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Correction of cellular and humoral links immunity in piglets under the weaning condition

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and subpopulations of T-lymphocytes in piglet blood samples collected before and at specific intervals after weaning. The analysis of the quantitative composition of T- and B-lymphocytes and the functional activity of T lymphocytes in the blast transformation reaction was conducted using immersion microscopy of smears. The statistical processing of results was carried out through variational nonparametric analysis using biometric methods. The findings revealed that weaning from sows caused a reduction in the number of T-cells of varying degrees of avidity, a decrease in the relative number of T-suppressors in piglets' blood, and an increase in the number of certain subpopulations of T-helper cells. Conversely, the administration of the liposomal preparation to piglets enhanced the number of various subpopulations of total and active T-lymphocytes and B-lymphocytes in their blood after weaning and increased the activity of lymphoid cells in the blast transformation reaction with phytohaemagglutinin. The observed immunomodulatory effect of the tested drug is attributed to the synergistic combination of fat-soluble vitamins, mineral elements, and arginine in its composition, which effectively prevented the development of stress-induced immunodeficiency in piglets following weaning

Keywords: immunodeficiencies; liposomal preparation; lymphocytes populations; weaning; stress; arginine

INTRODUCTION

Pigs are highly suitable subjects for studying the effect of stress on the body as a factor contributing to immunodeficiency. It is well established that animals of this species exhibit exceptionally high sensitivity to stress factors. Due to the demands of modern production systems, technological stress affects pigs throughout their entire development. One of the unavoidable stressors in the life of piglets is weaning from sows. Weaning is accompanied by activation of the sympathoadrenal and hypothalamic-adeno-hypophysis-adrenocortical systems and disruption of the pro-oxidant-oxidant balance in the piglets' bodies. This imbalance reduces natural resistance and immune reactivity during weaning, serving as a key factor in developing acute and chronic diseases in piglets (Jia *et al.*, 2020; Hao *et al.*, 2021). According to A. Luppi *et al.* (2023), weaned piglets are predisposed to diseases of both infectious and non-infectious aetiology, with toxic liver damage (hepatosis and hepatitis), gastroenteritis, and respiratory diseases (bronchitis and bronchopneumonia) being the most common.

Moreover, B. Jahangiri *et al.* (2022) demonstrated a functional relationship between these diseases. For instance, in respiratory diseases, liver activity is suppressed due to tissue hypoxia, inhibition of aerobic processes, intensification of anaerobic reactions, and the development of acidosis in the liver parenchyma. These processes lead to dystrophic and necrotic changes, reducing the liver's synthetic and detoxification functions. Chronic hypoxia also causes functional insufficiency in haematopoietic and immunoregulatory cells, leading to secondary immunodeficiencies. B. Foglia *et al.* (2021) highlighted that non-specific gastroenteritis in weaned piglets is associated with hyperchromic anaemia and hyperenzymopathy, along with a significant reduction in the De Ritis ratio, indicative of parenchymal liver damage and structural changes in the pancreas. Similarly, as shown by C. Lauridsen *et al.*, (2021), the transition from a milk-based diet to concentrated feed reduces lipid assimilation in piglets, leading to

insufficient digestion of nutrients and resulting in dystrophic processes in the liver.

The above discussion highlights the need to stimulate piglet immunity to prevent potential disorders caused by weaning from sows. Modern methods of regulating immunogenesis involve the use of medicines that target immunocompetent cells, enhance their functional properties, and activate interactions with antigenic material. In this context, fat-soluble vitamins – particularly retinol, tocopherol, and calciferol – along with arginine and several macro- and microelements, are noted for their powerful protective effects on the body (Rufino-Moya *et al.*, 2020; Buchko *et al.*, 2024). M. Khariv *et al.* (2017) observed that liposomal preparations, which remain in the body for extended periods, can serve as a significant alternative to conventional medicinal forms. Encapsulation of active substances, particularly hydrophobic ones, in liposomes, increases their bioavailability and allows modification of their release. Under certain conditions, liposomes are absorbed by cells and merge with cell membranes, enabling the direct transport of their contents into the cell (Nsairat *et al.*, 2022). However, despite their high efficacy, liposomal preparations are rarely used in veterinary medicine. In this context, studying the state of the cellular and humoral components of piglet immunity during weaning and developing effective complex preparations in the form of liposomal emulsions for its regulation is a pressing area of scientific research.

Thus, the experiments aimed to develop and evaluate the effects of an improved dosage form of an immunotropic preparation – based on fat-soluble vitamins, macro- and microelements – on the bodies of piglets during weaning from sows.

MATERIALS AND METHODS

The experiment was conducted at the “Flora-S” enterprise in the Sokal District of the Lviv Region, using two groups of Large White breed piglets, each comprising nine animals. Two days prior to weaning, the control

group of piglets was parenterally injected with 0.1 mL of NaCl, while the experimental group received an equivalent amount of liposomal preparation. The composition of 1 L of this preparation included the following: vitamin A – 900-1100 IU, vitamin D3 – 1100-1300 IU, vitamin E – 0.9-1.1 mg, arginine – 1.8-2.2 mg, zinc acetate – 0.9-1.1 mg, sodium selenite – 0.090.11 mg, cobalt acetate – 0.2-0.4 mg, and magnesium sulphate – 0.9-1.1 mg. During the study, all animals were fed “Prestarter for Piglets TN” (Trouw Nutrition, Belgium), a combined feed designed for piglets aged 5-65 days.

Blood samples from piglets were collected from the cranial vena cava two days prior to weaning and on the 1st, 5th, and 10th days following weaning. Lymphocytes were isolated from the blood using a ficoll-verographin gradient with a relative density of 1.077. The ficoll-verographin gradient was prepared by adding a 9% solution of Ficoll (Pharmacia, Sweden) to a 34% solution of Verographin (Spofa, Czech Republic). Lymphocytes were incubated in Basal Medium Eagle (Sigma, USA) with ram erythrocytes. During incubation, erythrocytes adhered to T-cells, forming rosette-like structures. B-lymphocytes were identified based on their membrane receptors for C3b-C3d complement components and the Fc fragment of immunoglobulin. Ram erythrocytes treated with trypsin (Biopharma, Ukraine) were used as indicator cells. Lymphocytes transformed into blasts through activation by phytohaemagglutinin (Lectynotest, Ukraine) from their inactive forms. Cytological preparations were fixed in methanol (ChemStore, Netherlands), stained using Romanowsky-Giemsa (RG) stain, and observed under a binocular microscope (J-CM2000B, Zenith Lab, China).

The number of T-lymphocytes in the blood of piglets was counted according to a modified and improved method (Vishchur *et al.*, 2007). Active rosette-forming lymphocytes, with receptors capable of attaching sheep erythrocytes without incubation, were isolated, and the number of helper lymphocytes forming rosettes after incubation with theophylline was also determined. To determine B-lymphocytes, the EAS system (erythrocytes sensitised with antibodies and complement) was prepared by adding haemolytic serum to sheep erythrocytes.

Rosettes were counted using smear microscopy under immersion. Based on the number of sheep erythrocytes attached, lymphocytes were classified as follows: undifferentiated (zero) – not attaching a single erythrocyte; low-avidity – attaching 3-5 erythrocytes; medium-avidity – attaching 6-10 erythrocytes; and high-avidity (morula) – attaching more than 10 erythrocytes.

T-suppressors were determined by calculating the difference between the number of helper T-lymphocytes and the total number of T-lymphocytes. The immunoregulatory index (IRI) was calculated based on the ratio of T-helpers to suppressors. The functional activity of T cells was assessed using the lymphocyte blast transformation (LBT) reaction, with phytohaemagglutinin used as a mitogen (Vlizlo *et al.*, 2012). The percentage of blasts was calculated relative to the total number of T cells. The data were analysed using the Statistica 7.0 software package (StatSoft Inc., USA). Results were statistically evaluated by calculating the arithmetic mean ($\bar{x}$) and its standard deviation (SD) using ANOVA. The significance of mean differences was assessed with Student's t-test. All interventions complied with the international principles outlined in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986), ISO/IEC 17025:2005 (2006), and the Law of Ukraine No. 249 (2012). Ethical approval for the study was obtained according to Protocol No. 93 (03.06.2021) from the Bioethical Commission of the Institute of Animal Biology of NAAS.

RESULTS

According to the obtained data, weaning from sows exerts an inhibitory effect on blood lymphocytes, particularly on the total populations in the control piglets (Table 1). This effect was most pronounced in the first days following weaning. For instance, on the 1st day after weaning, compared to the period before weaning, a 1.14-fold reduction in low-avidity T-total lymphocytes and a twofold reduction in high-avidity T-total lymphocytes were observed in the blood of piglets in the control group. By the 5th day, a 1.93-fold decrease in medium-avidity forms was recorded.

Table 1. Total T-lymphocytes and their functional activity in the piglets' blood ($\bar{x} \pm SD$; %; $n = 9$)

Indicators	Animal groups	Research periods			
		2 days before weaning	after weaning		
			1st day	5th day	10th day
T-general, attached 0 erythrocytes (undifferentiated cells)	C	52.01 $\pm$ 2.32	54.62 $\pm$ 2.91	55.69 $\pm$ 2.40	55.05 $\pm$ 2.31
	E		51.67 $\pm$ 3.52	53.30 $\pm$ 2.93	57.10 $\pm$ 2.34
attached 3-5 erythrocytes (low avidity)	C	37.14 $\pm$ 1.19	32.34 $\pm$ 0.65 ⁰⁰	36.57 $\pm$ 1.76	35.23 $\pm$ 3.06
	E		35.32 $\pm$ 1.24*	36.08 $\pm$ 1.17	32.03 $\pm$ 1.22
attached 6-10 erythrocytes (medium avidity)	C	9.08 $\pm$ 1.21	11.23 $\pm$ 0.66 ⁰	4.62 $\pm$ 0.69 ⁰⁰	7.37 $\pm$ 1.34
	E		10.01 $\pm$ 1.16	7.12 $\pm$ 1.18*	7.14 $\pm$ 1.17

Table 1. Continued

Indicators	Animal groups	2 days before weaning	Research periods		
			after weaning		
			1 st day	5 th day	10 th day
Morulas (high avidity)	C	3.22 ± 0.22	1.50 ± 1.01 ⁰	3.00 ± 1.18	2.49 ± 0.64
	E		3.03 ± 1.18	3.67 ± 2.39	4.22 ± 1.19
% relative amount	C	48.31 ± 2.35 ^a	45.38 ± 2.85	44.32 ± 2.42	45.31 ± 2.32
	E		48.31 ± 3.47	46.59 ± 2.95	43.18 ± 2.29

Note: C – control, E – experimental. * – probable differences compared to the control: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$; 0 – in relation to the period before weaning: 0 – $p < 0.05$; 00 – $p < 0.01$; 000 – $p < 0.001$; 0 – undifferentiated cells
Source: developed by the authors

For the experimental group of piglets, compared to the control group, a 1.09-fold higher number of low-avidity T-lymphocytes was noted in the blood on the 1st day after weaning. Additionally, the number of medium-avidity T-total lymphocytes increased by 1.49 times on the 5th day post-weaning. In terms of high-avidity forms and the relative number of total lymphocytes, the experimental group consistently demonstrated a tendency towards increased values compared to the control across nearly all post-weaning periods of the study.

On the 1st and 10th days after weaning from sows, a tendency to a reduction in the number of functionally

undifferentiated T-active lymphocytes was found in the blood of piglets in the control group. However, on the 5th day post-weaning, a decline in the number of low-avidity cells was observed (Table 2). The relative number of active T-lymphocytes in the blood of the control group piglets, compared to the pre-weaning period, increased at all stages post-weaning due to an elevation in the number of medium-avidity forms. In contrast, an increase in the number of low-avidity forms of active T-lymphocytes was observed at all stages of research in the experimental group, along with a rise in medium-avidity cells by the 10th day after weaning.

Table 2. Active T-lymphocytes and their functional activity in the piglets' blood ($\bar{x} \pm SD$; %; $n = 9$)

Indicator	Animal groups	2 days before weaning	Research periods		
			after weaning		
			1 st day	5 th day	10 th day
T-active, attached 0 erythrocytes (undifferentiated cells)	C	81.19 ± 2.32	78.59 ± 3.52	81.22 ± 2.03	78.70 ± 0.62
	E		79.61 ± 3.48	78.13 ± 2.42	76.37 ± 3.51
attached 3-5 erythrocytes (low avidity)	C	18.06 ± 1.20	18.48 ± 1.76	17.12 ± 3.07	18.03 ± 2.05
	E		19.11 ± 3.06	20.07 ± 1.18	18.31 ± 1.78
attached 6-10 erythrocytes (medium avidity)	C	1.52 ± 1.03	2.72 ± 1.81	2.31 ± 0.71	3.04 ± 1.14
	E		1.35 ± 0.68	1.69 ± 1.34	5.32 ± 1.75
% relative amount	C	19.04 ± 2.29	21.41 ± 3.46	19.35 ± 0.69	21.05 ± 2.30
	E		20.29 ± 3.54	21.68 ± 2.45	23.66 ± 3.52

Note: C – control, E – experimental. * – probable differences compared to the control: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$; 0 – in relation to the period before weaning: 0 – $p < 0.05$; 00 – $p < 0.01$; 000 – $p < 0.001$; 0 – undifferentiated cells
Source: developed by the authors

In the blood of piglets from the control group at the final stage of research after weaning, an increase in the relative number of T-helpers and their medium-avidity forms was observed against the backdrop of a decrease of undifferentiated cells (Table 3). Conversely, the relative proportion of T-suppressors in the control group

decreased after weaning compared to the pre-weaning period. In comparison to the control group, the number of low-avidity T-helper forms in the experimental group decreased by 1.31 times at the beginning of the study, while T-suppressors demonstrated a similar reduction at the end of the research.

