aimed at increasing the investment attractiveness of agribusiness.

For the first time among Ukrainian scientists, the concept of actuarial accounting in the context of the triple-entry system of accounting and improvement of internal control was covered in a joint study with V. Evdoschak and Y. Manachynska (2015). The innovative approach to improving the accounts by separating actuarial force accounts is based on calculating the prospective development potential of an agribusiness in the context of its sale as an integral property complex (IPC). Considering this, actuarial accounting is designed to factor in the force of changes in both the internal and external environment of an agricultural enterprise.

O.V. Stashchuk (2022) evaluated modern approaches to the concept of value-based financial management and suggests that it requires a comprehensive transformation of financial modelling algorithms for cash flow management in the context of the development of a new paradigm of financial management. The author's conclusions indicate that there is an urgent need to manage not only the finances of an economic entity, but also the overall management of an agricultural enterprise within the financial management system, which is transformed into a value-based approach to financial management of business processes. The interaction of modern digital and information accounting technologies and their active implementation in the company management system results in a comprehensive reorientation of the financial flow management system in business processes to the principles of business valuation in the financial management system as a whole.

O. Fomina et al. (2020) emphasise the importance of managing the valuation of agribusinesses based on the application of a multivariate actuarial model using the adjusted net asset method. The authors focus on the relationship between the accounting information disclosed in the financial statements of agricultural enterprises under international financial reporting standards (IFRS) and actuarial management reporting. The latter, in the context of the Conceptual Framework for Financial Reporting, facilitates the foreign investor's management decision-making on the expediency of investing temporarily free financial resources in the development of the agricultural sector of Ukraine.

L. Shapoval and Ju. Kolotii (2019) analyse the problems of financial modelling of companies' cash flow management. They defined the basis for the OCF model and proposed an algorithm for its construction. It is proposed to use the model for assessing the efficiency of cash flows of companies in practice, which will help to identify the key factors influencing the efficiency of financial and economic activities and will allow calculating the degree of this influence. This integrated approach based on financial modelling and cash flow forecasting is important for improving the financial position and minimising the risk of cash shortages. In the context of the post-war recovery of the national economy, the analytical assessment and modelling of trends in the net cash flow of an agricultural enterprise in the future should be based on actuarial accounting and reporting data. This will not only increase the investment attractiveness of Ukraine's agricultural sector but will also create a foundation for the food and financial security of domestic farmers.

According to I.I. Pasinovych and V.M. Hutak (2023), financial security of an enterprise is an important component of economic and corporate security. Financial security includes budgetary, banking, insurance, monetary, currency, stock, and investment components. The latter shows the possibility of attracting, investing, and using investment resources in the development of an agricultural enterprise. Scientists identify financial security with a certain level of financial standing and associate it with the ability to develop one's own financial strategy. The term "corporate security" is gradually gaining ground, which, along with economic and financial aspects, focuses on corporate governance mechanisms. Corporate governance itself is formed from the internal systems used to manage and control the agricultural holding. In essence, corporate governance involves the exercise of power in corporate-type agricultural enterprises. In other words, corporate governance forms the legal framework for determining the rights and responsibilities of different parties to an agricultural holding, as "agency conflicts" may arise in the course of business activities between the factual owners of agribusiness (real shareholders), top managers, and creditors. The actuarial concept of accounting, which forms a proper system of financial monitoring of top management activities to overcome "managerial opportunism", can overcome agency contradictions in agricultural holdings. According to the US approach, corporate governance helps to assess the ways in which factual and potential investors or other capital providers ensure a return on investment in agricultural production. The information content of the actuarial financial statements, specifically the Actuarial Cash Flow Balance Sheet (Form No. 3-a), serves as the basis for assessing the economic value of agribusiness and is based on the net present value of future cash flows, which ensures that the "conflict of interest" is levelled in the context of implementing the corporate finance theory.

I.V. Tomashuk and E.A. Borbolyuk (2023) assessed the importance of the agricultural sector of the economy in ensuring food security in Ukraine. The researchers emphasised that strengthening the country's food security requires a comprehensive interaction of all parameters of the development potential of domestic agricultural actors with economic, social, and environmental aspects that guarantee this security. The authors describe the structure of food security in Ukraine, which at the regional level includes the creation of a food fund through budgetary funding and loans. Ensuring food security requires a comprehensive approach and the implementation of modern strategies. Among the priority approaches, scientists identify investments in agriculture to ensure an adequate level of production growth and food reserves in the country. The range of strategic guidelines that ensure food security includes: providing preferential loans to farmers to restore equipment and production facilities damaged or destroyed as a result of hostilities; attracting foreign investment to restore the infrastructure for storing, transporting, and processing agricultural products; and conducting ongoing environmental and economic analysis. The active use of the latter will ensure a comprehensive and comprehensive analytical study of the implementation of the Sustainable Development Goals of Ukraine until 2030 and careful environmental management in the context of achieving food security and promoting inclusive agricultural development.

S.V. Stepanenko (2023) emphasises the importance of investment support for the inclusive and sustainable development of agribusinesses. This is an important aspect that affects the strategic development of the agricultural sector and rural areas to ensure their competitiveness. Investments create a strategic potential for addressing the challenges of sustainable agricultural production and improving the quality of life of the country's population. Notably, the financially secure functioning and protection of agricultural enterprises under martial law requires the introduction of more innovative technologies for making management decisions in the context of multidimensional space (n-D-format of business processes) with an appropriate actuarial accounting and analytical foundation. It is actuarial accounting that allows assessing the prospects for changes in cash flows, which will help increase the investment attractiveness of agribusiness and ensure its financial security. Based on the information from the actuarial accounting system, it is possible to create an image of the investment attractiveness of an agricultural market entity based on actuarial reporting and conventional methods of financial (economic) analysis. The information basis for actuarial reporting and economic analysis tools allow reflecting the potential for agribusiness development, both in the short and long term, which helps to attract investment from foreign capital providers.

Generally, various aspects, such as accounting and analytical support for financial modelling of cash flow management of an agricultural enterprise, affect the investment attractiveness of agribusiness. The researchers define the importance of clarity of accounting and reporting information for analytical assessment of the prospects for changes in the economic potential of agribusiness by real and potential foreign investors, as well as other capital providers. Objective accounting and analytical support for managing the growth of economic potential in the medium term plays a crucial role in attracting the necessary amount of financial investment in the development and post-war recovery of agribusiness.

CONCLUSIONS

This study covered the actuarial information support for the management of financial resources of an agricultural enterprise. Financial modelling of cash flows based on accounting actuarial and reporting content allows real and potential foreign investors to make an analytical assessment of the prospects for changes in the economic potential of agribusiness. The key stages of the procedure for financial modelling of cash flows in the financial security system, such as determination of the forecast net cash flow, calculation of the discounted value of assets, distribution of cash flows among the assets of the enterprise, reflection of the discounted value of all assets in the actuarial statements, and assessment of the potential for changes in the value of agribusiness are considered. The influence of the discount rate on the assessment of changes in the value of an agricultural enterprise through the lens of such forms of actuarial reporting as the "Actuarial Balance Sheet (Actuarial Statement of Financial Position)" and the "Actuarial Balance Sheet of Cash Flows" was studied. The expediency of documenting the procedure for selecting the discount rate is substantiated, specifically, with mandatory fixation in the accounting certificate and consolidation in the accounting policy. The study analysed the forecast net cash flow of PJSC "Agrofirma "Provesin" for 2024-2027 and determined that the amount of free cash flow should be identical both from the standpoint of an agricultural enterprise and from the standpoint of a real investor. The study emphasised the significant influence of information content of actuarial financial statements according to the valuation of assets at discounted value on the investor's decision to invest temporarily free financial resources. It was highlighted that the difference in the valuation of assets and liabilities of an agricultural enterprise at book and discounted values should be recorded in the system of actuarial accounting and actuarial 3D force accounts. The study proposed the possibility of opening a separate class 10 "Actuarial Accounts" in the Plan of Accounts for Accounting for Assets, Capital, Liabilities and Business Transactions of Enterprises and Organisations. To increase the investment attractiveness of agricultural enterprises and the post-war recovery of the agricultural sector of Ukraine, the procedure for modelling monetary assets in the financial security system based on the data of actuarial accounting and reporting was substantiated.

Further research will focus on investigating and analysing the best digital actuarial accounting technologies in the management of the value of agricultural enterprises and their possible implementation in Ukrainian accounting practice. It is necessary to analyse digital software products for the agricultural sector to improve the efficiency of agricultural enterprises, as well as to analyse the advantages and possibilities of using cloud services to digitise the accounting and analytical support for management in agriculture.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Фінансове моделювання управління грошовими потоками для безпеки агробізнесу: обліково-аналітичний аспект

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Анотація. Дослідження пов’язане з грошовими потоками сільськогосподарських підприємств, є досить актуальним, оскільки аграрний сектор є однією важливих галузей національної економіки, а продовольча безпека України стає стратегічним пріоритетом в контексті повоєнної відбудови та вимагає ефективних управлінських підходів для підвищення інвестиційної привабливості та сталого розвитку у сфері сільськогосподарської діяльності. Мета дослідження полягає в аналізі, розробці та вдосконаленні вже наявних пріоритетних підходів до управління грошовими потоками для фінансової безпеки агробізнесу з урахуванням обліково-аналітичного аспекту. Серед використаних методів було застосовано статистичний метод, методи прогнозування та фінансового моделювання, аналітичний метод та метод дисконтування. В процесі дослідження було проведено аналітичну оцінку і моделювання перспектив зміни чистого грошового потоку аграрного підприємства в середньостроковому періоді, визначено необхідність застосування актуарного обліку та звітності для залучення необхідного обсягу іноземних інвестицій в розвиток галузі в контексті фінансової безпеки аграріїв. Досліджено роль актуарного облікового забезпечення в управлінні вартістю агробізнесу для його фінансової безпеки. Було змодельовано процес управління потоками грошових коштів на сільськогосподарському підприємстві ПрАТ «Агрофірма «Провесінь». Досліджено актуарне інформаційне забезпечення із системи актуарного обліку для здійснення оцінки перспективної зміни майнового потенціалу агробізнесу. У ході дослідження було зазначено, що управління грошовими потоками у сільському господарстві передбачає використання інноваційного обліково-аналітичного забезпечення управління, яке б сприяло підвищенню іміджу інвестиційної привабливості на зовнішніх ринках. Практичне значення дослідження полягає в розробці конкретних практичних рекомендацій для топ-менеджменту аграрних підприємств з метою ефективного залучення необхідного обсягу фінансових ресурсів для сталого розвитку та повоєнного відновлення агробізнесу

Ключові слова: грошові активи; інвестиційна привабливість; сільське господарство; актуарна звітність; продовольча безпека

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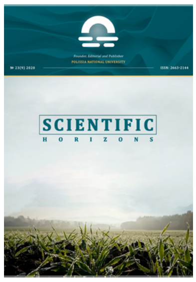

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Smart agriculture for urban regions: Digital transformation strategies in the agro-industrial sector for enhanced compliance and economic growth

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Abstract. Research on this topic is becoming relevant due to the growing pressure on agriculture in urbanized regions due to population growth, climate change and the need for sustainable production, which requires the introduction of smart technologies to increase productivity and sustainability. The purpose of this paper is to investigate the impact of growing pressures on agriculture in urbanized regions due to population growth, climate change and sustainable production needs. The methods used include the analytical method, classification method, functional method, statistical method, synthesis method, and others. The study found that modern digital transformation technologies have great potential to improve agricultural productivity and sustainability in urbanized regions, particularly in Latvia and the European Union. The use of data analytics allowed identifying key trends and patterns, which contributed to informed decision-making in the agricultural sector. The study results confirmed the need to implement comprehensive strategies to achieve compliance with sustainable development standards and ensure economic growth. The introduction of modern agricultural technologies, such as aeroponics and hydroponics, has focused on innovative methods to increase productivity and ensure sustainability. The use of the Internet of Things (IoT), data analytics, and modern agricultural technologies has led to

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an increase in the efficiency of urbanized agriculture. The practical significance of the work lies in the development of specific recommendations aimed at optimizing the functioning of agriculture, which will increase the efficiency of resource use and ensure sustainable economic growth in urbanized regions

Keywords: agrotechnology; advanced technologies; agribusiness development; environmental; monitoring

INTRODUCTION

In today's world, amidst rapid urbanization, agriculture is becoming a key sector that is under increasing pressure and requires innovative strategies to effectively enter a new stage of development. Against the backdrop of population growth, extreme climate change and the growing need for sustainable production, the agricultural sector in urbanized regions is facing increased pressure. Current challenges, such as the EU's agricultural reform, which focuses on green initiatives and technological transformation of production, provide an important context for researching digital transformation strategies (Fama & Corrado, 2023). This research is critical for the development of innovative strategies aimed at optimizing production, rationalizing resource use, and achieving sustainable development in the agricultural sector.

From the perspective of smart agriculture for urban regions, the urban environments that employ interconnected technology to enhance the efficiency, viability, and sustainability of urban agriculture practices – are smart cities. In smart cities, agriculture is not just about producing food but doing so in a way that is deeply integrated with the urban ecosystem (dos Santos, 2016). This means efficiently using limited urban space through vertical farming and hydroponics, deploying sensors AI to precisely manage water and nutrient cycles, and using data to understand and predict urban food demands. The goal of smart agriculture in smart cities is to create a closed-loop system that minimizes waste and maximizes resource use. It's a system where the production of food is tailored to the consumption patterns of the city's inhabitants, where transportation distances are minimized, and where the impact on the environment is considered in every farming decision (Volianska-Savchuk *et al.*, 2023).