Table 3. The number of T-helpers and T-suppressors, their functional activity, immunoregulatory index, and lymphocyte blast transformation in the piglets' blood ($\bar{x} \pm SD$; %; $n = 9$)

Indicators	Animal groups	2 days before weaning	Research periods		
			after weaning		
			1 st day	5 th day	10 th day
T-helpers, attached 0 erythrocytes (undifferentiated cells)	C	80.12 ± 2.29	76.98 ± 2.35	77.31 ± 4.80	75.94 ± 1.18 ⁰
	E		79.65 ± 3.51	74.08 ± 3.46	73.02 ± 3.46

Table 3. Continued

Indicators	Animal groups	2 days before weaning	Research periods		
			after weaning		
			1 st day	5 th day	10 th day
attached 3-5 erythrocytes (low avidity)	C	20.23 ± 2.31	21.89 ± 1.17	18.65 ± 3.05	20.07 ± 2.33
	E		16.58 ± 0.88**	21.34 ± 1.77	22.99 ± 1.14
attached 6-10 erythrocytes (medium avidity)	C	1.57 ± 1.04	1.52 ± 1.02	4.02 ± 2.30	4.01 ± 1.21 ⁰
	E		3.64 ± 1.76	4.68 ± 1.80	4.05 ± 1.29
% relative amount	C	20.04 ± 2.45	23.05 ± 2.29	22.67 ± 4.82	23.89 ± 1.19 ⁰
	E		20.32 ± 3.46	25.96 ± 3.47	26.95 ± 3.46
T-suppressors, %	C	27.99 ± 0.21	22.37 ± 0.53 ⁰⁰⁰	21.68 ± 2.39 ⁰⁰	21.11 ± 3.02 ⁰
	E		27.94 ± 6.12	20.62 ± 5.34	16.03 ± 0.58*
Immunoregulatory index	C	0.71 ± 0.08	1.03 ± 0.07 ⁰⁰	1.06 ± 0.32	1.16 ± 0.11 ⁰
	E		0.76 ± 0.31	1.31 ± 0.45	1.70 ± 0.19*
Lymphocyte blast transformation	C	44.97 ± 2.13	39.97 ± 1.14 ⁰	44.33 ± 2.41	41.33 ± 2.41
	E		41.62 ± 0.59	46.66 ± 3.52	46.71 ± 1.76*

Note: C – control, E – experimental. * – probable differences compared to the control: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$; 0 – in relation to the period before weaning: 0 – $p < 0.05$; 00 – $p < 0.01$; 000 – $p < 0.001$; 0 – undifferentiated cells

Source: developed by the authors

The immunoregulatory index increased in the control group throughout all periods following weaning compared to the pre-weaning period. At the same time, for piglets in the experimental group, the value of the immunoregulatory index exceeded the control value by 1.46 times on the 10th day after weaning. The more pronounced increase in the immunoregulatory index in the experimental group, compared to the control, during the specified periods post-weaning can be attributed to an increase in the relative number of T-active lymphocytes and T-helpers, along with a decrease in the number of T-suppressors. Apparently, the stress experienced by piglets during weaning from sows results in a weakening of the ability of lymphocytes to transform into blasts, as evidenced by a significant 1.13-fold reduction in lymphocyte blast transformation in the blood of the control group on the day following weaning. Administration of the liposomal preparation to piglets led to

increased lymphocyte blast transformation in the blood compared to the control group, with the effect being particularly pronounced at the end of the study.

The results shown in Table 4 indicate that the weaning of piglets from sows does not significantly affect the number and functional activity of B-lymphocytes but only leads to a tendency for their low-avidity and high-avidity forms to decrease in the blood on the 1st day after weaning. Conversely, for piglets in the experimental group, the relative number of B-lymphocytes exhibited a tendency to increase throughout all post-weaning periods compared to the control group. In general, compared to the control, the number of low-avidity forms in the blood of piglets in the experimental group increased by 1.11 times at the end of the study, while the number of medium-avidity forms of this lymphocyte population rose by 1.55 times on the 5th day after weaning.

Table 4. The number of B-lymphocytes and their functional activity in the piglets' blood ($\bar{x} \pm SD$, %, $n = 9$)

Indicators	Animal groups	2 days before weaning	Research periods		
			after weaning		
			1 st day	5 th day	10 th day
B-lymphocytes, attached 0 erythrocytes (undifferentiated cells)	C	54.93 ± 2.31	54.67 ± 4.06	55.96 ± 2.32	53.64 ± 1.77
	E		51.07 ± 3.45	52.88 ± 3.47	50.06 ± 2.73
attached 3-5 erythrocytes (low avidity)	C	28.76 ± 1.17	26.11 ± 2.25	26.69 ± 2.41	29.95 ± 1.18
	E		26.38 ± 2.42	22.93 ± 1.19	31.02 ± 1.15*
attached 6-10 erythrocytes (medium avidity)	C	9.02 ± 1.12	13.96 ± 2.03	10.35 ± 0.64	10.13 ± 1.38
	E		16.64 ± 2.91	16.07 ± 0.98***	11.20 ± 1.22
Morulas (high avidity)	C	7.11 ± 1.28	5.35 ± 1.34	6.95 ± 1.18	8.35 ± 0.69
	E		6.02 ± 1.18	8.07 ± 1.21	8.09 ± 1.58
% relative amount	C	44.89 ± 2.40	45.34 ± 4.08	43.91 ± 2.35	46.30 ± 1.76
	E		49.08 ± 3.46	47.12 ± 3.39	50.08 ± 3.40

Note: C – control, E – experimental. * – probable differences compared to the control: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$; 0 – in relation to the period before weaning: 0 – $p < 0.05$; 00 – $p < 0.01$; 000 – $p < 0.001$; 0 – undifferentiated cells

Source: developed by the authors

The obtained data indicate that weaning of piglets causes them to experience a stressful state, leading to a decrease in the number of total T-lymphocytes of varying avidity, a reduction in the relative number of T-suppressors, and an increase in certain subpopulations of T-helpers in their blood. In contrast, the liposomal preparation demonstrated a stimulatory effect on the functional activity of immune cells. This preparation had a normalising effect on the number of various subpopulations of T- and B-lymphocytes in the blood of piglets during different stages of weaning. In particular, parenteral administration of a liposomal preparation containing vitamins A, D3, and E, along with arginine, zinc acetate, sodium selenite, cobalt acetate, and magnesium sulphate, contributed to an increase in the number of total and active T-lymphocyte and B-lymphocyte subpopulations, as well as the activity of lymphoid cells in the reaction of lymphocyte blast transformation with phytohaemagglutinin.

DISCUSSION

There are two main forms of specific immune response: cellular and humoral. T-lymphocytes constitute the cell-mediated component of the immune system, recognising antigens through specific receptors located on their surface (Ohorodnyk *et al.*, 2017). Lymphocytes are cells that perform not only the specific functions of immune protection but also act as elements of a unified information system. This system accurately reflects the state of the body at a given time or under the influence of certain factors and enables the evaluation of the therapeutic effects of studied preparations (Cerdeira *et al.*, 2022). The obtained data indicate that weaning from sows exerts an inhibitory effect on the total population of T-lymphocytes in piglets' blood. This effect is evident from the first day after weaning, with a significant decrease observed in the number of low-avidity ($p < 0.05$) and high-avidity ($p < 0.05$) forms of T-total lymphocytes. On the 5th day, this is further evidenced by a reduction in the number of their medium-avidity forms ($p < 0.05$). The post-weaning decline in the relative number of total T-lymphocytes in the blood of piglets from the control group is attributed to the action of prostaglandins and interleukin-2 (IL-2). This is because the inhibition of the T-cell response to an antigen occurs during the IL-2-dependent stimulation of T-lymphocytes (Lykhoviy *et al.*, 2023). Under stress, the activation of receptors for this cytokine on the surface of T-lymphocytes is reduced (Niederlova *et al.*, 2023; Raeber *et al.*, 2023). It appears that weaning from sows also affects the transcription factor NF- κ B, whose activity leads to T-lymphocyte dysfunction, a reduction in their proteolytic activity, and the emergence of signs of immunodeficiency (ParraLlorca *et al.*, 2023).

In the blood of piglets in the experimental group on the first day after weaning, an increase in the number of low-avidity forms of lymphocytes ($p < 0.05$) was

observed, as well as an increase in the number of medium-avidity forms of T-total lymphocytes on the 5th day. During nearly all periods of observation following the weaning of piglets, there was a consistent tendency towards an increase in high-avidity forms and the relative number of total lymphocytes. It should be noted that the increase in the number of high-avidity forms and the relative number of total lymphocytes in the blood of piglets after weaning, as influenced by liposomal preparation, represents a positive outcome. This is because it is known that almost all mature T-lymphocytes express CD3 marker molecules on their surface, which are involved in transmitting signals from the T-cell receptor into the cells, thereby stimulating activation and proliferation processes. The reduction in the number of T-suppressors in the blood of piglets after the administration of the liposomal preparation on the 10th day after weaning suggests that immune aggression processes were stabilising within this period. This stabilisation concludes with the interaction of T-killer receptors with target cells in the presence of CD8. Cytotoxic T-lymphocytes with the CD8 phenotype regulate antibody production and the formation of immune tolerance. They also induce the death of infected cells and act on infectious agents. Due to their immunosuppressive function, CD8 prevents autoaggression and influences the development of immune responses, directing them towards either a humoral or cellular response (VieyraLobato *et al.*, 2018; Xie *et al.*, 2021).

The implementation of the humoral link of immunity in the body is carried out by B-lymphocytes, which, under the influence of an antigenic stimulus, differentiate into plasma cells that produce antibodies (Rastogi *et al.*, 2022). It is evident that stress factors inhibit antibody formation processes, while the production of lymphocyte-activating factors by macrophages is induced, and corticosteroids are released. These corticosteroids bind to specific lymphocyte receptors forming complexes that disrupt the production of lymphokines. Simultaneously, due to a reduction in cooperation between T- and B-lymphocytes, T-dependent antibody production is inhibited, and the differentiation of B-lymphocytes as well as the synthesis of IgM and IgG are impaired (Rodriguez-Mogeda *et al.*, 2024). Under the conditions of this study, the weaning of piglets from sows does not significantly affect the number and functional activity of B-lymphocytes but only shows a tendency towards a decrease in low-avidity and high-avidity forms in their blood on the 1st day after weaning. Meanwhile, in piglets of the experimental group, the relative number of B-lymphocytes showed a tendency to increase throughout all post-weaning periods.

The observed changes in the functional state of the receptor apparatus of T- and B-lymphocytes in the blood of piglets in the experimental group suggest that the combined application of all components of the liposomal preparation is an effective means for preventing

immunodeficiencies. This preparation activates the cellular and humoral mechanisms of the immune response in their bodies after weaning from sows. To fully understand the immunomodulatory effect of the liposomal preparation on the body of piglets post-weaning, it is essential to examine in detail the various components it contains, particularly the fat-soluble vitamins, L-arginine, zinc, selenium, cobalt, and magnesium. Retinol is a crucial vitamin for the formation of the body's defence mechanisms (Liu *et al.*, 2019). By increasing the secretion of IL-2 through RAR receptors, it stimulates cellular reactions and the proliferation of T-lymphocytes, activates the humoral link of the immune response to T-dependent antigens, enhances the activity of cytotoxic lymphocytes, and reduces the synthesis of IL-10 and IL-12 (Ouyang & O'Garra, 2019).

One of the key roles in lymphocyte formation is played by calciferol, the active form of which – $1.25(\text{OH})_2\text{D}_3$ – influences natural resistance and specific immunity, stimulates lymphocytes with concanavalin A, and induces cathelicidin and IL-1 gene expression (Ao *et al.*, 2021). Vitamin D_3 enhances phagocyte numbers, promotes interferon synthesis and T-lymphocyte activity, and stimulates enzyme systems involved in the generation of reactive oxygen species in immunocompetent cells (Sirbe *et al.*, 2022). Receptors for $1.25(\text{OH})_2\text{D}_3$ have been identified in 5% of activated T-lymphocytes and 95% of B-lymphocytes (Cyprian *et al.*, 2019). Specific receptor proteins for $1.25(\text{OH})_2\text{D}_3$ have been detected in immature thymus cells, with a significant proportion found in CD8^+ and CD4^+ . The vitamin D_3 cells hydroxylase activity of immunocompetent cells enables them to produce hormonally active forms of vitamin D_3 . Activated macrophages express receptors for vitamin D_3 , monocytes and lymphocytes express a 50 kDa receptor protein, and lymphocytes express an 80 kDa cytosolic protein (Liang *et al.*, 2019). The signals from these proteins affect TGF, which regulates the differentiation and maturation of progenitors of stem cells, monocytes, macrophages, and lymphocytes capable of synthesising cytokines, growth factors, and calcium-dependent mediators of immunogenesis (Fisher *et al.*, 2019).

At the same time, the tocopherol present in the preparation stimulates the transformation of lymphocytes into blasts. Vitamins E and D_3 inhibit the activity of the nuclear factor NF- κB , which is involved in the development of immunodeficiency and the regulation of genes responsible for forming adhesion molecules (VCAM, ICAM), TNF, IL-1, IL-2, IL-6, IL-8, and γ -IFN. These vitamins prevent the dissociation of the NF- κB complex with activator protein-1 (AP-1) (Mussbacher *et al.*, 2019). This complex regulates the proliferation and interaction of cells during immune response development. Normally, it is blocked by the cellular inhibitor AP-1, but under stress conditions, it dissociates, allowing the released NF- κB to be transported into the nucleus, where genes that cause apoptosis are expressed.

Zinc restores the levels of Th1 and Th2 cells and affects IL-6, which increases the cytotoxic effect of T-lymphocytes. It also acts on the immunostimulatory cytokine IL-1 (Prasad, 2020). IL-1, in turn, influences the hypothalamus, which is responsible for coordinating neuro-endocrine-immune interactions in the body that potentiate the immune response during the inductive phase. Overall, the action of these interleukins involves enhancing B-lymphocyte differentiation, increasing antibody production, protecting cells from apoptosis, and stimulating haematopoiesis processes (Da Silva Lima *et al.*, 2023). IL-1 and IL-6 act as co-stimulators of T cells and thymocytes, increasing neutrophil chemotaxis and the release of lysozyme from cells, thus contributing to the normal phagocytosis of antigens (Rybtsova *et al.*, 2023).

The trace element selenium also exerts a stimulatory effect on lymphocyte activity in the blood of piglets in the research group after weaning from sows. This effect is attributed to its ability to mitigate the destructive impact of stress on lymphocytes (Da Silva Duarte *et al.*, 2022). In turn, the cobalt preparation included in the study in small quantities inhibits oxidation processes (Kosiorek & Wyszowski, 2019), which consequently prevents the formation of lipid peroxidation products in piglets after weaning. Stress conditions are known to lower magnesium levels in the body; however, supplementation with magnesium has been shown to increase the synthesis of CD3, CD16, CD22, IL-1, and immunoglobulins (IgG, IgA, IgE). Magnesium also supports the differentiation of T-killers into mature effectors and reduces the formation of reactive oxygen species (Shimasaki *et al.*, 2020). According to M. Pedrazini *et al.* (2024), arginine enhances the functional activity of lymphocytes. Moreover, it is known that in young animals and under stressful conditions, the levels of this amino acid in the body decline sharply, making it essential. Thus, the inclusion of arginine in the studied preparation likely had an immunotropic effect on the piglets' bodies, increasing their antioxidant status and reducing the intensity of free radical oxidation, thereby limiting the impact of stress. Therefore, the injection of the liposomal preparation containing vitamins A, D_3 , E, arginine, zinc acetate, sodium selenite, cobalt acetate, and magnesium sulphate eliminates the stress state in piglets caused by weaning from sows.

CONCLUSIONS

The studies indicate that weaning from sows results in an increase in the number of T-helpers in the blood of piglets on the 10th day after weaning, a decrease in the number of T-suppressors throughout the studied periods, and a reduction in the transformation ability of T-lymphocytes into blasts on the 10th day after weaning. In contrast, the combination of fat-soluble vitamins, arginine, zinc, selenium, cobalt, and magnesium in the liposomal preparation enhanced the immunoregulatory index, increased T-lymphocyte activity in the blast

transformation reaction, and reduced the number of T-suppressors in the blood of piglets on the 10th day after weaning from sows. The positive effect of the investigated liposomal preparation on the activity of the cellular and humoral links of the immune system in piglets is attributed to the synergistic action of vitamins, trace elements, and Larginine, which effectively mitigated the stress induced by weaning from sows. Further research is recommended to examine the effect of the immunotropic liposomal preparation on antioxidant protection markers, the nitric oxide system, and lipid and mineral metabolism in the bodies of piglets.