The problematic of this study was to analyse not only from a technological point of view, but also taking into account the social, economic and environmental aspects of implementing digital strategies. This required examining the interaction between modern technologies and production processes, as well as their impact on consumers, rural communities, and the environment. It was important to consider the diversity of geographical, climatic, and economic conditions in urbanized areas to develop context-specific strategies. These issues are relevant not only for this study, but also for the governments of the European Union (EU) countries implementing their agricultural policies.

The authors H. Azadi *et al.* (2021) redefined sustainable agriculture from climate-smart to vulnerable. Their work was to study and evaluate the strategies used in the implementation of climate-smart agriculture and their transformation in the context of growing challenges that lead to vulnerable agriculture. However, this work did not consider the impact of the studied strategies on social and economic aspects, such as employment, farm income and access to food.

The study by L. Vistarte *et al.* (2023) shows a significant positive impact of the new common agricultural policy of Latvia on the climate resilience of the sector. The authors found that Latvia's new policy aimed at climate neutrality was successful. However, in order to maximize the positive impact, it is important to continue to improve technologies, involve farmers in the process, and take into account social and economic aspects in solving problems.

In their research, J.A.J. Mendes *et al.* (2022) conducted a study on the development of smart rural regions, their challenges, and opportunities. Their work has expanded the understanding of the dimensions of digital transformation in agriculture, providing important insights for improving strategies aimed at increasing the efficiency and sustainability of this important sector of the economy. However, the paper did not provide a detailed consideration of emerging technologies, such as blockchain, sensors, and their potential in digital transformation. These emerging technologies have notable effect for the smart rural regions like smart cities, as shown by other researchers (Popova & Cernisevs, 2023; Cernisevs & Popova, 2023).

The work of N. Griškjāne (2020) is dedicated to the assessment and development of the smart economy in Latvia. Her research concluded that the smart economy is important and promising for Latvia. With the proper implementation of strategies, the country can achieve sustainable economic growth, improve the quality of life of citizens and increase competitiveness in the global market. However, the author did not pay enough attention to the possibilities of using innovations in the field of social services and human capital development.

The research by D. Vasilevska and B. Rivza (2022) is aimed at studying the priorities and obstacles in the digital transformation of agriculture. Their work identified priorities in digital transformation for agriculture, analysed obstacles and provided guidance on how to overcome them. She also developed recommendations for agriculture and government agencies. However, the authors did not develop a clear plan for scaling up the initiatives so that their benefits and innovations could be effectively implemented in other areas.

The purpose of this study is to analyse and identify strategies for improving agricultural production processes to achieve higher levels of compliance and economic growth for urbanized regions.

MATERIALS AND METHODS

This study was carried out using methods that reveal the theoretical and practical content of the object. The analytical method in this study carried out a thorough analysis and processing of the collected data to identify trends in the use of digital technologies in agriculture and determine their impact on the production process, yields and economic indicators. This method allowed for a detailed review, which included a detailed examination and processing of various numerical data to identify the main trends characterizing agriculture in urbanized regions. It revealed the effectiveness of implementing smart strategies and made it possible to identify the benefits and challenges faced by the agricultural sector in the context of digital transformation. The analytical method also provided the basis for objective conclusions and recommendations for the development of smart agriculture in urbanized regions.

The classification method allowed systematizing and grouping the collected data, identifying common characteristics, and establishing links between different aspects of the use of digital technologies in agriculture in urban areas. The method helped to create a structured approach to understanding the diversity and specificities of digital transformation, which is important for formulating objective conclusions and recommendations. The functional method was used to structure and describe the functionality of different elements of the smart agriculture system for urbanized regions. The use of this method allowed for the analysis of the functions and interactions of these elements, but not in the context of direct analysis or evaluation. It identified the main functional tasks that need to be addressed for the effective implementation of digital technologies in agriculture in urbanized regions.

The statistical method in this study was used to systematize, process, and analyse numerical data. This method allowed transforming the collected data into numerical forms using the methods of sorting, grouping, and aggregation. By applying statistical measures such as mean, median and standard deviation, representative characteristics of the data set were obtained. By examining statistical trends, including changes over time, regional characteristics, and other factors that influence agricultural development in urbanized areas, a deeper understanding of the dynamics of this process was gained. Also, using a comparative analysis with statistical methods, various aspects of digitalization and its impact on agriculture were assessed, making clear the differences and similarities between different aspects of the topic under study. The synthesis method in this study was used to merge and synthesize various data and concepts to form an integrated and holistic understanding of smart agriculture in urbanized regions. This method contributed not only to the synthesis of existing knowledge, but also to the creation of new ideas and concepts that contribute to a deeper understanding and development of the research area. As a result of the synthesis method, a holistic view of the challenges and opportunities facing smart agriculture was developed, providing a deeper and more comprehensive understanding of the research subject.

RESULTS

Population growth in urbanized regions affects agriculture in a number of ways (Almadani & Mostafa, 2021). Firstly, population growth leads to a significant increase in demand for agricultural products, which poses challenges for the agricultural sector to ensure efficient and sustainable production and supply. The need to develop efficient supply systems to ensure reliable and rapid supply of agricultural products in urbanized areas is becoming increasingly important. Population growth is also creating a competitive environment among agricultural producers, requiring them to innovate to differentiate themselves and compete in the market. In the face of increasing demand and competition, the analysis showed that efficient production requires a shift to sustainable farming, using environmentally friendly methods and green technologies (Bunge *et al.*, 2022).

The use of smart agricultural technologies in the EU is becoming increasingly important, necessitating the introduction of the Internet of Things (IoT) and advanced agricultural technologies. This is essential for increasing productivity and optimizing agriculture, especially in the context of limited land space in large cities. An integrated approach takes into account the challenges and opportunities arising from population growth in urbanized regions of the EU and identifies ways to improve agriculture in urban areas. It recommends the creation of integrated networks of agricultural devices for automation and control over production, including automatic irrigation and plant growth monitoring systems.

Climate change and the need for sustainable production are having a significant impact on agriculture in urbanized areas (Dang *et al.*, 2021). On the one hand, climate change leads to unpredictable weather conditions, which creates challenges for the agricultural sector. In particular, the increase in extreme weather events, such as droughts or floods, requires the development of adaptation strategies and the use of technologies for balanced production in the face of unpredictability. On the other hand, the need for sustainable production in the EU drives the need to adopt environmentally friendly practices and technologies in agriculture. The results emphasize that the use of smart agricultural technologies and management systems helps to optimize resource use, reduce environmental impact, and ensure sustainable development of the agricultural sector.

The analysis revealed that the needs for sustainable production include two key recommendations. First, it is necessary to ensure sustainable development, which involves the introduction of efficient agricultural technologies and methods aimed at preserving natural resources and ecosystems. This includes the development of new agricultural technologies aimed at maximizing productivity with minimal use of resources and reducing the environmental impact of agriculture. Secondly, it is important to use environmentally friendly technologies to optimize production and reduce the negative impact on nature. These include the introduction of smart agricultural technologies and management systems aimed at increasing productivity and efficiency, while reducing the environmental footprint of agriculture. The development of organic and ecological farming methods is also identified as a key element for ensuring high quality products and maintaining ecological balance in the agricultural sector. Taking these aspects into account in agricultural planning and implementation is strategically important for achieving sustainable development in urbanized regions.

Figure 1 illustrates the impact of growing pressures on agriculture in the EU's urbanized regions due to population, climate change and the need for sustainable production. The figure shows the percentage distribution of pressures between key factors identified as resource efficiency, climate resilience and innovation in sustainable production in the EU.

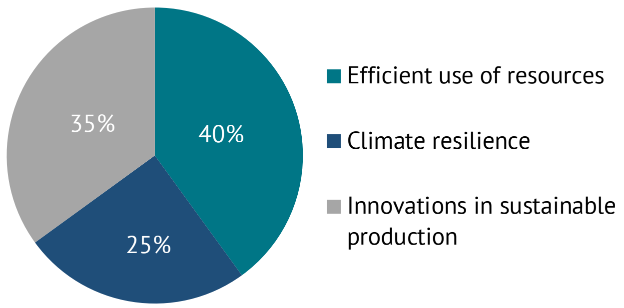

Figure 1. *Pressure factors on agriculture in the urbanized regions of the EU*
Source*: M. Agovino et al. (2019)*

The results of the study revealed a number of key challenges in agriculture for urbanized regions. Firstly, the identified challenges relate to production efficiency, where it is important to increase productivity and optimize the use of resources. These include improving agricultural practices, adopting new technologies and developing effective management systems. The second aspect of the challenges relates to resource management, where it is important to use land, water, and other materials efficiently. Ensuring the sustainable use of these resources includes water conservation strategies, the use of highly efficient land cultivation methods and the development of recycling technologies. The third aspect of the issue relates to the resilience of the industry to change, including changes in climate conditions and market trends. Ensuring the industry's flexibility and adaptability is key to overcoming the challenges posed by environmental change. This includes introducing innovative technologies, adapting to new climate conditions, and developing market adaptation strategies. These could include the development of specialized hydroponic farms and vertical farms that would allow for growing food in limited land space, reducing transportation and storage costs; setting standards for organic farming, providing subsidies for farmers using environmentally friendly practices; creating online markets where farmers can present their products and city residents can easily order fresh and sustainable food; organizing seminars, trainings, and access to modern agrotechnologies to improve the skills of farmers and stimulate their interest in innovation.

Digital transformation also includes the use of modern agricultural technologies as an important aspect of strategies (Quy *et al.*, 2022). The introduction of advanced agricultural equipment, including automated irrigation systems, drones for vegetation monitoring, and artificial intelligence systems for soil analysis, can increase productivity and efficiency. Drones are being used for area-based monitoring of farmland in Latvia, which allows problems such as disease or insufficient watering to be quickly identified and the necessary measures taken. In the EU, farmers use drones and satellite imagery to collect data on the condition of their fields. This allows them to obtain detailed information about plants, their diversity, and condition, which in turn helps them to more accurately determine fertilizer needs and regulate irrigation. The use of such technologies helps to reduce costs and maximize yields.

Modern agricultural technologies take into account the use of advanced agricultural equipment, such as automated irrigation and production control systems, which helps to optimize production processes and reduce resource consumption (Sinha & Dhanalakshmi, 2022). Aeroponics and hydroponics, as innovative methods of plant cultivation, make it possible to grow crops without using soil, ensuring efficient and environmentally friendly production. These agricultural technologies help to adapt EU agriculture to the conditions of urbanization, where limited land space and high demand for products put increased pressure on production processes. The introduction of such innovations helps to increase productivity and ensure the sustainable development of agriculture.

Data analysis is a key strategy for digital transformation in the agricultural sector. The use of analytical tools allows for the efficient processing of a large amount of information obtained from sensors and other sources used in smart agriculture (Totin *et al.*, 2018). At the EU level, data analysis is aimed at studying global trends in agriculture and market conditions, which helps to adapt policies and strategies to changes in agriculture and the market environment. In Latvia, the analysis of crop and product quality data helps farmers to make decisions on the optimal time of harvesting, inventory management and consumer research. The overall goal of data analysis is to improve the efficiency, resilience, and sustainability of production through the rational use of resources and the introduction of advanced technologies.

The concepts of making agriculture more compliant with sustainability and product safety standards include a number of strategies and initiatives. The strategy of improving production processes and introducing environmentally friendly methods of land cultivation and plant growing contribute to the compliance of agriculture with environmental standards. The use of advanced agricultural technologies, such as precision farming and automated irrigation systems, helps to reduce resource use and minimize the negative impact on the environment. Adopting high standards of product safety and quality, including traceability and quality control systems, ensures that agricultural products meet the highest standards and are safe for consumers. The introduction of certification systems, such as organic production or quality management systems, helps to confirm that products meet established standards and ensures consumer confidence. Educational initiatives among farmers on the importance of responsible and sustainable production help to promote awareness and adoption of modern farming practices. These strategies are aimed at achieving a high level of compliance of agriculture with modern requirements, which is an important aspect for ensuring sustainable development and competitiveness of the industry in urbanized regions. A look at economic growth in the EU and Latvia reveals several aspects that point to opportunities to improve the efficiency and competitiveness of agriculture in urbanized areas.

The introduction of modern technologies, such as IoT, agrotechnology and digital management systems, has contributed to increased productivity and reduced production costs. The effective use of technology has allowed agriculture to become a more efficient and profitable industry (Pilvere *et al.*, 2022). In the EU, IoT technology is used to monitor the condition of animals, determine important parameters of animal husbandry, and automatically detect diseases. Large farms use sensors to monitor temperature, humidity, movement, and other parameters in animal quarters, ensuring optimal conditions for their health and growth. This approach not only increases the efficiency of the farm, but also helps to reduce losses through early detection of problems and quick response to them. In Latvia, the use of monitoring systems includes control over milk production, the quality of its suitability for consumption and the health of livestock. Farms use modern sensors and data analysis systems to check the quality of milk, determine its composition and identify any possible anomalies that may indicate animal health problems or incorrect housing conditions. This allows farmers to respond quickly to potential problems, ensuring high product quality and animal health. When anomalies are detected, monitoring systems can automatically notify owners or veterinarians to take the necessary measures to improve animal conditions and treatment. This approach helps to increase the efficiency of agricultural production and ensure high quality agricultural products. The study identified opportunities to reduce losses in production processes, ranging from efficient use of resources to supply chain optimization and minimization of losses, which contributed to increased gross production and resource savings. The introduction of technological innovations and efficient resource management contribute to economic growth in the agricultural sector in urbanized regions.