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CONFLICT OF INTEREST

The authors declare that the research was conducted without any commercial or financial relationships that could be interpreted as a potential conflict of interest. All authors have read and approved the final manuscript.

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Анотація. Профілактика розвитку імунодефіцитів у поросят при відлученні від свиноматок можлива шляхом парентерального введення жиророзчинних вітамінів, макро- і мікроелементів та дослідження їх впливу на динаміку змін кількості й функціональної активності субпопуляцій Т- і В-лімфоцитів. Відповідно метою цих досліджень було дослідження впливу комплексного ліпосомального препарату на клітинну і гуморальну ланку імунної системи поросят після відлучення від свиноматок. Для визначення у зразках крові поросят, відібраної до та в окремі періоди після відлучення від свиноматок, кількості різних популяцій і субпопуляцій Т-лімфоцитів використовували уніфіковану методику. Аналіз кількісного складу Т- і В-лімфоцитів й дослідження функціональної активності Т-лімфоцитів у реакції бластної трансформації проводили шляхом мікроскопії мазків під імерсією. Статистичну обробку отриманих результатів виконували за допомогою варіаційного непараметричного аналізу з використанням біометричного методу. Як показали дослідження, відлучення від свиноматок спричиняє зменшення у крові поросят кількості Т загальних лімфоцитів різного ступеня авідності та відносної кількості Т-супресорів й зростання чисельності окремих субпопуляцій Т-хелперів. Натомість введення поросятм ліпосомального препарату сприяло збільшенню в їх крові у період після відлучення кількості різних субпопуляцій загальних і активних Т-лімфоцитів та В-лімфоцитів й викликало зростання активності лімфоїдних клітин у реакції бластної трансформації з фітогемаглютиніном. Отриманий імуномодулюючий вплив досліджуваного препарату зумовлений комплексним поєднанням у його складі жиророзчинних вітамінів, мінеральних елементів та аргініну, що дозволило ефективно запобігти розвитку в організмі поросят імунодефіциту, спричиненого дією стресу, внаслідок відлучення від свиноматок

Ключові слова: імунодефіцити; ліпосомальний перпарат; популяції лімфоцитів; стрес; аргінін



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Evaluation of the dynamics of fattening of lactating cows of the newly created Ukrainian red dairy breed

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Abstract. The purpose of this study was to determine the dynamics of fattening of lactating cows of the newly created Ukrainian red dairy breed during the current lactation and at different lactation ages by eye assessment. Based on an industrial dairy complex-reproducer for breeding cattle of the Ukrainian red dairy breed, a scientific and industrial study was conducted to assess the dynamics of fattening of

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lactating cows ($n = 391$) using a modified eye-measured scoring scale and an innovative methodological Model Cow approach to comparing the findings with the visualisation. The study employed structural-comparative, analytical, and statistical methods. The findings of this study revealed that lactating cows of the newly created Ukrainian red dairy breed had a steady downward trend in fertility rates compared to the optimum levels recommended by experts. Thus, the fatness of the first-born cows was on average 35.35-58.25% lower than the recommended level, of the second lactation cows – 18.91–25.67%, and of the third lactation cows – 21.82-28.33% ($P < 0.001$). In cows of the fourth and older lactations (4.43 lactations on average), the dynamics of fattening dynamics was closer to the recommended optimum indicators throughout the current lactation. On average, in the middle of lactation (134.96 days, $n = 59$), the fattening was 2.39 points, which was 89.91-79.67% of the recommended levels ($P < 0.001$). According to the observations during the current lactation, it was found that the fattening of lactating cows did not have time to reach optimum levels at the end of the lactation period, which indicated significant metabolic disorders in the body of females, especially of young age, which require finding ways to correct the system of feeding the dairy herd of the newly created breed. An innovative approach to assessing the fatness of dairy cows revealed the potential of the methodology to use research findings to regulate herd management and feeding behaviour of cows under intensive milk production technology

Keywords: lactating cows; newly created Ukrainian red dairy breed; feeding behaviour; visualisation; model cow

INTRODUCTION

Over the past decades, in response to an urgent demand from agrobusiness, Ukrainian breeders have carried out large-scale breeding work to create new Ukrainian specialised dairy breeds adapted to operation in large industrial complexes. To fulfil the tasks of forming highly productive dairy herds of animals with unified external parameters according to the conditions of industrial production and quantitative and qualitative indicators of milk that meet the requirements of modern processing enterprises, local populations of dairy cattle were used and large-scale breeding and biotechnology measures were applied, specifically, the frontal use of the method of artificial insemination of cows and heifers with cryopreserved semen of valuable foreign sires, mainly of Holstein breed.

According to J. Bezdicsek *et al.* (2020), D.P. Berry and R.D. Evans (2022), depending on the influence of paratypic factors, the findings showed significant variability and depended heavily on the management factors of each particular herd, the qualifications of veterinary medicine specialists, training of service personnel, etc. Considering the latest trends, dairy farms have generally moved away from the previous negative practice of a deficient feed base with uncontrolled ration composition. Modern dairy enterprises mostly use a stable feed base of their production using software to adjust daily rations of the dairy herd according to the requirements of scientifically based zootechnical standards for meeting the physiological needs of lactating and dry cows and sustainable production of the amount of milk required to ensure the cycle of processing raw materials into various dairy and fermented milk products (Ruban *et al.*, 2022).

F. Weik *et al.* (2021) found that the great level of milk yields and their planned growth were influenced by several factors, among which the most influential was the implementation of improved feeding systems

for the breeding stock based on the digitalisation of feeding technology, maximum mechanisation of feed preparation processes, and the widespread use of high-tech feed additives. Furthermore, a considerable pressure on the formation of newly created dairy breeds was the introduction of a strict selection system during breeding and the selection and culling of cows that do or do not meet the requirements of dairy breed standards. According to large-scale studies by S.S. Yurochka *et al.* (2023), 15-21% of full-grown cows were culled annually in leading farms, considering milk production, linear classification of exterior type, and live weight. According to experts, it was a balanced, integrated approach to the formation of dairy herds with first-born cows selected for their origin and individual qualities, combined with an improved feeding system, which led to an exponential increase in the milk production of herds of newly created Ukrainian breeds.

There is a consensus among scientists and livestock practitioners on the effectiveness of breeding and selection work with the herd, which is aimed at consolidating animals in the desired type according to the main useful economic traits (Brito *et al.*, 2021; Buonaiuto *et al.*, 2023). A significant factor that characterises compliance with both selection and ration optimisation requirements is the assessment of lactating cows' fatness at different stages of the lactation cycle (considering only sires with a stable feed base, to maximise the exclusion of the influence of breed factors on the implementation of breed factors and reduce the pressure of paratypic factors). According to modern scientific concepts, fatness is an essential indicator that helps to assess the metabolic status of a dairy cow in real time and trends in the dynamics of changes. D. Liu *et al.* (2020) defined fatness as the degree of development of muscle tissue and subcutaneous fat deposits. Thus, in the researchers' opinion, fatness reflects the state of fat reserves in

the animal's body, which increase the level of natural adaptability of the organism and the controllability of industrial dairy production processes.

There is an urgent need to improve the methodology for assessing the fatness of dairy cows, considering the intensity of the key production processes (parturition, peak lactation, end of lactation) to obtain a generalised quantifiable score profile that can be quickly and efficiently compared with the previous or subsequent one to develop operational measures to regulate the feeding system, urgent measures for individual animals or groups, and overall optimisation of herd management. Considering the insufficiency and controversy of data on the factual state of fatness of cows of different breeds in the literature, it is still important to find ways to increase milk production aimed at fulfilling the genetic potential of the population of newly created herds operated in very different regions of Ukraine and requiring the formation of a database for comparison.

The purpose of this study was to determine the dynamics of fattening of lactating cows of the newly created Ukrainian red dairy breed during the current lactation and at different lactation periods by means of eye assessment.

MATERIALS AND METHODS

The experimental part of the study was conducted during 2022-2024 in a breeding reproducer (Odesa region) for the breeding of a new domestic dairy breed of productivity – Ukrainian Red Dairy (URD), which was created based on the genotype of the red steppe breed of the southern fat-milk population, with which, according to the development of specialists of Ukrainian scientific institutions, long-term breeding work was performed to improve productive qualities through Holsteinisation using sperm from outstanding bulls obtained from leading breeding centres in Europe (ABS Genus) and the USA (CRI, CRV).

The farm had a stable feed base of its own production, daily rations were developed using the DairyPlan software with the formation of monofeed composition for each production Group of cows (parturition, peak lactation and mid-lactation, second half of lactation, end of lactation). The balance of diets was corrected by introducing feed additives to the diets to balance the composition of amino acids, macro- and microelements, vitamins, and other ingredients according to the requirements of the physiological state of the animals. Feed was distributed twice a day on feed tables, and specialists ensured that feed was raked in time for each cow to reach the area where it was available. The farm followed the technology of producing complete mono-feed, with the introduction of freshly cut green fodder in the summer (no more than 15% of the total nutritional value of the diet). The conditions of keeping, operation, and care of the animals met modern

zoohygienic requirements (untethered housing, individual cow rest boxes, machine milking with individual milk production records). Technological groups of cows were formed according to factual milk yield, time of fertility, and lactation period: Group 1 (104 cows) – 1st lactation, daily milk yield 37.30 ± 2.97 kg, lactation period 123.33 ± 42.06 days; Group 2 (99 cows) – 2nd lactation, daily milk yield 29.29 ± 3.41 kg, lactation period 153.68 ± 55.62 days; Group 3 (107 cows) – 3rd lactation, daily milk yield 36.92 ± 2.65 kg, duration of the lactation period 146.95 ± 53.77 days; Group 4 (81 cows) – 4th lactation, daily milk yield 42.90 ± 3.91 kg, duration of the lactation period 134.96 ± 42.22 days.

All livestock were covered by veterinary support and a programme of infectious disease prevention (vaccinations) according to current guidelines. No harm was done to the health of the animals during the study.

To conduct a visual assessment of lactating cows' fatness, a working scale for comparing fatness indicators in points was developed based on the recommendations of Ukrainian scientists (Vinyukov *et al.*, 2021) and modified to factor in the conditions of intensive production at the study farm. Due to the developed scheme of the experiment, a fixed time for visual inspection of dairy cattle was determined, and the evaluation methodology was established by the commission, considering the exterior type of the newly created URD breed of this population. The animals were not probed due to the lack of separate specialists for such research formats. To increase the standardisation of the results, the experts selected a 'model animal' from the given population for comparison, which met the requirements for the scoring on the working scale. The efficiency of the assessment was organised by inspection at a fixed time of daytime milking in the milking parlour ("Yalynka" type milking machine) with lighting of at least 200 lux. Viewing angles were chosen based on the design features of the viewing platform: from both sides during milking, at the same distance from the animal, examining the general shape of the body, exterior features: the sides of the cow, spinal vertebrae, gluteal tubercles, the presence of a hungry pit, protruding parts of the spine and spinal vertebrae, macules, and overall body roundness. The condition of the skin, limbs, and hoof horn were also considered. The data were recorded in the corresponding format in the report, after which they were subjected to generalisation, structuring, and biometric processing (Table 1). Structural-comparative, analytical, and statistical methods were employed. Zootechnical indicators were selected from the farm's computer database.

The commission data were quickly compared with the visualised example of the 'Model Cow' and the working scale. The research data were generalised, summarised, statistically processed, and presented for analysis in the form of tables with illustrative photographs of the most typical conditions of lactating cows.

The farm has implemented a modern system of feeding the dairy herd with monofeed from juicy forages and

concentrates with a moisture content of 50–60% with constant feed quality control.

Table 1. Working scale for assessing cow fatness in various periods of lactation

Production cycle and evaluation period	Terms, days	Fatness score, points		
		minimum	optimum	maximum
Early lactation	8-90	2.75	3.00	3.25
Peak lactation and mid-lactation	91-150	2.50	2.75-3.00	3.25
Late lactation	151 and more	3.00	3.25	3.50

Source: developed by the authors of this study

The analytical part of the study was conducted at the Department of Surgery, Obstetrics and Small Animal Diseases of Odesa State Agrarian University, where structural-comparative and statistical methods were applied using biometric processing of the results (Petrovska *et al.*, 2022). All experimental studies were conducted following modern methodological approaches and according to with relevant requirements and standards, specifically, they met the requirements of DSTU ISO/IEC 17025:2005 (2006). Animals were kept and all manipulations were performed according to the

provisions of the Procedure for conducting experiments and experiments on animals by scientific institutions (Law of Ukraine No. 249, 2012), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986).

RESULTS AND DISCUSSION

The summary results of the visual assessment of lactating cows ($n = 391$) of the newly created URD breed following the objectives of the study scheme are presented in Table 2.

Table 2. Dynamics of fattening of lactating cows of the URD breed during lactations, $n = 391$

Lactation	n	Fatness, score	Duration of the lactation period, days*	Factual daily milk yield, kg*
1	104	1.94 ± 0.02	123.33 ± 42.06	37.30 ± 2.97
2	99	2.23 ± 0.19	153.68 ± 55.62	29.29 ± 3.41
3	107	2.15 ± 0.19	146.95 ± 53.77	36.92 ± 2.65
4 and older	81	2.39 ± 0.07	134.96 ± 42.22	42.90 ± 3.91
M ± m	391	2.18 ± 0.09	139.73 ± 6.69	36.60 ± 2.79
Biometric indicators				
Q		0.187	13.397	5.589
CV		8.595	9.588	15.270
td		20.533	20.533	12.311
P <		0.001	0.001	0.001

Note: * – average for the entire period of observation during lactation; ** – factual milk yield at the time of assessment. td – criterion of reliability of the difference between the mean values of indicators, $P <$ reliability coefficient of fattening indicators, CV – coefficient of variation, Q – Cochran's C test

Source: developed by the authors of this study

The analysis of the dynamics of growth of indicators prompted the structuring of observation groups by the number of lactations: from the first to the fourth and older. In the older group, there were cows with 6-7 lactations, but the average age was 4.43 lactations and, as the data show, the number of such cows was only 20.72% of the total main dairy herd and indicated a strong level of excessive departure of females in the first or second lactation, as the authors of the present study have shown in previous publications.

Generalised data on the fatness of cows in the first lactation with an average lactation period of 123.33 days revealed a considerable deficit in the fatness of

firstborn cows (35.34-58.25% less than the recommended optimum level, $P < 0.001$). Even the comparison with the minimum acceptable assessment of the fatness of the firstborn cows provided only 71.14% ($P < 0.001$) of the average score of the fatness of the first-calf heifers, which requires the attention of the company's specialists to adjust the technology of feeding young cows in the early stages of lactation, which, as is well known, substantially affect not only the entire course of the current lactation, but also the entire duration and efficiency of the cow's production operation. The cow feeding management system is presented in Figures 1-3.