In the EU, manufacturers are introducing hydroponic systems for growing plants without soil (Ravis & Notkin, 2020). Hydroponics is the cultivation of plants in aqueous solutions with the necessary nutrients, rather than in traditional soil. This allows for optimal use of water and fertilizers, providing plants with all the necessary elements for growth. Hydroponic systems typically reduce water consumption compared to traditional agriculture and allow for precise control of the environment for optimal plant growth. In Latvia, farmers are developing and implementing vertical farms, which allow plants to be grown in vertical layers. This approach makes the most of limited space and increases production per unit area. Vertical farms typically use light-emitting diode systems to provide plants with the necessary light for photosynthesis. This method of cultivation is effective in urban environments and allows for the production of high-quality agricultural products without a large land area. Both approaches demonstrate innovation and respond to the challenges of modern agriculture, such as efficient use of resources, increased productivity, and adaptation to the limited space available in large cities.

Urbanized areas and agriculture interact in population growth and urban expansion. The link between urbanization and agriculture lies in the impact of urban structures on rural areas and the interaction between urban and rural aspects. First, urbanization leads to a reduction in available rural land space due to the growth of urban areas, which necessitates the use of limited rural land resources in a more efficient and sustainable manner. Second, urbanized areas create demand for rural products within the city. There is a growing need to grow food closer to consumers, which promotes the development of modern farming methods, such as vertical farming or other innovative techniques. Third, urbanization also affects agriculture through pollution, emissions, and other aspects of urban infrastructure. Waste management and green urbanization are becoming key elements for sustainable agriculture. Thus, urbanized areas determine the specifics of agriculture through interaction with urban structures, requiring agriculture to adapt to the urban environment, increase efficiency and ensure sustainable production.

To achieve sustainable development and optimization of agriculture in urbanized areas, it is recommended to implement a set of strategies and measures. It is important to develop and implement improved production processes aimed at balancing economic growth with environmental sustainability, including the use of advanced technologies to optimize production processes and reduce losses. The use of digital technologies, such as monitoring, data analytics and automation systems, will help reduce costs, improve the supply chain, and increase overall production efficiency. This also includes the adoption of best practices to optimize resource utilization and increase economic competitiveness. Developing and implementing strategies to improve the safety of agricultural products is an important aspect. This can be achieved through standardization and systematization of production processes. Establishing a system for systematic monitoring and adaptation to changes in the environment, climate, and market conditions will allow for effective monitoring and timely response to various challenges.

Further development of technologies includes continuous innovation activities aimed at improving agriculture (Majore & Majors, 2022). It includes the introduction of advanced agricultural technologies, the development of the latest tillage methods and modern automation tools that will help increase productivity and optimize production processes. Expanding the network of partners involves active cooperation of agricultural enterprises with representatives of other industries and partners. This includes the creation of integrated solutions that bring together the agricultural sector with technological, scientific and trade partners to jointly implement projects and solve common problems. Joint innovation and experience in digital transformation helps to develop a sustainable and efficient agricultural sector in different countries. Increased competitiveness reflects the expected increase in production efficiency and quality, which, in turn, will help strengthen the market position of urbanized agricultural regions.

DISCUSSION

This research article addressed the key topic of smart agriculture for urbanized regions in the EU and Latvia, focusing on digital transformation strategies in the agricultural sector, which is a relevant and promising topic of current challenges and opportunities. The discussion of this topic covered various aspects that affect the efficiency and sustainability of urbanized agriculture. The high level of urbanization requires the agricultural sector to improve and adapt to new realities. Modern technologies, such as IoT, sensors, data analytics, and artificial intelligence, are opening up new opportunities to increase productivity, manage resources, and create environmentally sustainable agricultural processes. The use of digital technologies has made it possible not only to monitor production in real time, but also to improve product quality and safety control (Samoilenko *et al.*, 2022). Automation and monitoring ensured compliance with standards and market requirements, increasing competitiveness. Digital technologies have been used to rationalize the use of resources such as water, fertilizer, and energy, which is important for sustainable production. Automated systems respond to changes in climate conditions by optimizing irrigation and fertilization processes. The implementation of digital strategies has become a catalyst for economic growth in agriculture. The creation of new business opportunities, the development of innovative products and services, and the expansion of sales markets have all been made possible by digital transformation (Kryvovyazyuk, 2023).

Study by A. Renner *et al.* (2020) examined the expected biophysical consequences of this process and their impact on the environment. The findings allowed developing specific strategies and recommendations for achieving sustainable agricultural development, balanced between economic benefits, social responsibility, and nature conservation. The introduction of internal use has helped to create conditions for biodiversity by reducing the use of pesticides and other chemicals that have a harmful impact on the ecosystem. The introduction of environmentally friendly technologies and practices had a positive impact on the health of the local population and contributed to the development of green jobs in rural communities (Lada *et al.*, 2023). Both studies are aimed at improving production processes and achieving efficiency in the agricultural sector. Smart agriculture, using digital technologies such as IoT and data analytics, aims to optimize agriculture and ensure sustainable development in an urban environment (Rexhepi *et al.*, 2023). On the other hand, research on environmental pressure considers internal use as a tool to reduce the negative impact of the agricultural system on the environment. Internal use helps to reduce the negative impact on groundwater and ensures optimal use of natural resources. Regarding the positive impact of environmentally friendly technologies, it is important to recognize that this opens up prospects for improving the quality of life of the local population. Reducing the use of chemicals in agriculture reduces environmental pollution and contributes to a safer and healthier environment for communities to live in and develop (Wrzecińska *et al.*, 2023).

The study by G. Gagliardi *et al.* (2021) aimed to examine the impact of IoT technologies on smart agriculture and identify the key benefits and challenges associated with the implementation of IoT in agriculture. As a result, using IoT, farmers can monitor various production parameters, such as soil moisture, temperature, nitrogen levels, and others. This allows them to optimize irrigation, apply fertilizers only where they are needed, and ensure the best conditions for plant growth. Both studies consider various aspects of the impact of digital technologies on the agricultural sector, but from different perspectives. This paper is distinguished by its overall strategic perspective, which provides an in-depth analysis of digital transformation strategies aimed at increasing compliance and ensuring economic growth in the agricultural sector. The study by the researchers specializes in the application of IoT technologies in agriculture, providing specific insights and success stories. The focus on the application of IoT technologies makes it more specific and detailed, analysing specific uses of these technologies in agricultural production.

The aim of the study by I. Pilvere *et al*. (2022) was to conduct a comprehensive assessment of the impact of the European Green Deal policy on Latvian agriculture, taking into account support payments. The analysis shows that the European Green Deal policy has already had a significant impact on the structure of agriculture in Latvia. Increased requirements for environmental sustainability and fair use of resources have led to restructuring of production processes. At the same time, support payments have become more focused on environmentally sustainable production, which encourages farmers to adapt new methods. Both studies address relevant aspects of agriculture, but have different approaches. The work of the researchers is more focused on the impact of the new EU policy on agriculture in a particular country (Latvia). Support payments aimed at environmentally sustainable production can encourage farmers to adopt new methods. This will help ensure a smooth transition to more sustainable and environmentally friendly agriculture. This study highlights the use of digital technologies in agriculture in urbanized regions, particularly for increasing compliance and economic growth. Both studies are important for understanding the current challenges and opportunities in agriculture.

The study by A. Rehman *et al*. (2022) was aimed at reviewing IoT technologies used for monitoring and control in smart agriculture. As a result, IoT technologies have great potential to improve the efficiency and sustainability of the industry. The integration of data, collection, and analytics has enabled farmers to make informed decisions, which is an important step towards sustainable and efficient agriculture. The central goal of both studies is to increase agricultural productivity through the introduction of innovative technologies and strategies. Both studies emphasize the use of modern technologies to optimize agriculture. They recognize the importance of implementing IoT, data analytics and digital strategies to achieve productivity gains. They also consider the importance of data collection and analysis for making informed decisions. However, their differences lie in the specific topics and target groups they address. Taking both approaches into account, comprehensive strategies can be developed to improve agriculture in both traditional and urbanized areas.

Paper by J. Yang *et al*. (2021) reviewed the basics and benefits of using IoT in the context of smart agriculture. The introduction of IoT in agriculture has made it possible to create automation systems for machinery. Tractors, seeders and other machines can work autonomously, taking into account the needs of a particular field. IoT is also used to monitor the condition of animals. Both studies have a similar focus on the use of modern technology to improve agriculture. However, this paper looked in detail at the application of IoT in specific aspects of production. IoT for animal monitoring is really useful in agriculture. It helps farmers in tracking animal health and welfare, which in turn has a positive impact on productivity and product quality. However, it is also important to consider the possible challenges related to data privacy and security of IoT in agriculture to ensure that its potential and limitations are fully understood.

The article examined how digital transformation is affecting the development of agriculture in urbanized regions of the EU and Latvia. One of the key ideas was the use of modern technologies, in particular, the IoT, to optimize production processes, which included the introduction of sensors and data collection and allowed for effective monitoring and management of various aspects of agricultural activities. The study also highlighted the importance of digital transformation to improve the industry's compliance with modern standards and requirements. The use of analytical tools and technologies has allowed businesses to adapt to changes in legislation, ensuring high quality and safety of products. Digital transformation strategies, as shown in the study, contributed to economic growth. This was achieved by optimizing production processes, reducing losses, and increasing productivity. In addition, the study highlights the importance of using innovative agricultural technologies to ensure sustainability and competitiveness.

Overall, the study not only addresses the urgent issue of smart agriculture for urbanized regions, but also provides a valuable contribution to the field of digital transformation strategies in the agricultural sector. It offers specific strategies, taking into account various influencing factors. Its results serve as a basis for further developments and implementations in the field of modern technologies in the agricultural sector.

CONCLUSIONS

The study found that in Latvia and in the EU, modern digital transformation technologies have great potential to improve the productivity and sustainability of agriculture. The use of the IoT has proven to be an important and promising area for optimizing production and increasing the efficiency of the industry. The introduction of animal welfare monitoring systems, soil and plant analysis, and automation of production processes using IoT has enabled accurate and efficient management of various aspects of agriculture. The use of data analytics stands out in its ability to identify key trends and patterns, which has contributed to informed decision-making. Modern agricultural technologies, such as aeroponics and hydroponics, have become a strategically important step towards achieving sustainable and efficient production in urbanized regions.

The results of the study highlighted the need to implement comprehensive strategies to achieve compliance with sustainable development standards and ensure economic growth in Latvia and in the context of the EU. The analysis of the needs for sustainable production and recommendations for the use of modern technologies indicate opportunities to improve the efficiency and competitiveness of agriculture in the context of urbanization. In summary, the study highlighted that digital transformation in agriculture for urbanized regions has great potential to address current challenges. The findings suggest that the use of digital technologies is a promising and effective approach. Another important aspect of the findings is the need to comply with sustainable development standards and the emphasis on economic growth strategies. The recommendations for continuous monitoring, adaptation and implementation of the latest technologies have become key points for the further successful development of digital agriculture in urbanized areas.

The study serves as a practical guide for agricultural enterprises, organizations, and authorities in Latvia and the EU seeking to implement digital innovations in their operations. The recommendations on the effective use of technology, sustainability standards and economic enhancement strategies can serve as a basis for the development of specific smart agriculture programmes and projects.

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CONFLICT OF INTEREST

None.

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Розумне сільське господарство для урбанізованих регіонів: стратегії цифрової трансформації в агропромисловому секторі для посилення відповідності та економічного зростання

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Анотація. Дослідження на цю тему стає актуальним через зростаючий тиск на сільське господарство в урбанізованих регіонах через зростання населення, зміну клімату та необхідність сталого виробництва, що вимагає впровадження смарт-технологій для підвищення продуктивності та стійкості. Метою цієї статті є дослідження впливу зростаючого тиску на сільське господарство в урбанізованих регіонах через зростання населення, зміну клімату та потреби сталого виробництва. Використані методи включають аналітичний метод, метод класифікації, функціональний метод, статистичний метод, метод синтезу та інші. Дослідження показало, що сучасні технології цифрової трансформації мають великий потенціал для підвищення продуктивності та сталості сільського господарства в урбанізованих регіонах, зокрема в Латвії та Європейському Союзі. Використання аналітики даних дозволило виявити ключові тенденції та закономірності, що сприяло прийняттю обґрунтованих рішень в аграрному секторі. Результати дослідження підтвердили необхідність впровадження комплексних стратегій для досягнення відповідності стандартам сталого розвитку та забезпечення економічного зростання. Впровадження сучасних сільськогосподарських технологій, таких як аеропоніка та гідропоніка, зосередило увагу на інноваційних методах підвищення продуктивності та забезпечення сталості. Використання Інтернету речей, аналітики даних та сучасних агротехнологій призвело до підвищення ефективності урбанізованого сільського господарства. Практична значущість роботи полягає в розробці конкретних рекомендацій, спрямованих на оптимізацію функціонування сільського господарства, що дозволить підвищити ефективність використання ресурсів та забезпечити стале економічне зростання в урбанізованих регіонах

Ключові слова: агротехнологія; прогресивні технології; розвиток агробізнесу; навколишнє середовище; моніторинг

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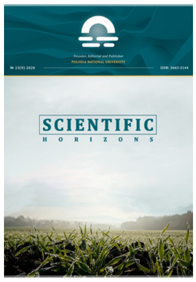

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Animal husbandry market in Kazakhstan: Dynamics and prognosis