Figures 1-2. Feeding management system for dairy cows in the studied farm

Note: the feeding management system of dairy cows in the studied farm was distinguished by the optimisation of manual shovelling of monofeed into the feed table area, which is comfortable for animal consumption

Source: compiled by the authors

After milking, the older cows immediately came to the feed table in the sections, and the first-calf heifers, even if there was a sufficient number of available feed places, stayed behind and did not try to stand next to the older cows. Such a sequence of feed consumption led to technological and then physiological malnutrition of the first-born cows (Figure 4 shows how the first-calf heifers with low live weight approach the feed table and begin to consume the remains of the sorted feed mixture, the nutritional value of which had already been reduced). They gradually lost weight and were even less suitable for typical feeding behaviour, which resulted in reduced milk production, increased vulnerability to diseases, especially of the reproductive system, and premature culling.



Figure 4. First-calf heifers in large sections come to the feeding table last

Note: first-calf heifers in large sections come to the feed table last and therefore eat a feed mixture with a lower content of concentrates and bio-additives

Source: developed by the authors of this study

Observations showed that the average fatness of cows of the second and third lactations was also below the recommended level, namely: the second – by 18.91-25.67%, and the third – by 21.82-28.33% ($P < 0.001$), which was a signal of deep metabolic disorders of



Figure 3. At a fixed time during milking in the milking parlour, the cows' fatness can be monitored

Note: under conditions of fixed time during milking in the milking parlour, the cows' fatness can be promptly monitored from both sides of the cows with optimum lighting and the choice of a fixed viewing area from two side angles

Source: developed by the author of this study

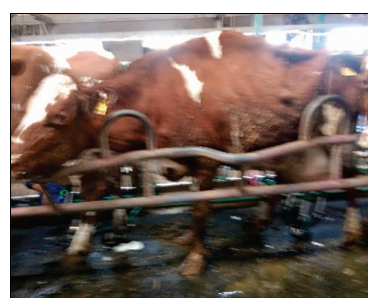


Figure 5. Animals with low fatness, extreme deficiencies, and locomotor defects were found among cows during different lactations

Source: developed by the authors of this study

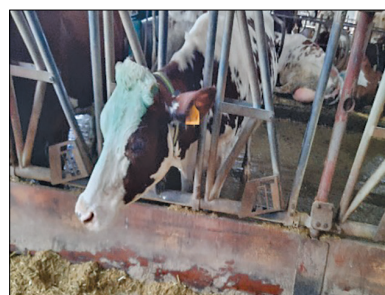


Figure 6. Photo and video materials were entered into the archive of the company's computer programme on a regular basis

Note: the company's computer programme archive was regularly updated with photos and videos describing the desired type of 'Model Cow' of the newly created breed

Source: developed by the authors of this study

lactating animals. Considering that the average daily milk yield of the first-born cows was greater than that of cows of the 2nd and 3rd lactations, the analysis of fattening revealed the need to correct both the composition of diets and housing conditions. Older cows (fourth

and higher lactations) showed a greater level of milk production among the entire dairy herd with a lower level of fatness loss: the average daily milk yield during 139.94 days of lactation was 2.39 points of fatness, which is 13.09-20.33% lower than the recommended norms ($P < 0.001$). The chosen methodological approach of eye assessment of fatness helped to compare the

dynamics of fat reserves recovery in cows during the current second and third lactations (Table 3), which accounted for most of the dairy livestock of the surveyed farm. This production period should potentially characterise the most typical physiological states of lactating cows at Ukrainian industrial enterprises, a clearly visualised signal of which is the assessment of animal fatness.

Table 3. Dynamics of fattening of lactating cows during the second and third lactations, $n = 196$

Lactation	Assessment of fatness by lactation days:								
	n	LP, days	Score ($M \pm m$)	n	LP, days	Score ($M \pm m$)	n	LP, days	Score ($M \pm m$)
2	14	7-90	1.96 ± 0.14^a	29	91-150	2.13 ± 0.14^b	46	>150	2.59 ± 0.10^c
3	13	7-90	1.86 ± 0.18^d	30	91-150	2.07 ± 0.07^e	64	>150	2.53 ± 0.08^i

Note: a-d; d-e ($P < 0.05$); a-b; a-c; d-e ($P < 0.01$); a-j ($P < 0.001$)

Source: developed by the authors of this study

As the findings of the study indicate, during the transition of cows from the terms of parturition to the peak of lactation and the udder secretory activity reaching the stage of reduced milk secretion, a steady

increase in the fattening of females is observed, namely: by 8.67% and 32.14% and by 11.29 and 26.48%, respectively, for the 2nd and 3rd lactations compared to the period of early lactation ($P < 0.05$; $P < 0.01$).



Figure 7. Fatness of cows from different angles compared to other animals in the section

Note: During the study, attention was paid to the fatness of cows from different angles compared to other animals in the section

Source: developed by the authors of this study



Figure 8. Visual comparison and correction of the cow fatness score

Note: The feature of the location of cows at a fixed milking time helps to conduct a visual comparison and correction of the fatness assessment scale among the group

Source: developed by the authors of this study

An illustrative analysis of the data on the factual timing of lactation in the Group of older cows, which biologically have the most stable indicators of adaptation to the technological operations of

this environment, where they were formed during both their ontogeny and several generations as the main biological objects of production, is presented in Table 4.

Table 4. Fatness of lactating URD cows of older age in different periods of lactation, $n = 59$

Number of cows	Lactation period at the time of assessment ($M \pm m$)	Fatness, score ($M \pm m$)	Lim of fatness assessment in points	
			min	max
7	60.67 ± 8.18^a	2.29 ± 0.35^d	1.50	3.50
21	137.34 ± 5.31^b	2.52 ± 0.19^e	1.50	2.50
31	206.08 ± 5.31^c	2.36 ± 0.19^i	2.00	3.50

Note: a-b; a-c ($P < 0.001$); d-e ($P < 0.05$); d-j ($P < 0.01$)

Source: developed by the authors of this study

In the early lactation period, cows had indicators of 75.33% of the optimum, in the most intensive period of milk secretion – 91.63-84.00%, and in the decline of lac-

tation – 72.62% ($P < 0.05$; $P < 0.01$), which reveals contradictory trends during metabolic processes in the body of dairy cows. The boundaries of the established fatness

in older cows also varied considerably even at the beginning of lactation: from 1.50 to 3.50 points (Fig. 8).

K.K. Cevik (2020) and H. Alem (2021) found that the key to fulfilling the high genetic potential of newly created cow breeds, maintaining health and reproductive capacity with minimal feed losses per unit of production is biologically complete energy-saving feeding, which ensures the optimum need of animals for nutrients, biologically active substances and a strong level of their absorption. I.J. Lean and P. DeGaris (2021) and S. Nagy *et al.* (2023) insisted on focusing on the development and study of the fullness of feed mixtures for feeding high-yielding cows, considering regional zones. The current study emphasised the need to create regional dairy cattle populations. The data obtained by I.J. Lean *et al.* (2023) indicated that in the current state, the intensification of the dairy farming industry, as the basis for supplying the internal market with sufficient raw milk and ensuring food security in each country, requires effective methods of managing the physiological state of dairy cows, which is formed by maintaining the animal's normal metabolism under the conditions of technological stress of industrial production.

Ukraine has already built large dairy complexes with intensive technologies that have been put into practice as a result of the Revived Livestock programme, which makes provision for round-the-clock free access to the feed table for each cow, the possibility of automated adjustment of the composition of diets, etc. Analysing the results of the programme implementation, Ukrainian researchers S.S. Yurochka *et al.* (2023) emphasised the necessity of considering the physiological characteristics of dairy animals, a characteristic feature of which is the ability to produce milk during certain periods of physiological activity at the expense of their body reserves, which leads to substantial changes in the condition of cows in various technological periods: with an increase in lactation and its decline, dry period. Particularly significant changes in fatness are typical for specialised dairy breeds with a prominent level of Holsteinisation, which was noted by W. Shi *et al.* (2023) as significant changes in animal condition. Considering the unresolved problems in advanced technologies for the rapid and stress-free reformation of similar groups of animals (by age, productivity, live weight, physiological condition, etc.), monitoring of fertility indicators of dairy cows stays a vital tool for managing herd productivity. Modern scientific research requires a deeper understanding of the conversion of feed into milk and the development of guidelines and manuals that will help implement this knowledge in cow feeding practices to improve the efficiency and sustainability of milk production. Therefore, the need to develop operational methods for assessing the real and dynamic fatness of dairy cattle in highly productive herds with the ability to individually adjust the diet for nutrient content, using additional feed to each cow's diet ac-

cording to the need by various means of mechanisation and automation, which increases the role of fatness assessment in the organisation of real production, is becoming increasingly significant (Dallago *et al.*, 2021; Marquez-Acevedo *et al.*, 2023).

Studies conducted by leading research institutions in Ukraine have provided detailed scientifically based recommendations on the methodology for assessing the fatness of cows operated in industrial enterprises to improve the skills of producers (Vinyukov *et al.*, 2021). The proposed methods are based on conventional approaches to animal grading using eyeball assessment of body shapes, individual articles, and methods of probing control anatomical points on the animal's body surface. For instance, muscle development is determined by the overall roundness of the body, the fullness of the hips, the density of muscle tissue when palpated, and how much the skeletal bones protrude. A well-fed animal has a rounded body with no protruding ilium, ischial tubercles, sacrum, and especially the spinal vertebrae. Animals with underdeveloped muscles have a less rounded body shape. Poorly fed cattle have an angular body shape, the lumbar region is flat, the hips are tight and almost not full, and the skeletal bones protrude quite strongly. The degree of development of fat deposits is determined by palpation of the animal's body in the places most characteristic of fat deposition.

Based on this methodology, J. Schillings *et al.* (2023) developed a series of recommendations for the boning process, organisation of the evaluation process (lighting of the visual inspection site, rules for choosing anatomical points – probes, etc.), which considerably increase the level of unification of indicators in points, measurability of qualitative characteristics. However, optimising the fatness assessment process by detailing indicators, especially those related to fat palpation, considerably slows down the procedure, which is technically inconsistent with the high pace of production processes in intensive enterprises. Furthermore, a substantial time lag in obtaining the analysis results from the factual physiological state of a particular cow or technological Group of livestock cancels out most of the positive developments of existing methods. The recommended methodological approaches require separate organisational and financial support, which in the current conditions of the Ukrainian agricultural sector does not have a basis for implementation.

Considering that, according to the observations and data of S. Nazhat *et al.* (2021), each farm may have cows that are very thin or overfed, the present study was characterised by a substantial decrease in fat resources in most of the lactating cows, subject to a stable feed base and stable filling of feed tables. The analysis of the production conditions of the studied enterprise revealed one of the crucial factors of significant loss of fatness of the first-born cows, namely: the technology of the farm was based on the principle of general

maintenance of dairy cows of all age groups in large sections (90-110 heads), where a negative trend in access to the feed table was observed according to the hierarchical structure of the herd, which was preserved in this population of the newly created breed. Thus, the animals gradually lost weight and were even less suitable for typical feeding behaviour, which resulted in impaired milk production, reduced immunity, and premature culling, which is consistent with the studies of a series of researchers (Poczta *et al.*, 2020; Newton *et al.*, 2021).

After and during the period of weight loss, cows need to receive more feed than their normal requirement to restore normal fatness. Each farm may have animals that are very fat or very thin for their period of lactation. An example of this is comparing the factual fatness of cows with a 'model cow' in real time or retrospectively. If such animals are not identified in time, conditions are created that negatively affect the rotation of the herd and the operational life of the dairy cattle. Specifically, the lack of attention to regulating the fatness of first-calf heifers has a substantial negative influence on the breeding progress in creating new Ukrainian breeds adapted to industrial technologies. Probably, the established signal of a significant decrease in the fatness of the first-calf heifers for this population should highlight the way to optimise the feeding system on the farm, considering the efficiency and availability of the selected innovative method of visual assessment with the comparison of a 'model cow'. L. Brito *et al.* (2021) showed the effectiveness of this methodological approach in intensive livestock production technologies.

According to the findings of the study, during the transition of cows from the terms of beginning lactation to its peak and to a decrease in the secretory activity of the udder, was observed a steady increase in the fatness indicators of females, namely: by 8.67% and 32.14% and by 11.29 and 26.48%, respectively, for the 2nd and 3rd lactations compared to the period of early lactation ($P < 0.05$; $P < 0.01$). The data obtained are in line with the findings of studies by many researchers, which indicated that the body of dairy cows tries to restore metabolic balance during the completion of lactation activity (Shi *et al.*, 2023; Marquez-Acevedo *et al.*, 2023). However, as the analysis revealed, the studied livestock does not restore fatness to the recommended levels, which proves the need for a more systematic consideration of the results to correct the methods of herd management, because the loss of the necessary resources of the cow's body contributes to vulnerability to diseases in the herd, which can result in large financial losses caused by treatment costs, reduced animal productivity and reproductive characteristics.

Research on feeding high-yield cows requires improving energy, protein-amino acid, carbohydrate, lipid, mineral, and vitamin nutrition, and developing

measures to ensure them, and controlling the effectiveness of organisational approaches is created by continuously monitoring the profile of fattening indicators of each herd. The methodological approach in the research and production study revealed that the task of training young specialists according to the dual principle of education is becoming a real production practice. The innovative methodological approach in the study allowed for parallel work in the demonstration and training module, which helped to implement the dual principle in the agricultural education curriculum. The training of higher education students in veterinary medicine showed young specialists the examples of positive practices in industrial livestock production, allowed them to learn about the complexities of the modern dairy industry and consolidate their practical skills. The use of modern office equipment helped to accumulate a list of examples of the 'Model Cow' of the desired exterior type in the farm's computer archive and to visualise the dynamics of changes in the recorded examples and live animals during production processes over several years.

CONCLUSIONS

It was experimentally established by eye (according to a modified method) assessment of the dairy herd ($n = 391$) at a fixed time that the fattening indices of cows of the newly created Ukrainian red dairy breed are significantly lower than the recommended optimum levels, namely in first-born cows, fatness is lower by 35.34-58.25% ($P < 0.001$); in cows of the 2nd lactation – by 18.91-25.67%, and in the 3rd lactation – by 21.82-28.33% ($P < 0.001$), respectively, which was a signal of deep metabolic disorders in females at the beginning of lactation, especially at a young age. In cows of the older Group (average age 4.43 lactations, $n = 59$), the smallest deviation of fattening indices from optimum levels was observed, namely: in the middle of the current lactation (134.96 days), the fattening was 2.39 points, which was 86.91-79.67% of the recommended optimum values ($P < 0.001$), indicating greater adaptability of animals that retain endurance for industrial operation for several lactations.

Changes in the fattening indices of cows of different age groups were marked by contradictory dynamics between the level of daily milk yield, the period of milk secretion and condition, which indicated the continuation of the processes of genotype transformation of the newly created population and, accordingly, the need for strict attention to the selection of the most desirable types of animals. An innovative methodological approach to the visual assessment of lactating cows' fatness as a signal for optimising industrial milk production technology helped to identify problems in the system of feeding of first-calf heifers during the beginning and peak of lactation, which contributed to optimising herd management. The methodology of eyeball

assessment of cow fatness at a fixed time using visualisation of the 'model cow' of a given herd will facilitate the introduction of practical skills for specialists and students of animal and veterinary engineering on the principles of dual education. The prospect of further research will be to improve the innovative methodological approach to assessing the fatness of cows at the end of lactation and during the dry period for the purpose

of selecting the most desirable types of cows of the newly created Ukrainian red dairy breed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Оцінка динаміки вгодваності лактуючих корів новоствореної української червоної молочної породи

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Анотація. Метою дослідження було визначення динаміки вгодваності лактуючих корів новоствореної української червоної молочної породи впродовж перебігу поточної лактації та за різного лактаційного віку шляхом оцінки. На базі промислового молочного комплексу-репродуктора із розведення худоби української червоної молочної породи було проведено науково-виробниче дослідження з оцінки динаміки вгодваності лактуючих корів ($n = 391$) за використання модифікованої оцімної бальної шкали та з інноваційним методологічним підходом порівняння результатів із візуалізацією «Модельної корови». Було застосовано структурно-порівняльний, аналітичний і статистичний методи. Результати дослідження показали, що лактуючі корови новоствореної української червоної молочної породи відрізнялись сталою тенденцією до зниження показників вгодваності у порівнянні з рекомендованими фахівцями оптимальними рівнями. Так, вгодваність первісток в середньому була нижче рекомендованої на 35,35-58,25 %, корів другої лактації – на 18,91-25,67 %; третьої – на 21,82-28,33 % ($P < 0,001$). У корів четвертої і старшої лактації (в середньому 4,43 лактації) було відмічено більш наближену до рекомендованих оптимальних показників динаміку вгодваності впродовж всієї поточної лактації. В середньому у термін середини лактації (134,96 днів, $n = 59$) вгодваність становила 2,39 балів, що складало 89,91-79,67 % від рекомендованих рівнів ($P < 0,001$). За даними спостережень впродовж поточної лактації встановлено, що вгодваність лактуючих корів не встигла досягти оптимальних показників наприкінці лактаційного періоду, що свідчило за значні метаболічні негаразди у організмі самиць, особливо молодого віку, які потребують пошуку шляхів корекції у системі організації годівлі дійного стада новоствореної породи. Інноваційний підхід до оцінки вгодваності молочних корів засвідчив потенціал методики для використання результатів досліджень з метою регуляції менеджменту стада та кормової поведінки корів в умовах інтенсивної технології виробництва молока

Ключові слова: лактуючі корови; новостворена українська червона молочна порода; кормова поведінка; візуалізація; модельна корова



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The role of microbial enzymes β -glucanase and α -amylase in optimising feed production for poultry

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Abstract. With the increase in world population and higher consumption of poultry meat, the need for efficient feed production to ensure high quality and quantity of products is increasing. The main objective of the study was to find out the effect of microbial enzymes β -glucanase and α -amylase on optimising the performance of

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poultry feed. The method of analysis, one-way analysis of variance, chemical analysis and quantitative analysis were used in the study. A review of the work showed that enzyme analysis in the experimental feeds confirmed the determined concentrations of 44, 67 and 88 kN. The treatments significantly reduced body weight gain and feed conversion ratio throughout the evaluation period. The effect was particularly marked at K-110 kcal/kg and enzyme concentrations of 250, 350 and 450 mg/kg. Enzyme supplementation resulted in partial recovery of performance evident at various intervals including 10-17 days, 17-30 days, and 30-37 days. The enzyme supplemented groups showed positive results in body weight gain, especially at K-110 kcal/kg and enzyme concentrations of 250, 350 and 450 mg/kg, compared to the control group. The results emphasised not only the high precision of the study, but also the effectiveness of the enzymes in improving feed utilisation and maintaining bird performance under altered dietary conditions. The study of enzyme complex in feeds is of practical importance to the poultry industry, as it confirms the efficacy of enzyme supplementation in improving feed utilisation and maintaining bird performance under altered diets. The findings provided a basis for optimising diets using specific energy levels and enzyme concentrations, which can lead to improved body weight gain and FCR, promoting more efficient nutrient uptake and reducing productivity losses in birds

Keywords: diet; active additives; broilers; productivity; body weight gain; feed conversion ratio

INTRODUCTION

The study of the use of supplementary enzymes in poultry diets represents an important aspect of modern science and agriculture. The results of the research conducted in recent years show a significant increase in interest in this topic. The increased need for research lies in the problems of determining the optimum conditions for the use of supplementary enzymes, selection of the most effective types of enzymes and their ratio in diets. It is also important to study the possible negative effects or limitations of using certain enzymes in order to prevent possible health problems in poultry. According to N.T. Rashidova and A. Fayziyeva (2023), the use of supplemental enzymes in poultry diets can significantly improve digestive efficiency and nutrient absorption. The authors note the increased relevance in today's emerging market and increased competition, where efficient poultry production plays a key role. According to the assessment of Z. Alimkulo *et al.* (2022) and also A. Temirbekova *et al.* (2023), research on this topic has important practical implications for agricultural enterprises and farmers. Understanding which types of enzymes have the best effect on digestive processes in poultry allows optimising diets and increasing poultry performance, which ultimately affects the economic outcome of the farm.

The Food and Agriculture Organization of the United Nations states that microbial enzymes such as β -glucanase and α -amylase play a key role in optimising poultry feed production, as they aid in efficient digestion and nutrient absorption. The intervention of these enzymes in feed additives helps to improve nutrient availability, increase bird productivity, and reduce feed intake (Smits *et al.*, 2021). In their research, A. Mendybayeva *et al.* (2023) concluded that α -amylase and β -glucanase, assist in the breakdown of complex carbohydrates such as starch and beta-glucans which may be difficult for birds to digest. This improves the digestibility of the feed, making it more suitable for

efficient energy utilisation. In addition, the authors note that the efficient breakdown of complex carbohydrates into simpler forms such as sugars and simple polysaccharides helps to improve feed intake efficiency. This is especially important for birds that frequently receive cereals in their diet.

Meanwhile, K. Mogilev *et al.* (2023) state that α -amylase and β -glucanase can help to overcome anti-nutrients such as phytates and other compounds that may reduce mineral absorption. This is especially important in diets with high grain content. The authors also note improved digestion and nutrient absorption, which are directly linked to improved bird performance. This can be in the form of faster growth, increased egg production (for laying hens) or improved body weight gain. In their work, M. Kurmanbayeva *et al.* (2023) noted the benefits of using β -glucanase in poultry feed. Among the positive aspects, the authors noted improved absorption of energy and other nutrients from the feed, which may lead to increased bird productivity, improved feed conversion and reduced digestive stress on the bird's digestive system. The results of a study by S.S. Muminova *et al.* (2023) show the role of α -amylase in the breakdown of complex carbohydrates, such as starch, into simple sugars. The authors observed an increase in energy availability from grain feeds, improved starch digestibility, reduced digestive load and increased feed intake efficiency. Since most cereals used in poultry feed contain starch, the addition of α -amylase helps improve the breakdown of starch into simpler forms that are more easily digested by the birds' bodies.

However, research lacks better data on dynamic changes in poultry growth and experiments with different concentrations of the microbial enzymes β -glucanase and α -amylase. Based on the above, the aim of the study was to analyse the effectiveness of supplementary enzymes in poultry diets to improve digestion and nutrient absorption. In particular, investigating the

role of microbial enzymes such as α -amylase and β -glucanase in improving digestion, studying their positive contribution in the breakdown of complex carbohydrates and overcoming anti-nutrients.

MATERIALS AND METHODS

Various methods were applied to solve the problems and to collect the necessary information to analyse the effects of protein additives in the production of meat products. Analysis method, one-way analysis of variance method, chemical analysis and quantitative analysis were used in the research. The method of analysis was applied to search for information resources and analytical processing of data in the research context of the selected topic. This enabled to identify patterns and study the dynamics of poultry development with microbial enzymes such as β -glucanase and α -amylase. The information for the study was derived from the data obtained from the experiments conducted by D. Shalginbaev *et al.* (2022) in agro-combinat "Astana" (Astana), A.T. Abylgazinova *et al.* (2023) in "KazAgroFood" (Pavlodar) and A.K. Saken *et al.* (2021) in "Altyn Taraz" company (Taraz city) of the Republic of Kazakhstan. Each of the researchers used the same methodology for rearing, preparation for slaughter, as well as slaughtering itself and analysing performance. This meant formulating diets based on soya and maize meal with careful consideration of nutrient and energy requirements of birds. The calculations made ensured that the birds' requirements were met or even exceeded, except for the total energy score. The diets were supplemented with an enzyme complex with a reduction of 120 kcal IEE/kg with the addition of 250 (U-110-250), 350 (U-110-350) and 450 (U-110-450) mg/kg of exogenous α -amylase+ β -glucanase.

Data were analysed using one-way analysis of variance using SAS 9.3 procedure. The significance level was set at 7% and mean differences were identified using Tukey's test for statistically significant model effect (Nanda *et al.*, 2021). Linear and quadratic effects of decreasing imaginary metabolic energy (energy absorption ratio) were investigated for diets without enzyme supplementation. Calculated values of imaginary exchangeable energy (IEE) for body weight gain (BWG) and feed productivity ratio (FPR) at each level

of enzyme addition allowed estimation of additional improvements in IEE with the enzyme complex at different points on the curve. Regression equations were fit and solved for the amount of energy intake and additional amylase intake (based on estimated data) in relation to specific variables. This was performed using both quadratic and linear models. The enzyme analogy estimation performed in this study followed the methodology outlined in the work by M.T. Hasan *et al.* (2023), where the equivalence of phosphorus released from phytase was evaluated. Poultry productivity was estimated using weekly measurements of BWG, feed intake (FI) and feed conversion ratio (FCR) corrected for dead weight of birds aged 1 to 45 days. Abdominal fat was weighed separately. Carcass yield was estimated considering the weight of the animal, while parts of meat for sale and abdominal fat were measured as a percentage of the total carcass weight. All sampling was conducted in accordance with the European Convention for the Protection of Pet Animals (1987) and the Universal Declaration on Animal Welfare (2007).

RESULTS

The widespread adoption in commercial practice of the use of exogenous enzymes in broiler diets has only recently occurred, although research on this topic has been ongoing for several decades. The practical use of phytase in broiler diets is now being successfully applied on commercial poultry farms, but the use of enzymes targeting other substrates remains less common. However, this trend is rapidly changing due to the increasing cost of ingredients, especially those that provide energy and have market linkages to bio-fuels. In the works of D. Shalginbaev *et al.* (2022) and also A.T. Abylgazinova *et al.* (2023), the compliance of α -amylase and β -glucanase concentrations with expectations in experimental feeds was confirmed by enzyme complex analysis. The set values were: 45, 65 and 85 kN units of α -amylase/kg; actually, recorded values: 44, 67 and 88 kN of α -amylase/kg, respectively. Exposure to treatment affected BWG ($p < 0.01$) and FCR ($p < 0.01$) in birds for all weeks evaluated, with a loss of productivity due to reduced IEE. However, a partial recovery of productivity due to enzyme supplementation was noted (Table 1).

Table 1. Effect of gradual reduction of imaginary metabolisable energy in broiler diets with or without the addition of α -amylase+ β -glucanase complex from days 1 to 40

Procedures, kcal/kg	Addition of enzymes, mg/kg	BWG, g					
		1-10 days	10-17 days	17-30 days	30-37 days	37-45 days	1-40 days
Control group	-	116	289	518	661	846	3,137
K-55	-	117	279	511	638	841	3,099
K-85	-	115	285	507	641	832	3,065
K-110	-	116	287	502	635	828	3,049
K-110	250	124	288	514	636	838	3,111

Table 1. Continued

Procedures, kcal/kg	Addition of enzymes, mg/kg	BWG, g					
		1-10 days	10-17 days	17-30 days	30-37 days	37-45 days	1-40 days
K-110	350	124	293	515	639	832	3,118
K-110	450	127	296	514	643	853	3,121
p-value		0.0399	0.0043	0.0223	0.0167	0.0369	0.0093
Procedures, kcal/kg	Addition of enzymes, mg/kg	Feed consumption, g					
		1-10 days	10-17 days	17-30 days	30-37 days	37-45 days	1-40 days
Control group	-	129	401	731	1.059	1.389	4,048
K-55	-	133	398	743	1.066	1.384	3,874
K-85	-	124	406	731	1.048	1.382	3,654
K-110	-	127	405	718	1.047	1.385	3,777
K-110	250	129	400	742	1.066	1.4	3,714
K-110	350	133	409	736	1.061	1.395	3,689
K-110	450	134	404	730	1.06	1.395	3,632
p-value		0.3501	0.2099	0.5811	0.9854	0.8592	0.9899
Procedures, kcal/kg	Addition of enzymes, mg/kg	Feed conversion ratio					
		1-10 days	10-17 days	17-30 days	30-37 days	37-45 days	1-40 days
Control group	-	1.079	1.341	1.387	1.622	1.647	1.599
K-55	-	1.117	1.402	1.437	1.667	1.658	1.638
K-85	-	1.091	1.4	1.439	1.657	1.668	1.644
K-110	-	1.12	1.388	1.4341	1.665	1.678	1.645
K-110	250	1.101	1.394	1.437	1.669	1.675	1.647
K-110	350	1.099	1.379	1.428	1.668	1.671	1.634
K-110	450	1.077	1.361	1.424	1.655	1.662	1.629
p-value		0.0051	0.000099	0.000099	0.0005	0.0053	0.0151

Source: compiled by the authors based on D. Shalginbaev et al. (2022), A.T. Abylgazinova et al. (2023), A.K. Saken et al. (2021)

The table shows the effects of different enzyme and energy levels in broiler chicken diets on live weight gain, feed intake and feed conversion ratio at different age intervals. Seven treatment groups, including a control group (K) and six diet variants with different enzyme concentrations and energy levels, participated in the experiment. Regarding body weight gain, as noted by D. Shalginbaev et al. (2022), the results show differences depending on the treatment at different age intervals (1-10 days, 10-17 days). In general, it appears that increasing enzyme levels, especially in groups with K-110 kcal/kg intake and enzyme concentrations of 250, 350 and 450 mg/kg, has a positive effect on body weight gain compared to the control group. Significant differences in BWG were observed between 10-17 days, 17-30 days, and 30-37 days, suggesting that enzyme supplementation may influence the early stages of growth. The table also presents feed intake data, indicating that there were no significant differences between treatments. Feed intake values are relatively similar between treatment groups, suggesting that changes in body weight gain are not related to differences in feed intake.

Treatments significantly affected feed conversion ratio, a key indicator for assessing feed utilisation efficiency. Lower FCR values were generally observed in groups with higher enzyme concentrations, especially in groups with K-110 kcal/kg at enzyme levels of 250 mg/kg and 350 mg/kg. This suggests that these

treatments are more efficient in converting feed to body weight, reflecting increased feed utilisation efficiency (Abylgazinova et al., 2023). Thus, the table shows the effect of enzyme supplementation and energy level on broiler chicken performance. Higher enzyme concentrations, especially in the group with K-110 kcal/kg, have a positive effect on body weight gain and feed conversion efficiency at certain growth stages. These results have implications for optimising broiler diets to improve growth performance and feed utilisation in poultry production.