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Abstract. Studies of livestock dynamics and productivity is an effective mechanism to monitor the possible risks to the breeding of animals of different species and a timely response to strengthen the food security of the Republic of Kazakhstan. The research aims to conduct an analytical review of the state of livestock breeding on the main species of animals that are bred in its territory and the forecast of development soon. The materials for the analysis were reports from the Bureau of National Statistics of the Republic of Kazakhstan, as well as information in Kazakh and foreign periodicals on the state of livestock breeding and current news in the agricultural sector of the country. The statistical materials on the number of animals of different species, gross output received from them, investment activities, and other indicators directly or indirectly related to the dynamic indicators in animal husbandry were used. The results of the analytical review revealed positive annual dynamics of increasing the number of livestock of most species of farm animals in the country. Thus, the growth of cattle population is 2-4% from year to year, a similar situation is observed in sheep (2-4%)

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and poultry (4-10%); the only exception is a decrease in pig population by 5-9%. Populations of cattle, sheep and goats, horses, and camels are mainly concentrated in small farms and individual households, so a significant part of products from them does not get to industrial processing, which leads to the need to import them to meet the needs of the urban population. The main mass of poultry, although represented by the livestock of specialized agricultural enterprises, the volume of output does not provide the necessary volume, which is reflected in an increase in purchases abroad. Therefore, to date, there are no visible prerequisites for reducing the number of animals of all species that are bred in Kazakhstan, and the state support of livestock breeding can contribute to the further growth of livestock

Keywords: gross output; epizootic situation; livestock breeding; subsidies; profitability; export of products

INTRODUCTION

Significant areas of natural pastures, as well as suitable climatic conditions, contributed to the development of livestock in the Republic of Kazakhstan. As of 2021-2023, under the Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (2024), there was a growth in the gross output of the agricultural sector. For 11 months of 2023 gross output from agricultural enterprises was 8,774.3 billion tenge, which is 8.5% higher than the level of the corresponding period of the previous year. This growth in livestock production is due to an increase in the production of eggs – by 4.9%, milk yield of raw cow milk – by 2.1%, and the volume of the slaughter of livestock and poultry in live weight – by 0.7%. This was achieved, in most cases, due to an increase in the livestock number. S.M. Yessengaliyeva *et al.* (2021) have also pointed to a steady increase in the number of farm animals over the years. This extensive approach is less effective and has shortcomings compared to the indicators of industrial livestock breeding. Following R.N. Zhangirova (2020), this is due primarily to the weak technical equipment of the industry and the lack of innovative approaches in Kazakh animal husbandry. A similar opinion is held by R.K. Konuspayev *et al.* (2020). Therefore, in the analytical review of the state of livestock breeding and the prospects for its development in the near future, it is necessary to consider the possibility of intensification of one or another branch. That is especially relevant in terms of breeding work with livestock, which is not given proper attention in the country.

But despite the active saturation of the market with the products of the agricultural sector and livestock directly, the food market remains import dependent. Thus, under the portal ElDala.kz for 2018-2020, exports of food products to Kazakhstan increased by 5.3 percent, from $3.1 billion to $3.3 billion. In 2020, its growth was 7 percent, which is 1.9 percent higher than the same figure for 2018 (Kazakhstan's food exports..., 2021; Pedigree cattle are..., 2022). This trend indicates a significant shortage of agricultural products of Kazakh origin, which reduces the food security of the republic. But simply increasing the productivity of animals or increasing their number will not solve this problem. In the opinion of N.B. Syzdykbayeva *et al.* (2021), it will be more effective to increase the competitiveness of Kazakh products in the local and world markets. These problems can be solved only with the stable growth of the agro-industrial complex of the country, as well as the continuous development of the industry with the help of new technologies and innovative approaches.

To eliminate this situation, the government provides several economic investments in the development and stimulation of agricultural and processing enterprises in Kazakhstan in the form of various state programs. Such support in recent years is expressed in the form of direct subsidies to agricultural producers. Thus, in 2022 more than 124 billion tenge were invested in the agricultural sector of the country for development (Subsidies to livestock..., 2022). Capital investments in cattle breeding have a rather long payback period and therefore need an analytical justification and constant monitoring of investment risks and possible ways to increase productivity in the industry and increase animal productivity. Therefore, the research aims to study the dynamics of livestock development, and productivity in different sectors of livestock breeding in the Republic of Kazakhstan and forecast the situation in them in the near future. It will allow to control of possible risks for animal breeding in the country and timely provide state support to owners of animals to increase the profitability of production and thereby strengthen the food security of the republic.

LITERATURE REVIEW

Livestock is the basis of the global food program, which provides sustainable development of all agriculture and provides jobs and food for almost 1.3 billion people (Moving towards sustainability..., 2021). The livestock market is quite specific and strictly structured both by animal species and geography (Meat industry size..., 2024). Production of such products varies by the type of animals raised, the purpose of their maintenance, the intensity of livestock production systems, and the resources they consume. In recent times, the market for animal products has been changing due to increased demand for different animal products, population and income growth, and changes in livestock technologies (Alders *et al.*, 2021; Bohatko & Utechenko, 2024).

One of the main factors that affect the intensity of the breeding of certain animals in a particular territory is the economic benefit from the sale of their products (Wei & Zhen, 2020; Adams *et al.*, 2021). This is the main goal of animal breeding, and it is characteristic of all countries, regardless of geographical and political foundations (Xu *et al.*, 2019;Abay & Jensen, 2020). X. Lan *et al.* (2021) and Z. Mehrabi *et al.* (2020) point out that it is the demand for certain animal raw materials or products that determines the priority species of animals that are bred in a particular region and determine their direction of productivity. Quite often, the demand for certain goods in neighbouring countries adjusts for the development of some direction of livestock breeding and the size of purchasing prices in the country. A similar situation is now observed in Kazakhstan, where prices for meat animals have increased, which was provoked by a shortage of meat raw materials in neighbouring countries, China and Russia (Meat in Kazakhstan..., 2023). The greatest increase in prices was observed precisely in the regions bordering these countries. This situation occurs in other countries as well and is a common reaction to various economic and social influences (Taipov *et al.*, 2017; Food and Agriculture..., 2022).

In Kazakhstan, as well as in Kyrgyzstan, given the presence of large areas that can be used for grazing animals, individual farms of the population are widespread (Yang *et al.*, 2022). Such agricultural enterprises are perceived differently within a single livestock market. Most authors point to the main role of such farms in the future (Wang *et al.*, 2020;Tirkaso & Hailu, 2022). They, the increase in individual farms, associate with the increase in employment of the rural population and less negative impact of them on the environment (Enahoro *et al.*, 2019). While the opposing viewpoints to the low productivity of animals in this category of farms and the low level of innovation in them (Baltenweck *et al.*, 2020). The low productivity of grazing, which is the main source of fodder for individual farms in Kazakhstan, also contributes to this. The analysis of pastures by T. Chen *et al.* (2020), R. Sabyrbekov (2019), and J. Umuhoza *et al.* (2021) points to the decreasing productivity of pastures in post-Soviet Central Asian countries. In addition, pasture productivity is significantly affected by weather conditions (Liang *et al.*, 2021). Lack of moisture and high temperatures during the growing season significantly reduce the efficiency of grazing and do not allow for optimal animal productivity.

Therefore, the state of any livestock industry in the country is influenced by a significant number of both internal and external factors. The more of them can be addressed during an analytical review of livestock market conditions, the more effective and accurate will be the obtained forecast of the development of this or that type of animal in the near future.

MATERIALS AND METHODS

The main source of materials for the analytical study was monthly and annual reports of the Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (2024), which are publicly available. For this purpose, statistical materials were used on the main indicators of the state and development of livestock in the republic, gross output received from it, investment activities in these sectors of agriculture, and other indicators directly or indirectly related to the dynamic indicators in the livestock sector of Kazakhstan. The studies did not include certain types of animals, the number of which occupies less than 1% in the country or are bred in a small area – rabbits, deer, bees.

In addition, reports and current news in the agricultural sector of the country, as well as the results of studies of several authors who analysed the impact of social, economic, weather, and other factors on the development of livestock in different countries were the materials for preparing the analytical review. Predominantly Central Asia studies were used, the conditions of which are similar to those of the Republic of Kazakhstan. Special attention was also devoted to the studies on the dynamics of animal population development and the established features of agricultural development in neighbouring post-Soviet countries, which also closely correlated with the trends in the Kazakh livestock sector of the agrarian business. The publication date of the study was also considered. In other words, studies from 2019 or newer were used in the research. The research results had to be conducted on animals, which are the main species in the agrarian sector of the Republic of Kazakhstan. Also, special attention was paid to the publications, which revealed the influence of various factors on the state and future development of animal husbandry, characteristic of the current political and economic situation in the world. The citation index was also used for the inclusion of studies in the research.

When preparing analytical materials, the main attention was focused on statistical information on the number and dynamics of livestock of different types of animals, their productivity in different types of farms, typical for Kazakhstan, and the volume of processing of animal raw materials at industrial enterprises. Special attention was also devoted to the analysis of exports and imports of both live animals and livestock products for the analytical period, which allowed to highlight the economically sound promising areas for further development of animal husbandry in Kazakhstan in the coming period. As a result of the system analysis causal and regular correlations between the indicators of the number of animals and economic (financial) prospects in each of the sectors of animal husbandry of Kazakhstan were determined.

Publication materials and presented research results selected for analysis were subjected to comparison and critical analysis methods depending on the number of animals on which research was conducted and the levels of statistical validity of the results obtained. Since most of the studies included in the analysis were conducted on the same animal species, but in different economic and geographical conditions, the method of analogy was used for their comparison to obtain ideas about general trends in the development of animal husbandry and the influence of various factors on its further development. At the stage of preparing intermediate and conclusions, the method of generalization was used, which helped to combine one's judgment with the conclusions of researchers who had conducted similar studies. In preparation for the forecast of the development of this or that branch of cattle breeding in the country's epizootic situation in neighbouring regions with Kazakhstan, factors of influence on the world economic situation of international conflicts and other global processes were also considered.

RESULTS

The dynamics of changes in the number of animal populations by species and direction of productivity were tracked by the materials of statistical information from website of the Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (2024). For 2020-2022 the information on the changes in the population of this or that animal species was used for the calendar year, as of December 31 of the current year. Information on animal population for 2023 was taken only for the first month of the current year to identify the main trends compared to the same situation in the previous year. Analysis of statistical materials shows an annual increase in the number of most species of productive animals from year to year (Fig. 1).

For a more detailed state of the livestock market in the Republic of Kazakhstan, it was decided to analyse the situation in each branch of livestock separately. The livestock of this species is represented by dairy, beef, and combined production animals. The main mass of animals is concentrated in farms and individual farms of the population (Table 1).

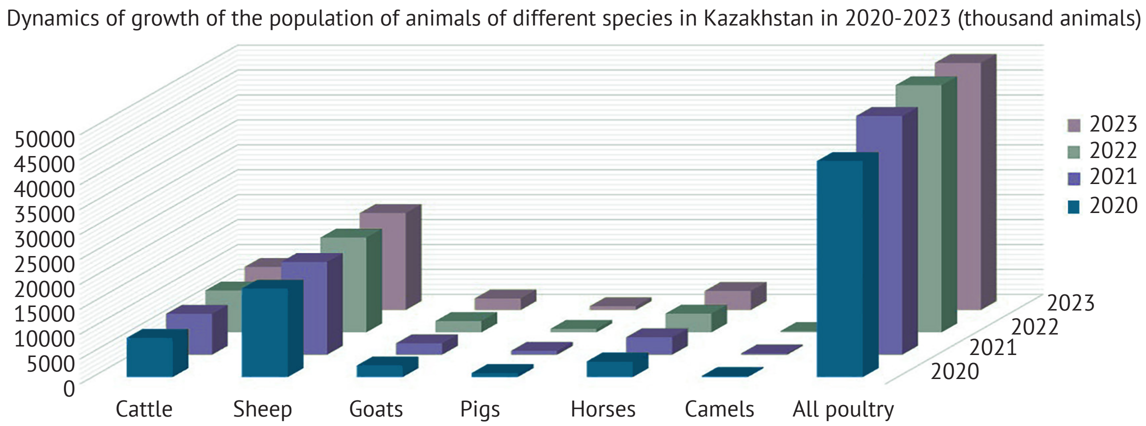

***Figure 1**. Change in the number of farm animals in 2020-2023*
***Source:** compiled by the authors based on the data by Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (2024)*

***Table 1**. Dynamics of change in the population of cattle in the controlled period in the Republic of Kazakhstan*

Cattle type	Year	Total, animals	Agricultural enterprises, %	Farms, %	Individual farms, %
Cattle	2020	7,850,045	9.6	36.4	54.0
	2021	8,192,415	9.4	38.2	52.4
	2022	8,395,031	9.6	40.2	50.2
	2023	8,638,307	9.4	39.5	51.1
	2024	8,651,105	9.5	40.0	50.5
Including cows	2020	4,008,270	7.3	39.9	52.8
	2021	4,235,659	7.1	41.5	51.4
	2022	4,501,585	6.9	42.0	51.1
	2023	4,504,845	7.0	41.7	51.3
	2024	4,506,823	7.1	42.0	50.9

***Source:** compiled by the authors based on the data by Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (2024)*

The data in the table indicate a positive trend in the country to increase the total number of cattle and cows. With a constant growth of the number of animals of this species, its distribution among different types of farms remains approximately at the same level. Insignificant fluctuations between adjacent years do not exceed statistical error. This positive trend allows to expect a slight increase in the next year, which is already confirmed by the figures for the first month of 2024. Population growth is + 4.5% for cattle and + 6.2% for cows through the same period in 2023. The number of cattle is mainly concentrated in individual farms and relatively small farms. These farms also contain the bulk of dairy and combined production animals. Households have 75.4% of dairy cows and 21.9% of dairy and meat cows. On farms – 42.3% and 39.6% respectively. Whereas in agricultural enterprises up to 52.9% of the livestock is represented by meat breeds. Due to this differentiation in cattle breeding, the bulk of raw milk is obtained in farms with a low level of manufacturability (Fig. 2).

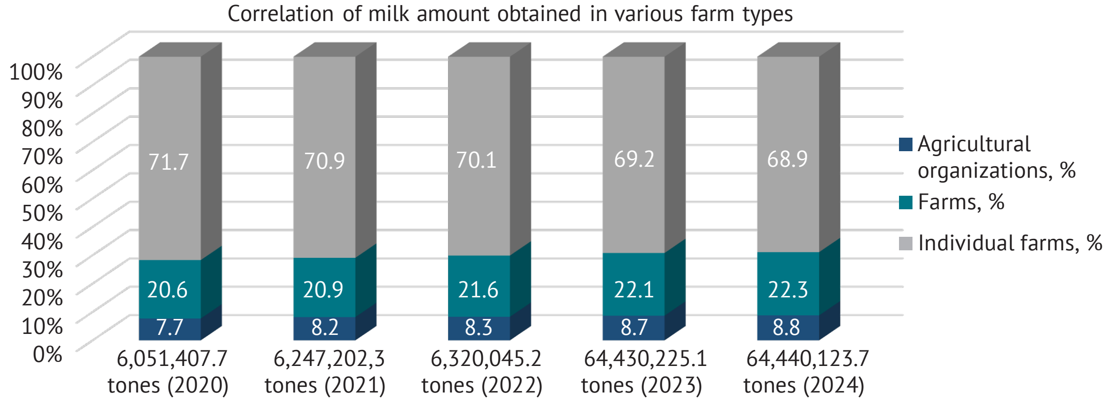

***Figure 2**. The structure of raw milk obtained in the Republic of Kazakhstan during the controlled period*
***Source:** compiled by the authors based on the data by Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (2024)*

Milk from individual farms, due to the lack of primary processing technology, has low-quality indicators and only part of it is used for further processing (up to 30%). The bulk of such milk is used in households or sold on the market after processing into other dairy products – sour cream, cottage cheese, and butter. This leads to a shortage of dairy products in the Kazakh market and the need to export them to meet the needs of the population. The main problem of cattle breeding in the country is low breeding work among livestock, so the milk yield of cows in farms and households is 1,865-2,465 kg of milk. It is a very low indicator and its increase in the level of animal productivity of agricultural enterprises (5,128 kg) could compensate for the lack of raw milk in the country. Unfortunately, it will not be possible to quickly raise the breeding level of local cattle in the near future.

This is primarily due to the impossibility of purchasing breeding animals in neighbouring countries because of the military conflict between Ukraine and Russia, and these countries were the main suppliers of breeding resources for cattle, and building up the productive capacity of cows by raising their replacement cattle requires a long period. Therefore, in the near future, there are no prerequisites to reduce the number of cattle in the country and a significant increase in their productivity. And the introduction of a new form of subsidizing the development of the agrarian complex is possible and will increase the number of cattle by 5%, as planned by the Ministry of Agriculture (Vidyanova, 2023). The breeding of these animals is traditional in Kazakhstan. As with cattle, the predominant concentration of animals of these species in farms and individual farms of the population (Table 2).

***Table 2**. Dynamics of sheep and goat number change in the Republic of Kazakhstan for the monitored period*

Cattle type	Year	Total, animals	Agricultural enterprises, %	Farms, %	Individual farms, %
Sheep and goats	2020	20,059,486	4.8	41.9	53.3
	2021	20,876,776	5.0	43.4	51.6
	2022	21,276,685	5.5	45.6	48.9
	2023	21,753,031	5.2	44.1	50.7
	2024	21,754,985	5.3	44.0	50.7

Table 2. Continued

Cattle type	Year	Total, animals	Agricultural enterprises, %	Farms, %	Individual farms, %
Incl. sheep	2020	17,751,517	5.5	42.3	52.2
	2021	18,595,263	5.7	43.7	50.6
	2022	19,014,009	6.0	47.1	46.8
	2023	19,438,210	5.8	45.5	48.7
	2024	19,439,123	5.9	46.1	48.0
Incl. goats	2020	2,307,969	0.9	30.8	68.3
	2021	2,281,513	1.0	31.3	67.7
	2022	2,262,676	1.0	33.0	66.0
	2023	2,314,821	1.0	32.0	67.0
	2024	2,315,862	1.1	31.9	67.0

Source: *compiled by the authors based on the data by Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (2024)*

As can be seen from the above table, the total number of sheep and goats in the country increases every year. At the same time, the growth between species is not proportional. So, if in sheep breeding there is a constant growth of 2-4% per year, the number of goats is practically at the same place with slight fluctuations of ± 1.5% between adjacent years. Thus, in January 2024, the sheep population grew 6.7% and the goat population was 3.8% less than in the same period in 2023. This disproportion is partly caused by increased demand for mutton in neighboring countries and the Middle East, where Kazakhstan exports its products (in 2023 alone 6,653 tons of meat were sold for export). This growth in exports of sheep meat during this period (sheep meat sales abroad increased by almost 3.5 times in 2023 compared to 2022) made it possible to compensate and cover export revenues from the sale of live sheep, which arose with the introduction of the ban on the sale of breeding animals, from $17 million in 2022 to zero in 2023. This lability allowed the sheep population to be maintained and show a positive trend in January 2024. The development of sheep breeding is also stimulated by the growth of purchasing prices for wool. The amount of wool sold from different forms of sheep farms is shown in Figure 3.

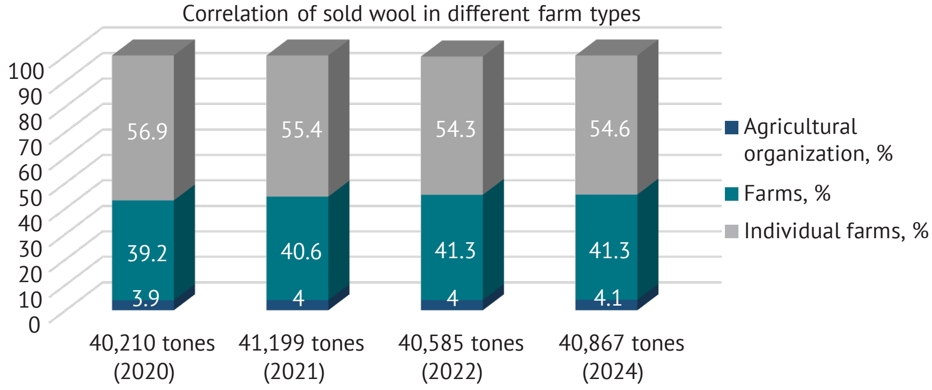

Figure 3*. Wool obtained and sold in the Republic of Kazakhstan during the controlled period*
Source: *compiled by the authors based on the data by Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (2024)*

Analysis of the quality of raw wool, which was sold, showed that the bulk of it – more than half, belonged to the coarse type, and fine and semi-fine wool, which in monetary terms is valued more highly was not more than a third of the entire mass. Based on the above mentioned, it can be expected that the number of sheep will continue to grow, and the structure of the population will change towards the breeding of fine-fleece sheep breeds. While the main product of goat breeding is milk productivity, and due to the specificity of this product, there is no reason to expect an increase in demand for this product (Tokysheva *et al.*, 2022). Therefore, no significant increase in the goat population should be expected in the near future. There are no economic or any other prerequisites for that.

In contrast to other animal species, raising and using poultry on an industrial scale involves significant investments in technological equipment and specialized feed. Therefore, the bulk of poultry is kept in specialized agricultural enterprises (Table 3).

***Table 3**. Dynamics of farm poultry number change in the Republic of Kazakhstan for the monitored period*

Cattle type	Year	Total, animals	Agricultural enterprises, %	Farms, %	Individual farms, %
All poultry types	2020	43,334,963	71.0	1.6	27.3
	2021	47,884,731	73.4	1.3	25.2
	2022	49,563,295	75.0	1.1	23.9
	2023	49,560,685	74.9	1.2	23.9
	2024	49,560,101	75.0	1.2	23 8
Including egg chicken	2020	20,783,218	59.6	1.9	38.4
	2021	23,037,044	63.0	1.6	35.3
	2022	21,498,958	63.2	0.8	36.0
	2023	21,308,313	65.6	0.8	33.7
	2024	21,308,998	65.5	0.9	33.6

***Source:** compiled by the authors based on the data by Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (2024)*

Given the high intensity of growth of specialized broiler poultry crosses and the relatively high profitability of its production, authors can predict a further increase in the number of poultry in the republic in industrial farms. This is confirmed by the increase in the number of poultry in 2024 in agricultural enterprises by 7% compared with the same period in 2023. At the same time, the number of poultry in other categories of farms is 3-4% short of last year's level. The decrease in the number of livestock may be due to the seasonality of poultry breeding in households. The maximum number of livestock in such farms is observed in the spring and summer periods. Therefore, there is no reason to worry about the reduction of the poultry population in households.

A similar situation is observed in the breeding of egg production chickens. The main of their livestock is placed in specialized enterprises, which will create comfortable conditions for poultry housing and productivity (Fig. 4).

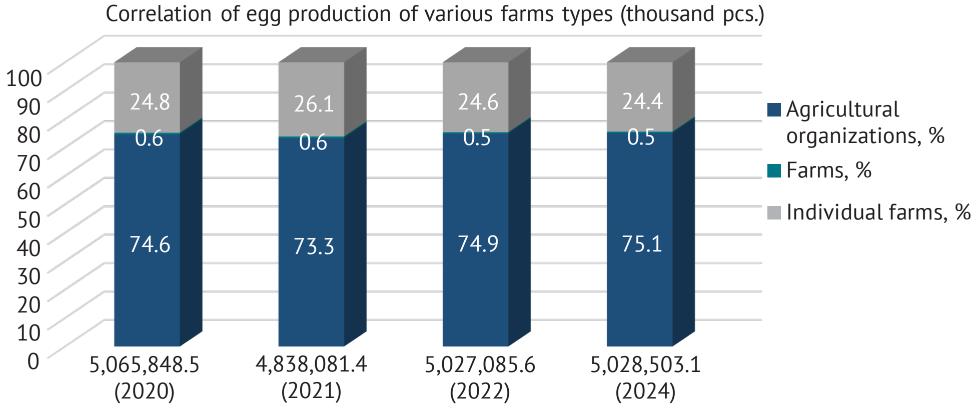

***Figure 4**. Production of eggs in Kazakhstan for a controlled period*

***Source:** compiled by the authors based on the data by Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (2024)*

Given that the egg-laying period is much longer than the time it takes to raise broilers, the prognosis for 2024 is optimistic. Much of the risk will depend on the epizootic situation for poultry diseases and the implementation of the necessary veterinary and sanitary measures. The decrease in the number of eggs obtained in 2021 was associated with a decrease in the number of egg birds due to the outbreak of avian influenza. Therefore, for this category of poultry, kept in significant numbers in small areas, the forecast remains cautious. Animals with small numbers of individuals whose products are used practically to meet the demand of the local population were included in this category (Table 4).

***Table 4**. Dynamics of farm animal number change in the Republic of Kazakhstan for the monitored period*

Cattle type	Year	Total, animals	Agricultural enterprises, %	Farms, %	Individual farms, %
Pigs	2020	816,736	31.2	8.9	59.9
	2021	776,117	33.4	10.5	56.0
	2022	706,519	32.5	9.7	57.8
	2023	709,796	31.7	9.2	59.1
	2024	710,986	31.9	9.0	59.1
Horses	2020	3,139,831	6.3	47.8	45.9
	2021	3,489,777	6.6	49.3	44.1
	2022	3,759,468	7.2	50.7	42.1
	2023	3,808,939	7.1	50.2	42.6
	2024	3,810,002	7.2	50.4	42.4
Camels	2020	227,703	7.4	41.7	50.9
	2021	243,365	7.0	43.0	50.0
	2022	254,595	6.7	43.9	49.4
	2023	257,303	6.7	44.3	49.0
	2024	258,987	6.8	44.0	49.2

***Source:** compiled by the authors based on the data by Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (2024)*

While the number of horses and camels increases slightly every year, the number of pigs is constantly decreasing. The main reason for this situation is the recent outbreak of African Swine Fever (ASF) in China when due to quarantine measures partial slaughter of animals in the border areas was carried out. In addition, the increase in the Muslim denomination in Kazakhstan will only reduce the consumption of pig meat. Therefore, from this point of view, and given the risks in the re-emergence of ASF, the likelihood of increasing the number of pigs in the near future remains unlikely.

As for the other two animal species, given their historical aspect in the life of the local population and the use of their products for the preparation of traditional national products, it is safe to assume that the number of these populations will remain at the same level from year to year, or will tend to a slight increase.

DISCUSSION

The main goals of the functioning of the livestock market in any country are to provide the population with food products and to improve the material well-being of owners of animal resources. Therefore, it is more appropriate to consider this sector of the economy in two positions: as a branch of agriculture, which is a priority of domestic state policy, and as a business project, since the main purpose of keeping animals, in any category of agricultural enterprises, is to make a profit. From these two positions, authors will try to build a forecast of the development of one or another branch of livestock farming in the republic for this and next years.