As soya meal contains minimal amounts, the main source of starch in the diets used by the farms for the study was maize. The proportion of starch in diets with a reduced IEE of 135 kcal/kg increased from 29.7% in the pre-starter diets to 44.5% in the finishing diets. The improvement in productivity was particularly marked with increasing age of birds, indicating a greater impact of adding the enzyme complex to diets with higher starch content, as shown in Table 1. Starch digestibility, which was investigated in this study, showed improved BWG and FCR in the enzyme supplemented birds as starch became a more significant part of their daily intake. This emphasises that the addition of α -amylase to the enzyme complex promotes starch digestion and increased availability of glucose, which is used as energy.

Despite the generally high starch digestibility in birds, the digestibility of maize starch in the terminal tubular intestine was only 85% and did not increase

with chick age. Resistant starch that avoids digestion in the small intestine may provide an opportunity to utilize exogenous α -amylase (Yadav & Jha, 2019). A.T. Abylgazanova *et al.* (2023) noted that the different treatments have no effect on the total carcass yield and marketable part of the carcass. Nevertheless, it is worth

noting a significant reduction in the proportion of fat in the abdominal part of the carcass in birds fed the K-110-450 diet compared to the group fed the K-110 programme (1.55% compared to 1.88%; $p < 0.0347$). Importantly, all other treatment options were intermediate in this parameter (Table 2).

Table 2. Effect of gradual reduction of apparent metabolizable energy and addition of α -amylase + β -glucanase complex on the yield of carcass, abdominal fat, and marketable cuts in broilers from days 1 to 40 (%)

Procedures, kcal/kg	Addition of enzymes, mg/kg	Bran	Abdominal fat	Fillet	Hip	Wings
Control group	-	76.8%	1.79%	32.1%	33.1%	11.1%
K-55	-	76.6%	1.75%	31.7%	32.7%	11.2%
K-85	-	76.5%	1.87%	32.3%	32.4%	11.3%
K-110	-	76.8%	1.87%	31.9%	31.4%	11.3%
K-110	250	78.7%	1.81%	32.4%	31.4%	11.2%
K-110	350	78.7%	1.82%	31.9%	31.6%	11.3%
K-110	450	78.5%	1.68%	31.7%	31.7%	11.3%
p-value		0.8601	0.0352	0.3412	0.5543	0.2349

Source: compiled by the authors based on D. Shalginbaev *et al.* (2022), A.T. Abylgazanova *et al.* (2023), A.K. Saken *et al.* (2021)

A.K. Saken *et al.* (2021), interpret this result as a potential effect of the C-110-450 diet on the biochemical composition of broiler carcasses, especially with respect to abdominal fat content. The data presented highlight the importance of considering diet composition when formulating dietary programmes for broilers to optimize their metabolic parameters and meat quality characteristics. A variety of interpretations have been proposed to explain the improvements achieved when exogenous carbohydrases are introduced into poultry diets, and these interpretations are not always related to a more intensive degradation of the target substrate of these enzymes. A more efficient breakdown of starch, and hence increased digestibility of this substance, can be seen when metabolised energy is increased when broilers are fed corn-soybean diets supplemented with amylases.

The use of α -amylase in combination with other enzymes such as beta-glucanase and xylanase resulted in an increase in metabolised energy content in corn-soy-based broiler diets and improved digestibility of all amino acids in the club intestine (Bakare *et al.*, 2021). In a study by D. Shalginbaev *et al.* (2022), field performance on diets with reduced energy and addition of α -amylase- β -glucanase complex also improved, although it did not fully compensate for the productivity losses caused by a 120 kcal/kg reduction in the diet

for 40 days. The enzyme complex includes α -amylase and β -glucanase. The β -glucan content of the corn-soya diets was low, approximately 0.2% in corn and 0.4% in soya meal. Although the improvements are probably due to the effect of α -amylase on starch, since its content exceeds that of β -glucans, the influence of the latter cannot be excluded either. It is generally believed that β -glucans have a negative effect on chicken performance due to their viscosity, and they are considered as an important anti-nutritional component in diets with barley, wheat, or rye. Despite the low content of β -glucans (8.1% in barley, 0.6% in wheat and 0.9% in rye), their negative effects on poultry performance are proportionally high (Moran & Bedford, 2022). Consequently, even considering their small proportions compared to other cereals, the impact of β -glucanase on maize-soya diets may be notable.

Broilers have been successfully adapted to starch-based diets as a result. However, the high feed intake of modern fast-growing broilers may create physiological constraints to starch digestion, leaving some dietary starch undigested and available for interaction with additional amylase. Table 3 shows that BWG and FCR responded linearly to the reduction in IEE in diets without enzyme supplementation ($p < 0.02$). At the same time, no effect on feed intake was detected in the weekly dynamics.

Table 3. Regression equations for body weight gain and feed conversion ratio in response to a decrease in imaginary metabolisable energy and relative estimates of the equivalence of IEE with the addition of an α -amylase + β -glucanase complex to broiler feeds from days 1 to 40

Components	Regression equation	Determination coefficient	p-value	Bioequivalence (mg/kg)		
				250	350	450
BWG, g						
1-10 days	A=-0.00003b+0.1193	0.2962	0.0065	7	7	7
10-17 days	A=-0.00009b+0.3002	0.2889	0.0076	10	9	66

Table 3. Continued

Components	Regression equation	Determination coefficient	p-value	Bioequivalence (mg/kg)		
				250	350	450
17-30 days	$A = -0.00020b + 0.5356$	0.3111	0.0003	56	59	61
30-37 days	$A = -0.00009b + 0.3152$	0.2167	0.0031	96	86	99
37-45 days	$A = -0.00011b + 0.2846$	0.1148	0.0297	49	67	69
1-45 days $A = -0.00091b + 3.2746$		0.4378	0.0002	41	48	57
FCR						
1-10 days	$A = 0.00024b + 1.0785$	0.2279	0.0354	49	41	62
10-17 days	$A = 0.00052b + 1.2498$	0.3137	0.0003	26	69	97
17-30 days	$A = 0.00055b + 1.3888$	0.4754	0.0002	67	83	92
30-37 days	$A = 0.00029b + 1.7368$	0.1843	0.0067	99	129	153
37-45 days	$A = 0.00034b + 1.9161$	0.0994	0.0507	20	55	57
1-45 days	$A = 0.00043b + 1.6146$	0.2903	0.0004	61	78	101

Source: compiled by the authors based on D. Shalginbaev et al. (2022), A.T. Abylgazina et al. (2023), A.K. Saken et al. (2021)

Table 3 presents the calculation of apparent metabolisable energy based on body weight gain and FCR data from broiler chickens subjected to different levels of enzyme supplementation. These results are then compared with the responses of birds whose diets had reduced apparent metabolisable energy and which received no enzyme supplementation. The calculated values of apparent metabolisable energy show variability across age intervals and enzyme concentrations in the feed, with the lowest values observed in the first week and the highest values in the fourth week of the observation period.

The conditional values of metabolised energy reflect the metabolic efficiency of the birds in utilising the provided feed for growth. The age-related changes emphasize the dynamic nature of energy utilisation patterns of birds as they progress through different developmental stages. The first week, characterised by lower conditional metabolisable energy values, may indicate a period of adjustment and adaptation to feeding conditions. Importantly, the mean values of the conditional apparent metabolised energy of the enzyme complex show weekly fluctuations, highlighting the influence of

both age and enzyme concentration on the metabolic processes of broiler chickens. Notably, as the birds matured and reached the fourth week of age, there was a significant increase in conditional AME values, indicating an increased capacity for energy utilisation.

Over the entire observation period, conditional estimates of metabolised energy as stated by A.T. Abylgazina et al. (2023) and A.K. Saken et al. (2021), show a positive correlation with increasing enzyme concentration in the feed. Specifically, the addition of 250, 350 and 450 mg/kg of enzyme complex was associated with corresponding conditional IEE values of 42, 48 and 59 kcal for body weight gain and 61, 79 and 102 kcal IEE/kg for feed conversion ratio. These results highlight the potential usefulness of enzyme supplementation for optimising energy utilisation and growth of broiler chickens. Linear corrections for BWG and FCR obtained from the beginning to the end of the study when birds were fed different concentrations of enzymes in the diet are presented on Figures 1 and 2. No effect of treatment was found on mortality, which was an overall mean of 4.15%.

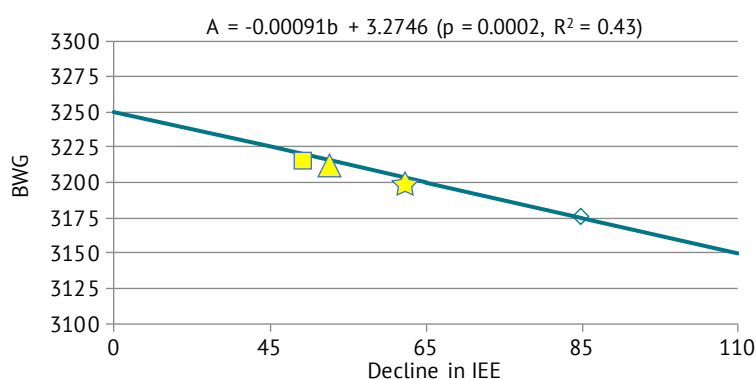


Figure 1. Body weight gain of broilers fed AME diets supplemented or not with α -amylase+ β -glucanase complex from day 1 to day 40, g

Note: square – 250 mg α -amylase + β -glucanase; triangle – 350 mg α -amylase + β -glucanase; star – 450 mg α -amylase + β -glucanase

Source: compiled by the authors based on D. Shalginbaev et al. (2022), A.T. Abylgazina et al. (2023), A.K. Saken et al. (2021)

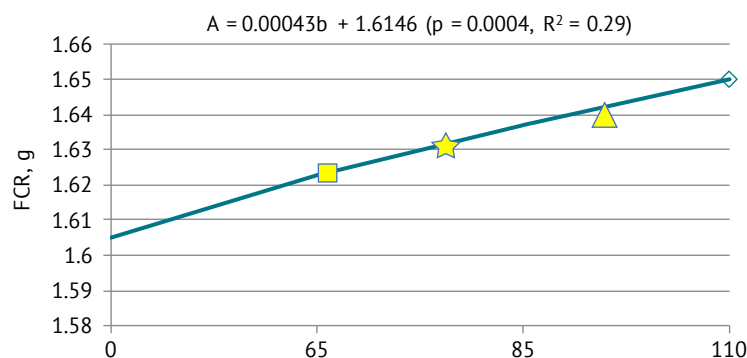


Figure 2. FCR values for broilers fed diets containing progressively lower apparent metabolisable energy levels, with or without α -amylase+ β -glucanase complex supplementation, from day 1 to day 40

Source: compiled by the authors based on D. Shalginbaev et al. (2022), A.T. Abylgazanova et al. (2023), A.K. Saken et al. (2021)

Exposure of complex substrates containing different types of molecules, after partial or complete degradation by an enzyme, may provide access to other enzymes that would otherwise be unable to interact with the original substrate. The concept of degradation of a new matrix is often invoked to explain these improvements. Some plant nutrients, such as starch and protein, are retained within insoluble cell walls (sometimes referred to as the cage effect), preventing poultry from accessing these nutrients. In addition, soluble fibres dissolve in the intestine, forming viscous gels that trap nutrients and slow the rate of etching and passage of feed through the intestine (Boukid *et al.*, 2023).

The use of different enzyme combinations under a wide range of conditions and with a variety of feed compositions has demonstrated improved broiler performance. The results of this study highlight that the addition of α -amylase- β -glucanase complex to diets may represent a promising alternative in the field of supplementation that can potentially optimize the efficient energy utilisation of broiler birds. This discovery emphasises the importance of finding innovative solutions to improve broiler feed efficiency. Supplementation of an enzyme complex such as α -amylase- β -glucanase may be an effective way to optimize the nutritional process and improve overall bird performance.

DISCUSSION

In conjunction with the global trend of optimising poultry feed production, microbial enzymes such as β -glucanase and α -amylase are gaining importance, attracting attention and wide application. The increasing world population and improved living standards in different regions are accompanied by an increase in meat and egg consumption, thus presenting the livestock industry with an urgent challenge to improve poultry productivity. In the context of this challenge, there is a growing trend towards more efficient feeding as producers seek to optimize the use of feed resources to reduce costs and increase overall productivity (Voronetska & Yurchuk, 2023). The use of microbial enzymes such

as β -glucanase and α -amylase stands out as one of the promising optimisation methods in this field. This approach represents not only a cost-saving strategy, but also an important component of a sustainable development strategy in the face of increasing demands for food resources (Daneshmand *et al.*, 2023). The results of studies on the role of microbial enzymes in poultry feed production show positive effects on various parameters, which opens new perspectives in improving feed efficiency and poultry productivity.

The observed weight gain of hens is an important indicator of the effectiveness of microbial enzyme administration (Szeląg-Sikora *et al.*, 2024). Such results are in line with the findings of authors A.A. Nascimeto *et al.* (2023), who also observed a positive effect on poultry growth when such enzymes were used. In this study, the inclusion of feed bard (DDGS) in the diet resulted in a significant increase in body weight. It is emphasised that the study revealed an increase in feed intake due to the introduction of DDGS in the diet. In addition, the authors note that DDGS significantly increased the palatability of the diet. The results suggest that DDGS contributes to increased weight gain not so much through favourable digestion and absorption of nutrients, but by increasing the palatability of the feed. This is supported by similar digestive enzyme activity, nutrient absorption levels and overall performance of the feed without DDGS supplementation.

The study confirms increased feed digestibility and energy metabolism efficiency with microbial enzymes. These results are in agreement with the study of scientists A. Daneshmand *et al.* (2023) who emphasised that enzymes promote better polysaccharide breakdown and nutrient digestibility, resulting in improved energy efficiency. The authors stated that enzymes can also improve the absorption of proteins, fats, vitamins, and minerals. This occurs by breaking down the cellular structures of plant material and activating processes that promote better absorption of nutrients in the gastrointestinal tract. Similar results are provided by P.E. Rupolo *et al.* (2023), in their work. In it, the authors

concluded that microbial enzymes have a positive effect on the function of the gastrointestinal tract, giving it a higher efficiency in digestion and assimilation of food. This process results in a shorter residence time of feed in the body, which in turn reduces nutrient losses and increases overall energy efficiency.

Studies conducted by N.K. Morgan *et al.* (2021) confirm the effects of microbial enzymes in stimulating the microbiota. In their research, the authors found that microbial enzymes exhibit the ability to create favourable conditions for the growth of beneficial bacteria in the gut, thereby contributing to an improved microbiotic balance. This important aspect of the observed effects of microbial enzymes has the potential to improve feed processing efficiency and nutrient absorption. The results obtained by J. Chauhan *et al.* (2023), highlight that the use of microbial enzymes can not only stimulate the microbiota, but also contribute significantly to the optimisation of physiological processes in the digestive system. Creating favourable conditions for the development of beneficial microorganisms represents an important mechanism to improve the overall health and digestive efficiency of birds (Daskalova *et al.*, 2023). These findings provide additional scientific justification for considering microbial enzymes as a promising component in optimising poultry nutrition.