The main goals of the functioning of the livestock market in any country are to provide the population with food products and to improve the material well-being of owners of animal resources (Bondarenko *et al.*, 2023). Therefore, it is more appropriate to consider this sector of the economy in two positions: as a branch of agriculture, which is a priority for the state. The state program of support of the agroindustrial complex of the country will be significantly revised this year in Kazakhstan. First, it will allow access to subsidies for all market participants, regardless of the type and number of animals kept. Before that, the funds were available only for agricultural enterprises, and it was not available for farms and private farms the population where most of the cattle were kept. In this case, an obligatory condition of granting funds for owners of animals was an annual increase of livestock by at least 5% and get not less than 80 heads of brood per 100 sows (Vidyanova, 2023). This approach alone will make it possible, if not to increase the number of livestock in the country, then at least to stabilize it at the achieved level. The only subsidized item for agricultural enterprises will be compensation for the purchase of pedigree resources from imports and cheaper artificial insemination services (What has changed..., 2023). Such an approach can partially increase the growth of animal productivity, and hence improve the state of livestock breeding. Other authors – M. Petrick (2021), G. Chi *et al.* (2020) – also point to the positive impact of state support on the development of the livestock industry in the economy of post-Soviet countries. The studies of these authors indicate that in all countries as a result of such support, there was an increase in livestock and (or) animal productivity.

Following A. Lavruk (2019) in 2018 in Ukraine 4.23 billion UAH were allocated from the budget, as a result, there was an increase in pork production by 108.9% and poultry meat by 613%, as well as an increase in egg production by 176%. Following M. Petrick (2021), agricultural enterprises for animal breeding in post-Soviet countries of Central Asia began to appear only recently and this process is partially encouraged by generous state support. Following the research of G. Chi *et al.* (2020), state support for livestock farming in Kyrgyzstan contributed to the increase in agricultural production through new technologies and equipment. Using these two positions, an attempt to build a forecast of the development of one or another branch of livestock farming in the country for this, and the next years will be made.

Another positive factor that contributes to improving the state of livestock in the country is the use of natural pastures, which are rich in Kazakhstan (Bulegenova *et al.*, 2019). This system of animal breeding allows to obtain products with minimal capital investment, which contributes to improving profitability and maximizing profits from livestock breeding. A group of researchers led by S. Robinson *et al.* (2021) shared the same opinion. They point to the advantage of farms and individual farms of the population for livestock production in Kazakhstan, given the significant areas used as natural pastures, but the lack of state support, in their opinion, hindered the development of this category of farms. Therefore, a new approach to subsidizing livestock in the country, introduced in 2023, will stimulate the development of this category of farms and increase their animal productivity. To assess the potential of livestock development in terms of business attractiveness, such factors as the export of certain types of animals and their raw materials, as well as the provision of the domestic market with livestock products were used.

The results of the analytical study indicate the import dependence of livestock breeding in Kazakhstan, which was reflected in the purchase of horses for 5.3 million dollars over the past two years, and cattle for 55 million dollars over the same period. These figures indicate a shortage of breeding stock in the country for these types of animals, as evidenced by the volumes of purchased livestock only from European countries. As of the end of February 2023 imported more than 57 thousand breeding cows (Kazakhstan and EU..., 2023). Unfortunately, this approach of improving the breeding qualities of local livestock, by partially replacing it with imported, carries several risks. These are problems with the acclimatization of imported animals to the new conditions of maintenance and productivity, and an increased percentage of their retirement from the herd due to the impact of stress factors on them (Krasnova & Funta, 2023). Another significant factor of such policy was the reduction of the breeding animal market, caused by a Russian invasion as these countries were the main suppliers of breeding cattle to Kazakhstan. This, perhaps, explains the decrease of one-third in the volume of purchased heifers in 2022. The purchase of animals in other countries is less profitable due to the high prices of breeding resources there. This situation can fundamentally change the established principle of improving the country's herd by intensifying targeted breeding work among local animals. It looks realistic, considering also the compensation of the cost of artificial insemination of animals under budget programs. Moreover, this approach is also used in other countries to improve the productivity of livestock in the individual farm of the population. In India, subsidizing artificial insemination of cows with sperm divided by herds with high genetic productivity potential allowed to increase in the productivity of local herds up to 4000 kg of milk per lactation (Ministry of fisheries..., 2022). A similar situation was observed in Ukraine (Resolution of the..., 2018). The result of a partial reduction in the price of artificial insemination of cows for all categories of enterprises was an increase in milk productivity of cows in 2018 in agricultural enterprises to 5898.7 kg, and in the population – 4893.5 kg (Popko, 2020).

The situation in pig and sheep breeding is radically different from the previously mentioned industries. In recent years, the number of exported animals of these species has exceeded the volume of animals imported into the country. Sheep breeding has the greatest potential in cattle breeding in Kazakhstan. Statistical materials on the number of sheep and the volume of sales of products are consistent with the results of research conducted in Kyrgyzstan by K. Tilekeyev *et al.* (2016) and confirm the dominance of this industry in all Asian post-Soviet countries. In their opinion, individual farms of the population through the simplified system of taxation prevail in this region and are traditionally engaged in sheep breeding, which is associated with the predominantly pastoral breeding of these animals and the predominance of mutton in the diet of the population of the Asian republics. The growing volume of wool sales from year to year, especially of fine-fleece animal breeds, also contributes to the strengthening of the economy of the industry. Therefore, considering the forecast of sheep breeding development for the nearest future, authors can only hope for annual growth in the number of animals of this type with a possible increase in the number of fine-fleece breeds. The situation in pig breeding remains less clear. Despite the positive balance of export operations from the sale of live pigs, there is an annual decrease in the number of livestock. And this is aggravated by the increase in the number of imported pigs purchased in recent years. This situation can only be explained by the fear of breeding pigs after the outbreak of African swine fever in China in 2020. G. Gongal *et al.* (2022) suggest that providing appropriate veterinary and preventive measures to protect animal health will reduce the likelihood of particularly dangerous diseases spreading in Asia. Therefore, authors hope to stop reducing the number of pigs in Kazakhstan already this year and to increase it in subsequent years.

A similar situation with a high risk of morbidity and mortality is characteristic of industrial poultry production. Since most poultry are located within relatively small areas. But, despite this possibility, the number of poultry farms and their livestock in Kazakhstan is only increasing every year (Bayantassova *et al.*, 2023). The economic prerequisites that contribute to this are the annual increase in the imported purchase of poultry meat and eggs to meet the demand of the population of Kazakhstan. At the same time total sum spent for the purchase of poultry products on import balances on 30-33 million dollars per year. Also, one should not discard other attractive sides of breeding this type of animal: short interval of meat poultry breeding, high egg productivity, and fast payback period of investments in this industry. Therefore, based on statistical information and economic analysis of the situation in poultry farming in Kazakhstan, it is safe to expect only further growth of the poultry population in the country.

Summarizing the results of the analytical review, it can be stated that today in Kazakhstan there are no visible prerequisites for a decrease in the number of animals of all species that are bred in its territory, and constant government support can contribute to further growth of the population.

CONCLUSIONS

Based on the results obtained during the analytical review of the livestock industry in Kazakhstan and short-term forecasts of further development of this market, the following conclusions and suggestions for future studies can be made: Breeding animals of most species represented in Kazakhstan tend to continually increase their numbers from year to year. This happens in all farms, regardless of their category or the volume of their livestock. The only animal species whose numbers have been declining for several years now are pigs. This phenomenon was preceded by an outbreak of a particularly dangerous disease of African swine fever in neighboring China. As the epizootic situation improves, it is expected that the number of pigs will recover to their pre-disease level. There are economic prerequisites for this – a positive balance in the export-import operations for pig products.

Revision of the domestic livestock subsidy policy of Kazakhstan in 2023 will allow access to state funding for all categories of farms engaged in livestock breeding. Given that the main livestock is concentrated in farms and individual farms of the population, which previously could not apply for a subsidy, and the fact that the main condition for providing such assistance is an annual increase in the number of animals by 5%, it is worth expecting growth of the entire livestock market in the country. A no less positive factor in the development of cattle breeding in Kazakhstan is its lability. The ban on the sale of breeding animals in 2022 could have brought down sheep breeding as an industry, but a quick change in priorities and an increase in exports of mutton several times allowed not only to keep the number of livestock at the same level but even increase it in January 2023 compared to the same period in 2022.

Based on the abovementioned facts it can be confidently stated that the livestock market in 2023 will not only maintain its position but also show an increase in the number of animals of most species that are bred in Kazakhstan. To make a more reasonable long-term forecast for the development of livestock in Kazakhstan in the near future plans to study the main trends in the industry in different regions of the country, mainly with a maximum concentration of animals of certain species, and to identify the main factors that may affect the dynamics of livestock.

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CONFLICT OF INTEREST

None.

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Ринок тваринництва в Казахстані: динаміка та прогноз

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Анотація. Дослідження динаміки поголів'я та продуктивності тварин є ефективним механізмом моніторингу можливих ризиків для розведення тварин різних видів і своєчасного реагування для зміцнення продовольчої безпеки Республіки Казахстан. Метою дослідження було проведення аналітичного огляду стану тваринництва за основними видами тварин, які розводяться на території країни, та прогноз розвитку на найближчу перспективу. Матеріалами для аналізу послужили звіти Бюро національної статистики Республіки Казахстан, а також інформація в казахстанських і зарубіжних періодичних виданнях про стан тваринництва і поточні новини в аграрному секторі країни. Використано статистичні матеріали щодо поголів'я тварин різних видів, отриманої від них валової продукції, інвестиційної діяльності та інші показники, які прямо чи опосередковано пов'язані з динамічними показниками у тваринництві. Результати аналітичного огляду виявили позитивну щорічну динаміку збільшення поголів’я більшості видів сільськогосподарських тварин в країні. Так, приріст поголів'я великої рогатої худоби становить 2-4 % з року в рік, аналогічна ситуація спостерігається у овець (2-4 %) та птиці (4-10 %); єдиним винятком є зменшення поголів'я свиней на 5-9 %. Поголов'я великої рогатої худоби, овець і кіз, коней і верблюдів переважно зосереджене в дрібних фермерських господарствах та індивідуальних домогосподарствах, тому значна частина продукції від них не потрапляє на промислову переробку, що призводить до необхідності її імпорту для задоволення потреб міського населення. Основна маса м'яса птиці, хоч і представлена поголів'ям спеціалізованих сільськогосподарських підприємств, але обсяги виробництва не забезпечують необхідного обсягу, що відображається на збільшенні закупівель за кордоном. Тому на сьогоднішній день немає видимих передумов для скорочення поголів'я тварин всіх видів, які розводяться в Казахстані, а державна підтримка тваринництва може сприяти подальшому зростанню поголів'я худоби

Ключові слова: валова продукція; епізоотична ситуація; тваринництво; субсидії; рентабельність; експорт продукції

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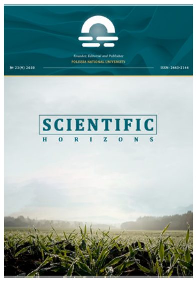

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Innovation enterprise development strategy in animal husbandry

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Abstract. The research relevance is determined by the need to create a strategy for the development of innovative entrepreneurial structures in livestock farming, the application of which can significantly increase the economic return on farms. The study aims to develop a strategy for the development of progressive livestock farms. The following general scientific methods were used for the study: analysis, synthesis, deduction, induction, and generalisation. Among the innovations considered were various genetic studies to improve the animal genome (the amount of meat and milk, the efficiency of feed assimilation), as well as the selection of genetically healthy embryos for breeding; the use of modern technologies in livestock farming, such as Radio Frequency Identification, Augmented Reality, Convolutional Neural Networks and Global Positioning System; technologies aimed at automating animal feeding processes. Particular attention was paid to various livestock monitoring systems created using advanced algorithms. A strategy for the development of innovative livestock farms was also presented, with drones, CCTV cameras, mixed reality glasses

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and scanner gloves as the main elements. Other necessary elements included a drone control module and software. Relevant calculations to prove the feasibility of implementing this strategy and its financial potential were also carried out. Provided that 82 units of various equipment are used, with a total cost of $187,970, the strategy provides for a profit of $1,072,030, with a 3-year implementation period and a payback period of less than 6 months. The practical significance of the information obtained is that it can be considered by researchers to further study the specifics of implementing innovative projects in livestock production, as well as to further develop various strategies for agricultural modernisation in Kazakhstan

Keywords: modernisation of farms; breeding traits; automated feeding systems; balanced rations; smart agriculture

INTRODUCTION

In Kazakhstan, agriculture plays an important role in the economic development of the country. There is still a relatively high level of private employment in livestock farming in some regions, and there are several farms specialising in the breeding of specific animals. Nevertheless, there is a certain chaotic approach and direction in the field of innovative projects related to this sector of agriculture, which undoubtedly slows down its development and prevents the active implementation of a comprehensive and systematic approach such as "smart" agriculture.

The topic of various innovative approaches in animal husbandry in Kazakhstan has been of interest to researchers such as O. Radchenko *et al.* (2023). Their study presents the concept of an automated pig feeding system that can improve feed utilisation, optimise feeding processes and increase the overall economic viability of farming. Another group of researchers, consisting of A. Dossanova *et al.* (2022) noted that West Kazakhstan, which is oriented in particular towards livestock production, is developing more slowly due to outdated farming practices. Agricultural enterprises in this region do not apply modern methods of animal housing and feeding, as well as technologies that could increase productivity and improve product quality. In a study conducted by E. Nasambaev *et al.* (2022), breeding farms in West Kazakhstan including Aisulu farm, Khafiz farm, Dongelek farm and Plemzavod Chapaevsky LLP were considered. The main objective of the study is to collect data on young Kazakh white-headed cows in different calving seasons. The development of young cows was studied monthly, including the morning weighing of calves before feeding and water supply. According to the data obtained, different calving results were observed on each farm depending on the time of year, which needs to be considered when planning more efficient herd renewal.