Increased productivity is related to feed conversion efficiency, which means that poultry produce more meat or eggs with the same or even less feed intake (Sychoy *et al.*, 2024). This aspect becomes extremely important in the context of high feed costs in industrial poultry production. The work of scientists P. Gulizia *et al.* (2023), also highlights the improvement in feed conversion when microbial enzymes are used, which confirms the importance of this factor in overall productivity. The authors argue that increased productivity also has a direct impact on the economic efficiency of poultry production. Reducing feed costs while increasing yields can significantly improve profitability of production. This is an important aspect to attract the attention of industrialised enterprises and farms. Meanwhile, C. Liu *et al.* (2022) note environmental benefits in the use of microbial enzymes. The authors note that increased productivity accompanied by more efficient feed utilisation may also have positive environmental aspects. Reduced feed intake results in a reduced need for agricultural inputs, which helps to reduce environmental impacts and supports the sustainability of poultry production. These findings emphasize the importance of considering microbial enzymes not only as a tool to increase productivity, but also as a strategy that can promote environmental sustainability in agriculture. Optimising the use of feed resources not only improves the economic performance of production, but also contributes significantly to reducing the negative impact of human activities on the natural environment (Turmagambetova *et al.*, 2015).

The observed improvement of the conditional equivalent after the first week of feeding is of interest. The initial deterioration of the conditioned equivalent in the first week may be due to adaptation of the organism to the new type of feed and activation of enzymes, which takes time to optimize their action. Subsequent improvement in this indicator suggests that the birds' digestive system is successfully adapting to the use of microbial enzymes, which in turn leads to increased efficiency of feed digestion and optimisation of energy balance (Montayeva *et al.*, 2023). Similar dynamics were also identified by researchers L. Bornaei *et al.* (2022), who suggested that enzyme activation during the initial phase of feeding may take time to reach optimal efficiency. The results showed a temporary deterioration in equivalence during the initial period of enzyme administration, followed by a gradual improvement with increasing duration of use. The authors attributed this to the gradual adaptation of the birds to the new biological processes induced by microbial enzyme administration. This indicates the importance of long-term studies to better understand the temporal aspects of the effects of microbial enzymes on birds.

Summarising the above, the results of the study on the role of the microbial enzymes β -glucanase and α -amylase in poultry feed production provide valuable information for optimising poultry production. The observed positive changes in hen weight, feed digestion, conditioned equivalent, and performance confirm the significance of introducing these enzymes into feed rations. The results obtained not only confirm previous studies, but also indicate the need for a more in-depth study of temporal aspects and interactions with microflora. These findings may provide a basis for the development of new strategies in poultry production to improve animal performance and resilience to changes in feed conditions. In the long term, understanding these mechanisms may provide impetus for the development of innovative solutions in agriculture, balanced in terms of economic efficiency and eco-sustainability.

CONCLUSIONS

The analysis of the enzyme complex in the experimental feeds confirmed that the concentrations of α -amylase and β -glucanase were as expected. The developed values were 45, 65 and 85 kN of α -amylase/kg and the actual values were 44, 67 and 88 kN, respectively. This indicates the high accuracy of the study in supporting the stated objectives. The effect of the treatment significantly affected the productivity of the birds. There were statistically significant decreases in both body weight gain (BWG index, $p < 0.01$) and feed conversion ratio (FCR index, $p < 0.01$) throughout the weeks evaluated. These productivity losses were associated with a reduction in energy in the diet, resulting in a decrease in IEE (expressed in kcal). Enzyme supplementation resulted in a partial recovery of productivity. The effectiveness

of this recovery is emphasised by data on weight gain and conversion ratio. For example, significant differences in body weight gain (BWG) were observed between 10-17 days, 17-30 days and 30-37 days, where enzyme supplemented groups, especially at K-110 kcal/kg and enzyme concentrations of 250, 350 and 450 mg/kg, showed positive results compared to the control group.

It was found that the treatments significantly affected the feed conversion ratio (FCR). Lower FCR values were recorded in the groups with higher enzyme concentrations, especially at K-110 kcal/kg and enzyme levels of 250 mg/kg and 350 mg/kg. These results indicate an increase in feed utilisation efficiency in the groups with enzyme supplementation. Based on the results of this study, the following recommendations for optimising broiler chicken performance are proposed: the introduction of enzyme supplements, especially α -amylase- β -glucanase complex, is a promising approach to improve bird performance. It is recommended to consider the inclusion of such additives in diets,

especially under reduced energy conditions. It is recommended that the concentration of enzymes in diets should be tuned for age-specific performance, which may help to maximize feed utilisation efficiency at different growth stages. Further research is recommended to determine the optimum enzyme concentrations in diets that maximize the positive effects on growth and feed conversion efficiency. The results emphasize the importance of starch treatment and effects on the fat component of the carcass. Further investigation of the effects of enzymes on these parameters and possible adjustments in rations is recommended.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Роль мікробних ферментів β -глюканази та α -амілази в оптимізації виробництва кормів для птиці

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Анотація. Зі збільшенням чисельності населення планети та зростанням споживання м'яса птиці зростає потреба в ефективному кормовиробництві для забезпечення високої якості та кількості продукції. Основна мета дослідження полягала в тому, щоб з'ясувати вплив мікробних ферментів β -глюканази та α -амілази на оптимізацію продуктивності комбікормів для птиці. У дослідженні використовували метод аналізу, однофакторний дисперсійний аналіз, хімічний аналіз та кількісний аналіз. Огляд роботи показав, що аналіз ферменту в експериментальних кормах підтвердив визначені концентрації 44, 67 і 88 кН. Обробка значно знизила приріст живої маси та коефіцієнт перетворення корму протягом усього періоду оцінки. Ефект був особливо помітним при К-110 ккал/кг і концентраціях ферменту 250, 350 і 450 мг/кг. Додавання ферментів призвело до часткового відновлення продуктивності, що було помітно через різні проміжки часу, включаючи 10-17 днів, 17-30 днів та 30-37 днів. Групи, які отримували ферментну добавку, показали позитивні результати у збільшенні маси тіла, особливо при К-110 ккал/кг і концентрації ферменту 250, 350 і 450 мг/кг, порівняно з контрольною групою. Результати підкреслили не тільки високу точність дослідження, але й ефективність ферментів у покращенні використання корму та підтримці продуктивності птиці в умовах зміненого раціону. Дослідження ферментного комплексу в кормах має практичне значення для птахівничої галузі, оскільки підтверджує ефективність ферментних добавок у покращенні використання корму та підтримці продуктивності птиці при зміні раціонів. Отримані результати є основою для оптимізації раціонів з використанням певних рівнів енергії та концентрацій ферментів, що може призвести до покращення приросту живої маси та КПК, сприяючи більш ефективному засвоєнню поживних речовин та зменшенню втрат продуктивності птиці

Ключові слова: раціон; активні добавки; бройлери; продуктивність; приріст живої маси; коефіцієнт конверсії корму



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Productivity of selection numbers of perennial ryegrass (*Lolium perenne* L.) in the nursery of competitive variety testing

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Abstract. Increasing the efficiency of perennial ryegrass cultivation is possible by improving breeding work and clear organisation of seed production, as the variety plays a decisive role in this process. The purpose of this study was to evaluate promising perennial ryegrass material for productivity in haymaking and pasture use and to select the best numbers for submission for scientific and technical expertise. The study was conducted during 2022-2024 at the Precarpathian Research Department of the Institute of Agriculture of the Carpathian Region of NAAS (Drohobych district, Lviv region). The selection of breeding material and phenological observations were performed following the "Methodology for breeding perennial legumes and cereals in the Carpathian region" (2015) and "Formation and conservation of genetic diversity of forage and lawn grasses in the Carpathian region" (2015). In the competitive variety trial for haymaking, 2 selection numbers

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of perennial ryegrass (PFZ 02082 and PFZ 02514) were distinguished by plant height; 4 numbers – by leafiness (PFZ 02082; PFZ 02136; PFZ 02320; PFZ 02514), 2 numbers (PFZ 02082; PFZ 02514), 2 numbers – by green mass yield (PFZ 02082; PFZ 02514), and 2 numbers (PFZ 02082; PFZ 02514) – by dry matter yield. Under the pasture method of use, the best were the numbers PFZ 02082 and PFZ 02514 in terms of plant height, PFZ 02082; PFZ 02081 and PFZ 02514 in terms of leafiness, PFZ 02082; PFZ 02081; PFZ 02514 in terms of green mass yield, and PFZ 02082; PFZ 02081; PFZ 02514 in terms of dry matter yield. The highest seed yields were provided by PFZ 02082; PFZ 02136; PFZ 02081; PFZ 02514 (0.41 t/ha, 0.39 t/ha, 0.37 t/ha, and 0.35 t/ha). The promising numbers PFZ 02082 and PFZ 02514 for hayfield and pasture use were identified, which will be submitted to the State Scientific and Technical Expertise after multiplication

Keywords: perennial ryegrass; selection; green mass; dry matter; seeds

INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.) is a lowland perennial, loosely bushy cereal. It grows best in areas with sufficient rainfall. When used for pasture, it grows quickly and forms numerous shoots. Generative organs are developed on shoots formed in summer and autumn. It is prone to lodging. It stays in the grass stand for three or more years, forming a dense turf. It reaches full development in the second or third year after sowing. The seed stand is used for one or two years, ripening in the first or second decade of July. The seeds are very crumbly when ripe.

The natural diversity of ecotypes is significant for grassland species. J. L. Blanco-Pastor *et al.* (2019) investigated the genetic structure and origin of perennial ryegrass in 481 populations, finding that its spread across Europe during the Pleistocene contributed to a decrease in tree dominance in favour of grasses. Modern cultivars are closely related to the natural diversity of northwestern Europe, but do not capture the wide genetic variation of natural populations. T. Keep *et al.* (2020) emphasise the importance of natural genetic diversity for breeding programmes aimed at climate adaptation. Using molecular markers, the researchers identified key associations of genetic traits with phenological characteristics that contribute to adaptation and ecotype differentiation. Genomic predictive models proved effective in breeding for adaptive and agronomic traits.

Over the past decades, the problem of climate change (specifically global warming) has become extremely relevant. Rising temperatures affect a series of physiological processes (including photosynthesis). The growth rate of perennial ryegrass plants, their productive longevity in grass stands and high fodder quality depend on meteorological and soil conditions. K. Jaškūnė *et al.* (2020) note that global warming will affect agriculture, which will suffer from reduced water availability. Due to changes in precipitation, moderate summer droughts are expected to become more frequent even in temperate regions. The researchers assessed the response to moisture deficit for perennial ryegrass genotypes using a high-precision phenotyping platform. The study revealed phenotypic variation in growth-related traits and major differences in leaf

growth under normal conditions in subgroups of lawn and pasture-type cultivars. Phenotypic data were combined with genotypic variants identified by sequencing genotyping to perform a genome-wide association study.

P. Beukes *et al.* (2021) predicted the yield of perennial ryegrass in New Zealand under climate change and identified optimal and risky areas for cultivation, taking into account changes since the middle of the last century, and emphasised the need to develop better adapted plant varieties for pastures that can cope with the challenges of climate change. S. Khulan *et al.* (2021) conducted a study to determine the effect of temperature and salinity on seed germination. The findings revealed that seed germination occurred over a wide range of salinities (0 to 100 mM NaCl) and temperatures (15–30°C), with the highest germination between 0 and 25 mM NaCl at 15–20°C. Plants were capable of germinating under moderate salinity conditions and germinating as normal when salinity conditions were removed, but at optimum temperatures. A. Lina and A. Escobar-Gutiérrez (2022) determined the germination of perennial ryegrass plants of various origins at varying temperatures. Temperature was the key factor controlling plant development. Overall, the relative growth rates of roots and shoots were slow at 5°C, peaked within 25–30°C, and then decreased sharply. These findings indicate high genetic variability in the source material in terms of the response to temperature at the initial stages of ryegrass plant development.

A prominent element in plant breeding is resistance to diseases and pests. K. Jaškūnė *et al.* (2022) point out that fungal diseases (in particular crown rust) can also lead to serious losses in seed yields as they affect plant growth and development. In some varieties, this can reduce yields by up to 30% and impair feed quality. As the disease also reduces plant vigour and competitiveness, the composition of plant mixtures is gradually changing. Increased tolerance and resistance to rust is a genetically determined trait that can be controlled by various breeding methods. The fastest and most efficient way to develop varieties is to combine phenotypic and molecular breeding. D. Chapman *et al.* (2023) conducted research on ryegrass plants and obtained

several highly efficient and widely adapted combinations of varieties and endophytes (*Epichloë*) for pasture production in New Zealand. The coefficients of increase in yield, nutritional value, phenological and morphological traits, as well as the economic value of these traits were estimated among the functional groups of ryegrass by date of cultivation, ploidy, etc. Z. Chen *et al.* (2020) stated that soil with low nutrient content is the key factor limiting the normal growth and development of plants. This study was conducted to determine the effect of the endophyte *Epichloë* on the growth, survival, and nutrient content of *Lolium perenne* L. under low fertility conditions. P. P. Freitas *et al.* (2020) also covered this issue while investigating the endophyte *Epichloë* sp. in perennial ryegrass plants.

Apart from varieties with greater yields, the demand for lawn varieties is constantly growing. K. Wolski *et al.* (2021) found that grass mixtures based on the dominance of perennial ryegrass are described by higher quality for football fields (and, accordingly, lawns) compared to grass mixtures based on red fescue (*Festuca rubra* L.). The values of the 6 studied traits (general visual appearance, turf, colour, leaf blade width, disease susceptibility, and overwintering) were statistically different depending on the year and mixtures. T. Głąb *et al.* (2021) investigated the effects of plant growth regulators, which are widely used in lawns to reduce mowing frequency and inhibit the development of generative shoots. However, they can also affect turf colour, density, and resistance to trampling. Competitive variety trials are a key part of the breeding process in the development of varieties. As of 2024, 40 varieties of perennial ryegrass were included in the State Register of Plant Varieties Suitable for Distribution in Ukraine. These include 30 varieties of foreign selection and 10 varieties of Ukrainian selection (Ruslana, Mriia, Dovbushanka, Aitera, Slavetna, Vinnytska, Kyivska 101, Obrii, Drohobyt'skyi 16, Osyp). Two varieties (Drohobyt'skyi 16 and Osyp) were selections of the Institute of Agriculture of the Carpathian Region of the National Academy of Agrarian Sciences (State Register of Plant Varieties., 2021).

However, despite the considerable number of varieties, further breeding improvement of this crop is necessary. Therefore, the purpose of this study was to investigate the selection numbers of perennial ryegrass in competitive variety testing and to identify promising numbers for economic and valuable indicators.

MATERIALS AND METHODS

Field studies were conducted during 2022-2024 at the Precarpathian Research Department of the Institute of Agriculture of the Carpathian Region of the National Academy of Agrarian Sciences (Drohobych district, Lviv region) on soddy-medium podzolic surface-alkaline medium acid loam soils formed on deluvial deposits. The key agrochemical parameters of the arable (0-20 cm) layer of these soils were as follows: humus content by Tyurin (DSTU 4289:2004, 2005) – 1.22 %, pH of salt extract (potentiometric method) (DSTU ISO 10390:2007, 2009) – 4.6; hydrolytic acidity (Kap-pen-Hilkowitz) (DSTU 7537:2014, 2015) – 4.23 mg-eq. per 100 g of soil; Hr (sum of absorbed bases) (DSTU 8806:2018, 2019) – 11.8 mg-eq. per 100 g of soil; mobile forms of phosphorus (according to Kirsanov) (DSTU 4405:2005, 2006) – 118 mg, exchangeable potassium (according to Kirsanov) (DSTU 4405:2005, 2006) – 82 mg, easily hydrolysed nitrogen (according to Kornfeld) (DSTU 7863:2015, 2016) – 108 mg per 1 kg of soil.