S. Bostanova *et al.* (2022) studied the dairy herd of Aina farm, and the milk produced. They found a tendency to decrease the fat/protein ratio to less than 1.1:1, which may be a consequence of consumption of an energy-rich but nutrient-deficient diet. Analysis of cow feeding on this farm indicates the predominance of a silage-concentrate feeding strategy, which mainly satisfies the energy and nutrient requirements of the animals. However, there is a deficiency of sugar, excess crude protein and starch and excess dry matter in the diet. It is observed that increasing the proportion of roughage and succulent forages in the herd's diet and improving the quality of haylage and silage in this case are necessary conditions for improving the quality of dairy production on the farm. Thus, the study demonstrates the possibility of an innovative approach to assessing the quality of cow nutrition through chemical analysis of milk composition. In a study conducted by M. Amandykova et al. (2023), the application of breeding trait analysis of meat-wool sheep in Kazakhstan was considered. The analysis identified regions in the genome structure associated with meat and wool quality, which is an important step in improving the breeding performance of the Kazakh fleshing sheep breed.

Despite significant research in various spheres of livestock production in Kazakhstan, specific strategies for the development of innovative entrepreneurial structures in livestock production in Kazakhstan are not often considered, although the creation and development of such strategies is one of the important directions of scientific research in the field of agriculture. Thus, the study aims to create a strategy for the development of innovative entrepreneurial structures in livestock farming.

MATERIALS AND METHODS

The following methods were used to investigate innovative entrepreneurial structures in livestock production: analysis, synthesis, deduction, induction, and generalisation. The analysis was used to examine in detail progressive methods and practices of livestock farming, including different approaches to the organisation of herd feeding, quality monitoring of animal health and comfortable stay of animals in pens, barns and other specially prepared buildings and premises. The analysis was also used to consider the economic feasibility and feasibility of particular technologies in the context of the active development of progressive livestock farms. Synthesis was used to bring together the techniques and innovations in livestock production identified in the analyses, as well as the specifics of their implementation in practice and related issues of efficiency, feasibility, and payback, for possible further application in the strategy. Synthesis was also used to structure information on the different approaches to livestock monitoring, which differ from each other in terms of the technical aspect of achieving the desired efficiency and accuracy in fulfilling the objectives of the system.

In the process of investigating innovative approaches, methods and technologies in livestock production, deduction was integral to a more in-depth analysis. Through the use of deduction, the innovations identified in the synthesis process were analysed in more detail. This method also allowed a closer look at the information on specific innovations and best practices applied in the organisation of production on farms, which was necessary for the development of the strategy. An important aspect is the adaptation of farms to current trends, which was also examined using this method. The individual pieces of information were systematised and grouped to provide a more structured overview of new approaches and technologies in livestock production. The use of induction in the study was aimed at developing an overview of trends in livestock production and related research and technologies. Induction was also applied in forming similar conclusions regarding the features of innovative approaches to feeding, breeding and selection of different animal species such as cows, pigs, sheep, and horses highlighted in the synthesis.

Induction allowed the systematisation and synthesis of the information obtained, which was subsequently used for strategy development. Consideration of the specific application of innovations in the care and breeding practices of different livestock species was also carried out using induction, which enabled the identification of general trends and perspectives for the industry as a whole. The application of generalisation was a crucial step in structuring all the identified features relating to progressive farms. This method was also purposefully used to generalise the vast majority of the characteristics associated with each of the aspects identified in the study on the actual implementation of the innovations considered in the development strategy of progressive entrepreneurial structures in livestock production. The generalisation also allowed for the creation of a systematic overview, identifying key features and general trends inherent in livestock innovation and development strategies for farms of this nature. In turn, this method of structuring the information contributed to a better understanding of the interrelationships between the different innovative aspects and their impact on the integrated development of enterprises in this area, based on the respective strategies.

The following formulas were also used in the study to make calculations: equipment cost per cow per year, costs for each element, total savings on all elements, profit, and the required payback period in months. Equipment cost per cow per year (1):

$$x = (a \cdot b)/100, \tag{1}$$

where a – cost of 1 unit of equipment; b – number of required equipment per 100 cows; x – cost per 1 cow per year.

Costs for each element (2):

$$y = (c \cdot d), \tag{2}$$

where c is the cost of one of the strategy elements; d is the amount of equipment needed for n heads; y is the cost.

Total savings on all elements (3):

$$z = e1 + e2 + e3, \tag{3}$$

where $e1$, $e2$, $e3$ – savings on different elements per 1 cow per year; z – total savings on all elements.

Profit (4):

$$f = z - g, \tag{4}$$

where z – total savings on all elements; g – total acquisition costs of all elements; f – profit.

Required recouping period in months (5):

$$h = g/(i/12), \tag{5}$$

where g – total acquisition costs of all elements; i – total savings on all elements per year; h – required period for payback in months.

RESULTS

A significant number of technological innovations have been successfully introduced in the livestock sector in recent years, but only the most relevant innovations in this area will be considered to create a strategy for the development of innovative livestock farms. It should be noted that due to the widespread use of mobile and stationary sensors installed both on farm equipment and on the animals themselves, modern livestock technology provides operators with access to a variety of data. The amount of time spent on herd monitoring tasks is driven by the need for operators to carry personal computers, tablets, or smartphones to access the farm database. Currently, SmartGlove is a prototype with Level 3 technological readiness (capable of performing the assigned functions and confirming the feasibility of the concept), which is capable of scanning the unique code of an animal from a Radio frequency identification tag (RFID tag) and transmitting it to the Augmented Reality Support Group (ARSG), where all necessary information related to a specific animal is displayed (Fig. 1).

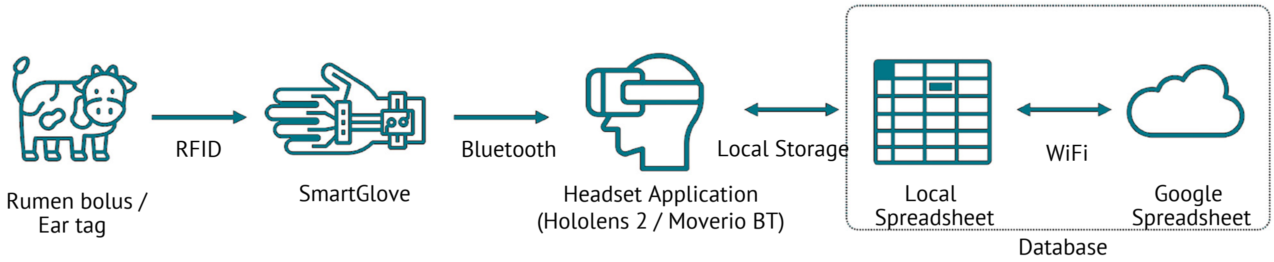

***Figure 1**. How SmartGlove operates*

***Source:** D. Pinna et al. (2023)*

The software solution includes three components: a database, a headset application, and a glove equipment manager. RFID tags placed in the rumen or ear are read using an RFID antenna attached to an Adafruit board embedded in SmartGlove. The animal's identification is transmitted to the headset via Bluetooth technology. When a new ID is received, the interface displays all available information about the corresponding animal stored in a database (Pinna *et al*., 2023). Thus, this technology can be used to provide better care for each animal in the herd.

At the same time, in modern livestock farm management systems, video monitoring is becoming an increasingly important tool for qualitative assessment of cattle behaviour to monitor their health, predict calving and provide early medical attention. Traditionally, animal behaviour has been identified and monitored using a variety of sensors, but video cameras offer a potential alternative to sensors attached to the animals' bodies as they do not stress them. However, identifying and tracking specific cattle, especially those of similar black and brown colour, can be challenging. Considering that texture and colour effectively determine the appearance of an object, a coincidence matrix (CM) should be used for texture representation and the optimal colour space should be explored to extract colour moment information. Ultra-fine neural networks (CNNs) are applied to extract detailed features from recent cow photos, and the extracted features are combined in the tracking process to improve the performance of the system. In addition, an efficient multi-object tracking system that considers multiple characteristic colours of cows and behavioural patterns to effectively track their movements is also significant in this issue. Monitoring of daily activities such as time spent sitting or restless, frequency of drinking and feed intake, and posture of cows in a sitting position can provide valuable information to assess the health status of cattle (Mar *et al*., 2023). Thus, video monitoring of herds using modern technology provides a unique opportunity to maintain herd health and performance.

It should also be noted that many embryonic developmental disorders in different species are predominantly due to genomic defects and anomalies. With the increasing number of equine embryos being created worldwide, it is now possible to analyse statistical data and evaluate, prioritise, and select individual embryos according to their genetic composition before transfer. While natural mating has long been the mainstay of traditional horse breeding, the rapid development of assisted reproductive technologies (ART) is having a significant impact on modern breeding tactics. The development of ART breeding in the late 19th century allowed the distribution and conservation of genetic material, and nowadays the rich gene pool is directly used in horse breeding, both in selection and breeding of specific breeds. Despite the development of technology, the efficiency of breeding with HRT is approximately 70%, but the use of preimplantation genetic testing (PGT), which involves analysing the genetic profile of the embryo before transfer, provides the opportunity to perform sufficient genetic analysis to detect defects in the genome. Preferring embryos with normal chromosomal and genomic composition for transfer selected by PGT for aneuploidies (PGT-A) may increase the frequency of foals. However, the use of PGT for structural chromosomal defects (PGT-SD), polygenic (PGT-P) or monogenic (PGT-M) traits and disorders allows the selection of only healthy embryos that have the desired characteristics before transfer (De Coster *et al*., 2024). Thus, with advances in genetic research, more efficient breeding of horses is possible.

At the same time, a significant proportion of costs in sheep breeding is devoted to feed and improving feed utilisation efficiency can reduce overall production costs and increase economic profitability. Sheep (*Ovis aries*), a large ruminant animal, provide significant economic benefits to pastoralists through the production of meat, wool and other products. The popularity of mutton worldwide is due to its high protein content, low-fat content and juicy, appetising flavour. As feed costs account for approximately 65-70% of the total costs of sheep production, the efficiency of large sheep farms depends directly on the rational use of feed. Farmers therefore need to optimise their feeding processes. First of all, this can be done by paying attention to the efficiency of feed utilisation, which is largely dependent on metabolism and fat accumulation. Two genes, PLIN1 and MOGAT1, which play a key role in lipid metabolism, were found to have a significant impact on these processes. In Qinchuan cattle, polymorphisms in the PLIN1 gene were found to cause different carcass characteristics such as breast depth, subcutaneous fat thickness and intramuscular fat. In turn, MOGAT1, by regulating the conversion of free fatty acids to triglycerides, controls the release of fatty acids from adipose tissue (Ma *et al*., 2024). Thus, genetic studies contribute to further breeding and selection of more appropriate and economically viable sheep diets.

Precision livestock farming uses technological advances, such as digitalisation, to provide products that meet safety and sustainability requirements. It enables agriculture to actively contribute to public welfare through innovations in animal care and farm management. With the development of awareness among farmers and their professional advisors of the benefits of information and communication technologies, it is possible to encourage them to upload a variety of data to central repositories. These data, including information on diseases, fertility, feed intake and meteorological parameters, provide valuable information for decision-making in animal health and production. At the same time, the development of animal behaviour data collection, also illustrated by the AutoPlayPig project, highlights the importance of using information technology to monitor animal welfare in pig breeding with technologies such as cameras, microphones and sensors used to assess health and track animal movements (Kopler *et al*., 2023).

The significant development of energy supply systems in agriculture has a significant impact on the development of this sector. Progress in the process of agricultural electrification is supported by modern technologies and is an important factor in the history of agriculture, where significant transformations have always depended on the emergence of new energy sources. Thus, one of the fundamental conditions for the regular and stable development of rural areas and agricultural production is the provision of electricity. The integration of agriculture and the energy industry is a viable business model with great potential for economic growth, contributing to the financial rewards of the sector. In the livestock sector, such a model can be applied to set up automated farms that utilise green energy. From a business perspective, this model reinforces the positive effects that agriculture has on the food supply processes of the population, while also caring for the environment (Fu & Niu, 2023).

In 2022, more than twenty digital farms and approximately one hundred and seventy innovative farms were active in Kazakhstan. To complete the process of digitalisation in the agricultural sector, the country's Ministry of Agriculture has expressed its intention to create another four thousand innovative farms and at least twenty digital farms by 2023. The main objective of this transformation is to achieve full automation of government services and operations in this sector. Although most farms in the country currently operate using conventional or outdated technologies, the process of digitalisation in agriculture is already well underway. This can be seen, for example, in the ability to automate the care and monitoring of livestock, thanks to the emergence of new livestock technologies such as Global Positioning System (GPS)-trackers that, when used with smartphones, simplify the process of finding lost animals and enable herd location tracking (Dambaulova *et al.,* 2023). Unfortunately, the process of modernisation and development of livestock farming in Kazakhstan is not often covered in studies, media publications and statements by government officials, so it is not possible to adequately assess the extent to which the objectives have been met and the state of the industry in the creation of digital and innovative farms.

Thus, to create a strategy for the development of innovative entrepreneurial structures in the livestock sector, a progressive research area such as digitalisation should be considered. Digitalisation consists of the creation of electronic databases with various animal characteristics in the region and related epidemiological conditions, as well as the application of remote monitoring systems for the herd using modern technologies, from GPS trackers and video cameras to animal analysis and identification systems based on ultra-precise neural networks. Based on the innovations and their directions, it is possible to propose the following strategy for livestock farms that have a basic set of equipment in the form of a computer and a stable connection to electricity and the Internet. It should also be noted that some of the elements can be used in a more comprehensive combination in the future, but this will require additional customisation and improvement. The strategy includes a plan to gradually build up the following elements over a period of three years: drones, drone control modules, glove scanners, mixed reality goggles, CCTV cameras, and software (Table 1).

Table 1*. Cost of individual elements of the strategy*

No.	Element description	Equipment costs	Cost per cow per year	Savings per cow per year
1	Drones (HZH C680)	$8,000	$80	-
2	Drone control module (Skynode X)	$1,600	$1.6	-
3	Scanner glove (SmartGlove)	~$40	$1.2	-
4	Augmented reality headset (HoloLens 2)	$3,500	$105	-

Table 1. Continued

No.	Element description	Equipment costs	Cost per cow per year	Savings per cow per year
5	CCTV Camera (8MP IP Camera POE H.265 CCTV)	$17	$0.17	-
6	Software (CattleEye)	-	$1	$420

Source: *compiled by the authors based on R. Price (2023) and the article "Holy cow! CattleEye reduces EC2 cost by over 60% with Spot Scaling" (2022)*

Appropriate quantitative ratios were used to effectively utilise the elements: 1 drone per 100 cows, 1 drone control module per 10 drones, 3 scanner gloves and mixed reality goggles per 100 cows, 1 CCTV camera per 100 cows, and 1 copy of the software for all CCTV cameras. The cost per cow per year assumes purchase according to the digitalisation plan over a period of 3 years. Savings per cow per year are only shown in one case due to the need for pilot testing. The digitalisation plan foresees the amount of equipment needed to supply an innovative livestock farm of 1,000 animals (Fig. 2).

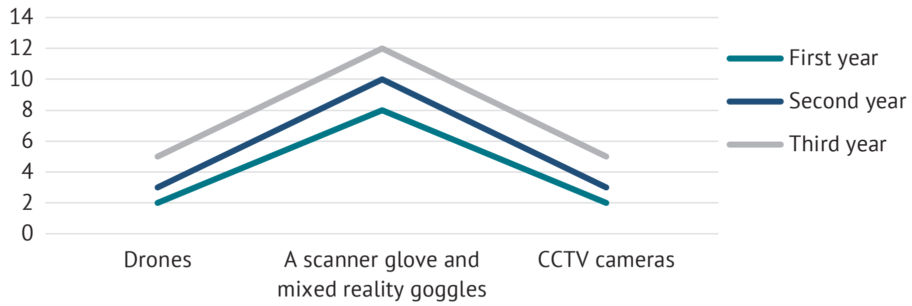

Figure 2*. Digitalisation plan for livestock farming over a period of three years*
Source: *compiled by the authors*

A total number of items required: 10 drones, 1 drone control module, 30 scanner gloves and mixed reality goggles, 10 CCTV cameras, and 1 copy of software for all CCTV cameras. The elements not mentioned in the visualisation of the digitalisation plan (drone control module, copy of software) are purchased in the first year of the strategy application. The total cost of the strategy, when applied to a farm of a thousand heads, is $187,970, for a saving of $1,260,000 (Table 2).

Table 2*. Detailing the cost of the strategy (for a farm of a thousand head)*

No.	Description of the element and its required quantity	Overall equipment cost	Overall savings
1	Drones (HZH C680) x 10	$80,000	–
2	Drone control module (Skynode X) x 1	$1,600	–
3	Scanner glove (SmartGlove) x 30	~$1,200	–
4	Augmented reality headset (HoloLens 2) x 30	$105,000	–
5	CCTV Camera (8MP IP Camera POE H.265 CCTV) x 10	$170	–
6	Software (CattleEye) x 1	–	$1,260,000

Source: *compiled by the authors*

Thus, the profit from the implementation of this strategy is $1,072,030, with an implementation period of 3 years and a payback period of less than 6 months. It should be noted that digitalisation is the most technologically and economically costly of the modernisation processes, yet it offers access to advanced systems for monitoring vital signs of the herd, which significantly reduces costs in terms of proper control of herd health.

DISCUSSION

Various technologies such as drones, CCTV cameras, glove scanners, drone control modules and camera software have been incorporated into the developed strategy. Several other innovative technologies can be used for this kind of strategy.

Among the research on such technologies, the study of D. Wu *et al.* (2023), considers automated respiratory monitoring of dairy cows and claims that it helps to increase the degree of automation of their health assessment and also reduces manual labour. The reasoning states that cows are often crowded on farms, and this creates difficulties for conventional health monitoring due to the physical limitation of free view and access to the cows. In turn, the automated method of tracking cows' breathing, in addition to measurements of blood pressure, blood oxygen level, body temperature and electrocardiogram, is one of the key indicators of animal health, which also serves as a significant indicator of the normal course of vital processes in the animal's body. The manifestation of these processes is the synchronous movement of the abdomen when breathing characteristics change – this phenomenon is known as "respiratory behaviour". Monitoring the respiratory behaviour of dairy cows is a difficult task due to less intensive respiratory processes, which makes their breathing more difficult to observe, especially when the number of observed cow increases. But these issues are addressed by introducing more advanced approaches to recognise the resting state of each cow (lying or standing position), for which CNNs and bi-directional long-term and short-term memory algorithms are integrated, using the You Only Look at Coefficients (YOLACT) algorithm for multi-cow image recognition and segmentation. In this way, ultra-precise neural systems are utilised in interaction with any advanced algorithms and applied to various animal vital signs monitoring systems using video cameras. As can be seen, algorithms for recognising certain data by analysing the image transmitted from cameras use different principles to perform certain functions efficiently, but common to them is the desire for more advanced and accurate analysis of video data, which determines the productivity and quality of each of them.

In turn, attention should be devoted to the study conducted by G.K. Dambaulova *et al.* (2023), which considered the possibility of improving the natural potential of animals, for which biotechnology, genomic analysis and DNA analysis are used in animal breeding to improve the genome of animals, as well as faster reproduction methods are introduced, which leads to the standardisation of cattle in general. In advanced countries that are major cattle producers, intensive breeding efforts have led to high results in the development of dairy cow breeds with milk yields of 8,000-10,000 kg or more and lactation lengths of more than 305 days. It was also noted that unmanned aerial vehicles are widely used in New Zealand agriculture to assist herders in herd management. These drones use loudspeakers and recorded dog barking sounds to assist in herd gathering. Likewise, with the application of more efficient land, forest and pasture management practices, smart farming techniques are helping to prevent soil erosion and avoid deforestation for pasture expansion, a prime example being the successful restoration of degraded farmland in Brazil, achieved through the Integrated Crop, Livestock and Forestry Systems (ICLFS) programme, in a country that produces more than 23% of the world's cattle. Thus, the scientific community is actively considering innovation and emphasising the opportunities offered by modern technologies in genetic research and digitalisation. Particular attention should be paid to the use of drones, as it is their use that makes it possible to respond effectively and in a timely manner to unfavourable situations that animals may get into during free grazing. They are also indispensable in the search for lost individuals, as they can provide a wide field of view from a height, but it is prone to be affected by weather conditions.

R.C. Chebel *et al.* (2024) note that along with improvements in nutrition and care, advances in genetics and breeding approaches have a positive impact on breeding efficiency. The researchers also note the role of automated monitoring systems that are used in the selection of cows for breeding on genetic and physiological parameters. It is also possible to note that a thoughtful approach to the introduction of innovative technologies is their important feature, as they are more sensitive to poor quality management, especially those that work in a system and disabling one of its elements does not allow to fully utilise the others or apply them at all.

In turn, N. Widaningsih *et al.* (2023) state that precision livestock management, which is a set of modern electronic tools and techniques used in livestock management to promote sustainable practices in agriculture and livestock production, when used in conjunction with automated livestock management systems, has a positive impact on the efficiency and productivity of livestock systems. It is difficult to disagree with this, as any of the innovations mentioned in the study has a positive impact on farm performance to a greater or lesser extent, but the main issue remains the amount of capital investment required for their implementation, which in general is the economic feasibility. Despite this, most of the innovative technologies, when properly implemented, show quite good payback periods.

According to C. Bader and H. Bernhardt (2023), due to the increasing global demand for energy resources, the latter is becoming an urgent problem for the entire agricultural industry, and this has a significant impact on the economic situation of livestock farms. In turn, biogas plants represent a potential solution capable of generating additional energy by processing manure. Another option is the installation of photovoltaic systems on the roofs of farm buildings. This approach allows us to efficiently utilise low-cost energy sources on the farm and to actively participate in the energy market by supplying the generated energy directly to the general grid. It is hard to disagree with this view, as it is a direct example of the use of green energy technologies. Since the energy costs of innovative farms are comparatively higher than those of conventional farms, the use of "green" sources of electricity can significantly reduce costs in this aspect.

At the same time, according to F.A. Kurniadi *et al.* (2023), with increasing farm size, there is a need for an effective livestock monitoring system, especially in cases where the animals are on pasture or in paddocks instead of in specific facilities. To address this problem, the application of YOLOv5 method is proposed to analyse drone images. To ensure detection efficiency, a training process is performed on a dataset consisting of cow photos and the hyperparameters of the algorithm are tuned to achieve optimal results. Thus, YOLOv5 is an object detection model designed to recognise cows on farms from drone images. Indeed, different algorithms can be applied not only on fixed video cameras but also directly integrated into drones, for better herd monitoring. The recognition of specific individuals can also be applied to further develop the automation of drone use, potentially offering a herd monitoring system completely independent of human intervention.

V. Naujokienė *et al.* (2022) noted that in the production of fodder concentrates, grass silage and maize silage they are significantly affected by fertilisers. The application of integrated smart management identified these critical points, after which bioproducts replaced 50% of nitrogen fertiliser as an experiment. The introduction of biopreparations has reduced the negative impact on grass, barley, hay, and maize used for silage. Thus, the concept of "smart" agriculture is also manifested in the harvesting of animal feed, which undoubtedly has a positive effect on the economic aspect of livestock production.

C. Manteuffel (2022) emphasises that the effective implementation of innovations in livestock production implies that these innovations must be economically justified. However, constraints related to biological, ethical, and environmental aspects limit the economic potential of a concept such as precision animal husbandry. At the same time, the utilisation of the solutions offered by this concept often requires the use of sophisticated sensor systems and evaluation methods, which increases the overall cost and payback periods of the technology. Yes, most modern technologies are indeed quite expensive, especially when it comes to implementing entire systems, but despite the long payback period of some technologies, their benefits can be utilised immediately after installation, and in some cases, innovative solutions can prevent significant costs associated with unforeseen on-farm situations such as epidemics.

CONCLUSIONS

Thus, a study of innovative entrepreneurial structures in livestock farming was conducted and a strategy for their development was proposed. In the course of the study, various innovative approaches to livestock farming, such as smart farming, were studied, and a variety of progressive proposals for the use of modern technologies in livestock farming were considered. The strategy developed was to gradually scale up innovative technologies within individual farms, regardless of their size. The main elements of the strategy are drones, sensor gloves, CCTV cameras and their mixed reality goggles, and CCTV cameras in the quantities required to optimally fulfil their functions. Most of the technologies involved require further experimental evaluation of their economic feasibility but based on the already available information about the effectiveness of some of them, as well as the cost of all the elements involved, it was possible to make appropriate calculations of costs, profits and payback period for a livestock farm of a thousand animals. The following figures should be noted: costs – $187,970, savings – $1,260,000, profit – $1,072,030, payback period – less than 6 months, implementation period – 3 years. Total equipment requirements, not counting the basic set: drones – 10 pcs, drone control module – 1 pc, glove-scanner – 30 pcs, mixed reality glasses – 30 pcs, CCTV cameras – 10 pcs, software – 1 copy.

Many of the innovative projects are not as financially costly for progressive farms but offer significant economic potential for cost reduction. It should be summarised that the topic of progressive farms and their development is of great importance for the development of this area in Kazakhstan, and also requires more detailed scientific substantiation and experimental application, as the improvement of the efficiency of livestock farming will have a beneficial impact on the economic situation in the state, bringing closer the full and widespread introduction of international standards and progressive technologies in agriculture in Kazakhstan.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Стратегія розвитку інноваційних підприємницьких структур у тваринництві

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Анотація. Актуальність даного дослідження полягає в необхідності створення стратегії розвитку інноваційних підприємницьких структур у тваринництві, застосування якої може значно підвищити економічну окупність господарств. Мета дослідження – розробити стратегію розвитку прогресивних тваринницьких господарств. Для проведення дослідження було використано такі загальнонаукові методи: аналіз, синтез, дедукція, індукція та узагальнення. Серед розглянутих інновацій було виокремлено: різноманітні генетичні дослідження задля покращення геному тварин (кількість м'яса та молока, ефективність засвоєння корму), а також відбору генетично здорових ембріонів для розведення; використання у тваринництві таких сучасних технологій, як: Радіочастотна ідентифікація, доповнена реальність, нейронні мережі та система глобального позиціонування; технології, спрямовані на автоматизацію процесів годівлі тварин. Окрему увагу було приділено різним системам моніторингу поголів'я, створених з використанням просунутих алгоритмів. Також було представлено стратегію розвитку інноваційних господарств у тваринництві, основними елементами якої стали дрони, камери відеоспостереження, окуляри змішаної реальності та рукавички-сканери. Іншими необхідними елементами були обрані модуль управління дронами та програмне забезпечення. Також було проведено відповідні розрахунки, які доводять доцільність впровадження цієї стратегії та її фінансовий потенціал. За умови задіяння 82 одиниць різного устаткування, загальною вартістю 187,970$, стратегія передбачає прибуток у 1,072,030$, з періодом реалізації 3 роки та окупності менше 6 місяців. Практичне значення отриманої інформації полягає в тому, що вона може розглядатися дослідниками для подальшого вивчення особливостей реалізації інноваційних проєктів у тваринництві, а також і для подальшого розвитку різних стратегій у сфері модернізації сільського господарства в Казахстані

Ключові слова: модернізація господарств; селекційні ознаки; автоматизовані системи годівлі; збалансований раціон; «розумне» сільське господарство

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