To evaluate the biological characteristics and productivity of perennial ryegrass in 2021, a competitive variety testing nursery was established in the summer sowing season. Accounting and phenological observations were performed in the second and subsequent years of the crop's life (2022-2024). The predecessor was pure fallow. Agricultural technology of perennial ryegrass cultivation in the experiment is generally accepted for the Western region of Ukraine. The material for the research in the competitive variety testing nursery was 6 selection numbers of perennial ryegrass. The standard was the Osyp variety of the Institute of Agriculture of the Carpathian region of NAAS (Table 1).

Table 1. Characteristics of selection numbers of perennial ryegrass used in the nursery of competitive variety testing

Institutional registration number	Sample
PFZ 00735(St)	St Osyp (standard)
PFZ 02082	individual selection from No. 1679 (hybrid population of Drohobyt'skyi 16× Alduva)
PFZ 02136	mass selection from No. 1112 (selection from Sviatoshytskyi)
PFZ 02081	individual selection from No. 1678 (individual selection from No. 907)
PFZ 02320	individual selection from No. 1098 (individual selection from the local Lithuanian population No. 3474 / 660)
PFZ 02514	long-term mass selection from No. 1684 (individual selection from No. 980 (Drohobyt'skyi 16)

Source: compiled by the authors of this study

The registered area of the plots for fodder productivity was 2.5 m², seed productivity – 5 m², sowing area – 10 m². Replication was fourfold. Yields of green mass, dry matter, and seeds were determined for hay-making and pasture use. The selection of breeding material and phenological observations were performed according to the “Methodology of selection of perennial leguminous and cereal grasses in Precarpathia” (2015) and “Formation and preservation of genetic diversity of forage and lawn grasses in Transcarpathia” (2015). The fodder productivity was recorded for haymaking (two mowings) and pasture (four mowings) methods of use. For haymaking use, mowing was recorded in the phase of earing – beginning of flowering, and for pasture use – at the beginning of pasture ripeness at a grass height of 15-25 cm.

The yield of green mass and dry matter was determined by mowing and weighing the grass, followed by the conversion of green mass to dry matter by the percentage of shrinkage of 1 kg test sheaves. Seed yield was recorded by threshing, wiping, cleaning, and weighing separately from each plot. The findings obtained during the analysis were expressed as a percentage, rounded to the nearest whole number, for each trait. The statistical processing of the data on fodder and seed productivity was performed by the method of analysis of variance on a PC using a special application program for Windows 98.

The weather conditions of 2022-2024 had a series of specific features, which helped to evaluate the

selection numbers for the main productivity indicators in a more comprehensive way. In 2022, during the growing season (March–August), the average monthly temperature was 14.1°C, with a long-term average of 12.3°C. The amount of precipitation during the growing season was 316.2 mm, compared to the long-term average of 509 mm. In 2023, the average monthly temperature for the growing season was 14.2°C. The amount of precipitation during the growing season was 737.7 mm. The average monthly temperature for the growing season in 2024 was 15.4°C, while the amount of precipitation was 493.7 mm. In conducting the study, the authors followed the standards established by the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS AND DISCUSSION

During the three years of observations, the average monthly temperature of the growing season of perennial ryegrass exceeded the long-term average. In terms of precipitation during the growing season, in 2022 it was insufficient (316.2 mm), in 2023 excessive (737.7 mm), and in 2024 close to the long-term average (493.7 mm). Thus, during the study, there were significant differences from the average long-term data of precipitation and temperature during the growing season, which helped to better assess the specific features of the development of fodder and seed productivity of perennial ryegrass (Table 2).

Table 2. Meteorological indicators of the vegetation period of perennial ryegrass for 2022-2024 (according to the meteorological station of Drohobych)

Year	Month					
	March	April	May	June	July	August
Average monthly air temperature, °C						
2022	2.5	7.3	15.1	19.5	20.3	20.0
2023	5.5	7.9	13.5	17.1	20.1	20.9
2024	6.1	11.5	14.5	19.4	20.9	19.9
Average long-term indicator	1.8	7.9	13.2	16.2	17.6	17.0
Total precipitation per month, mm						
2022	15.8	53.6	25.8	36.9	85.9	98.2
2023	85.2	71.4	46.1	187.9	217.3	129.8
2024	71.1	44.7	44.2	91.2	127.0	115.5
Average long-term indicator	38.0	53.0	97.0	119.0	110.0	92.0

Source: compiled by the authors of this study

On average, for three years of research in the nursery of competitive variety testing, the height of selection numbers of perennial ryegrass under hay-making method of use ranged from 75 cm (PFZ 02081) to 79 cm (PFZ 02082; PFZ 02514), daily growth ranged between 0.67-1.20 cm (PFZ 02320) to 0.68-1.21 cm (PFZ 02081), and leaf area was between 42.0%

(PFZ 02081) to 43.9% (PFZ 02082). In terms of green mass yield relative to the standard, the following selection numbers exceeded the standard: PFZ 02082 (+2.7 t/ha) and PFZ 02514 (+1.5 t/ha). In terms of dry matter yield, the numbers PFZ 02082 (+0.4 t/ha) and PFZ 02514 (+0.1 t/ha) exceeded the standard with LSD₀₅ at 0.23–0.35 (Table 3).

Table 3. Productivity of perennial ryegrass selection numbers in the nursery of competitive variety testing under haymaking method of use, average for 2022-2024

Samples	Plant height, cm		Daily growth	Leafiness, %		Green mass yield, t/ha		Dry matter yield, t/ha	
	average	± to St	average	average	± to St	average	± to St	average	± to St
St-Osyp	77	-	0.69-1.20	42.7	-	25.8	-	6.7	-
PFZ 02082	79	+2	0.69-1.13	43.9	+1.2	28.5	+2.7	7.1	+0.4
PFZ 02136	77	-	0.68-1.19	43.0	+0.3	23.8	-2.0	6.0	-0.7
PFZ 02081	75	-2	0.68-1.21	42.0	-0.7	23.7	-2.1	5.9	-0.8
PFZ 02320	76	-1	0.67-1.20	43.3	+0.6	23.4	-2.4	6.0	-0.7
PFZ 02514	79	+2	0.67-1.25	43.0	+0.3	27.3	+1.5	6.8	+0.1
LSD ₀₅ 2022	1.29			0.93		1.13		0.35	
2023	1.63			0.63		1.15		0.32	
2024	0.97			0.35		1.03		0.23	

Source: compiled by the authors of this study

Under the pasture method of use, the height of the selection numbers of perennial ryegrass ranged from 22.8 cm (PFZ 02136; PFZ 02320) to 24.3 cm (PFZ 02514), daily growth ranged between 0.67-0.70 cm (PFZ 02136; PFZ 02081) to 0.70-0.74 cm (PFZ 02514), and leaf area was between 71.4% (PFZ 02320) to 74.6% (PFZ 02082). In terms of green

mass yield relative to the standard, a significant excess was observed in three selection numbers: PFZ 02514 (+1.7 t/ha), PFZ 02081 (+1.2 t/ha), PFZ 02082 (+1.1 t/ha) with LSD₀₅ at 0.59-1.01. In terms of dry matter yield, the excess compared to the standard was in PFZ 02514 (+0.6 t/ha), PFZ 02081 (+0.4 t/ha), and PFZ 02082 (+0.3 t/ha) with LSD₀₅ at 0.25-0.32 (Table 4).

Table 4. Productivity of perennial ryegrass samples in the nursery of competitive variety testing for pasture use, average for 2022-2024

Samples	Plant height, cm		Daily growth	Leafiness, %		Green mass yield, t/ha		Dry matter yield, t/ha	
	average	± to St	average	average	± to St	average	± to St	average	± to St
St-Osyp	23.3	-	0.68-0.74	73.5	-	26.5	-	6.6	-
PFZ 02082	24.2	+0.9	0.69-0.72	74.6	+1.1	27.6	+1.1	6.9	+0.3
PFZ 02136	22.8	-0.5	0.67-0.70	72.5	-1.0	23.3	-3.2	5.8	-0.8
PFZ 02081	23.2	-0.1	0.67-0.70	73.6	+0.1	27.7	+1.2	7.0	+0.4
PFZ 02320	22.8	-0.5	0.68-0.74	71.4	-2.1	24.9	-0.6	6.3	-0.3
PFZ 02514	24.3	+1.0	0.70-0.74	74.1	+0.6	28.2	+1.7	7.2	+0.6
LSD ₀₅ 2022	0.88			1.02		1.01		0.25	
2023	0.20			1.01		0.74		0.32	
2024	0.99			1.05		0.59		0.26	

Source: compiled by the authors of this study

On average, over the three years of research, the number of generative stems was between 481 (PFZ 02320) and 682 (PFZ 02136); the number of seeds per ear was between 24 pcs. (PFZ 02514) and 26 pcs. (PFZ 02082; PFZ 02081); vegetation period – between 106 days (PFZ 02082) and 111 days

(PFZ 02081; PFZ 02320); thousand-kernel-weight was between 2.40 g (PFZ 02514) and 2.46 g (PFZ 02081). The following numbers exceeded the standard in terms of seed yield: PFZ 02136 (+0.08 t/ha), PFZ 02082 (+0.06 t/ha), PFZ 02514 (+0.04 t/ha), PFZ 02081 (+0.02 t/ha) (Table 5).

Table 5. Seed productivity and structural elements of perennial ryegrass selection numbers in the nursery of competitive variety testing, average for 2022-2024

Samples	Quantity, pcs.			Vegetation period, days	TKW, g	Seed yield, t/ha	
	generative stems		seeds in the ear			average	± to St
	average	± to St					
St-Osyp	554	–	25	110	2.41	0.33	–
PFZ 02082	642	+88	26	106	2.42	0.39	+0.06
PFZ 02136	682	+128	25	110	2.44	0.41	+0.08
PFZ 02081	584	+30	26	111	2.46	0.35	+0.02

Table 5. Continued

Samples	Quantity, pcs.		seeds in the ear	Vegetation period, days	TKW, g	Seed yield, t/ha	
	generative stems					average	± to St
	average	± to St					
PFZ 02320	481	-73	25	111	2.42	0.29	-0.04
PFZ 02514	619	+65	24	108	2.40	0.37	+0.04
<i>LSD₀₅</i> 2022	33.7		0.46	1.54	0.04	0.02	
2023	51.1		0.22	1.32	0.03	0.02	
2024	46.0		0.24	1.25	0.03	0.02	

Note: TKW – thousand-kernel-weight

Source: compiled by the authors of this study

The development of vegetative and generative organs depends on the supply of moisture and nutrients to plants. It is known that there is a direct correlation between yield, plant height, and green mass, as stems and leaves are the primary organs that transport organic and mineral substances. Researchers noted a direct correlation between leaf area and vegetative mass formation. A. Dzyubaylo *et al.* (2020) investigated the fodder productivity of sown grass, revealing the influence of the botanical composition of the mixture (specifically, perennial ryegrass) on productivity. G. Heineck *et al.* (2020) studied the relationship between fodder and seed production in perennial ryegrass plants, disease and pest resistance in the state of Minnesota, USA. V. Moisienko and T. Sladkovska (2019) found that high productivity of grasses can be obtained only in crops that effectively form the optimum leaf surface area capable of active work for a long time. A prominent factor that influenced the formation of leaf area of perennial ryegrass was the variety. I. Komar *et al.* (2020) pointed out that seed production is significant for the success of perennial ryegrass varieties but is limited by the complexity of yield components and low correlations between breeding and production environment, the study showed that seed yield per ear affects overall plant yield. The findings suggested that increased competition in the nursery and selection for high fertility can more accurately identify the best plant material compared to environments with less competition.

T. Sladkovska *et al.* (2022) investigated herbs with significant phytoremediation capacity (*Lolium perenne*, *Festuca rubra*, and *Poa pratensis*). These species are among the basic ones in lawn mixtures and can be used for phytoextraction of lightly contaminated agricultural soils or for phytostabilisation of soils with low or moderate concentrations of trace elements. Herbs have considerable potential for stabilising trace elements in soil, sediments, and wastewater. M. Scotton (2018) studied the seed production process of the major grass species in temperate pastures (specifically, perennial ryegrass). Investigating the seed production of herbaceous species can help to conserve grassland habitats and restore new high-value grasslands. J. Pranga *et al.* (2021) monitored the biomass yield of perennial ryegrass, although the reliability of the methods they developed

needs to be confirmed with independent data (other samples, locations, years, etc.). Scientists demonstrated that the combination of multispectral imagery and radio frequency modelling approaches can provide accurate yield forecasts for perennial grasses.

A series of scientists have conducted research on samples of other types of perennial cereals and legumes in breeding nurseries. O. Perehrym *et al.* (2020) investigated creeping clover samples in preliminary and competitive variety testing. The researchers identified No. 1076, which provided a yield of green mass of 38.6 t/ha, dry matter of 5.85 t/ha, seeds of 0.18 t/ha for haymaking, and 47.8 t/ha of green mass and 7.19 t/ha of dry matter for pasture. M. Chomiak (2019) studied 16 samples of *Dactylis glomerata* L. for the environmental parameters of plasticity and stability under the influence of environmental changes. The researcher identified valuable genetic sources with high indicators of the main environmental parameters of the green mass yield. Overall, all researchers noted the significance of investigating the selection numbers of crops in the context of climate change with the subsequent transfer of selected promising numbers for scientific and technical expertise. The findings obtained should be considered when creating varieties of perennial grasses for specific uses and selecting parental components for hybridisation.

CONCLUSIONS

As a result of phenological observations, it was found that the duration of the vegetation period of the studied ryegrass accessions from the beginning of the growing season to economic ripeness in 2022–2024 ranged from 106 days in selection number PFZ 02082 to 111 days in numbers PFZ 02081 and PFZ 02320. According to the yield of green mass, on average for three years of research in haymaking, the numbers PFZ 02514, PFZ 02081, PFZ 02082 stood out, which considerably exceeded the standard by 1.7 t/ha, 1.2 t/ha, and 1.1 t/ha, respectively. In terms of dry matter yield, the best were the selection numbers PFZ 02082 and PFZ 02514, which gave an increase of 0.4 t/ha and 0.1 t/ha, respectively.

For pasture use, the numbers PFZ 02514, PFZ 02081, and PFZ 02082 stood out, exceeding the standard in terms of green mass yield by 1.7 t/ha, 1.2 t/ha, and

1.1 t/ha, respectively, and dry matter yield by 0.6 t/ha, 0.4 t/ha, and 0.3 t/ha, respectively. Selection numbers PFZ 02136, PFZ 02082, PFZ 02514, and PFZ 02081 stood out for their seed yields, which were 0.41 t/ha, 0.39 t/ha, 0.37 t/ha, and 0.35 t/ha of seeds, which is 0.08 t/ha, 0.06 t/ha, 0.04 t/ha, and 0.02 t/ha greater than the standard. At the next stages of selection work, promising numbers (PFZ 02082 and PFZ 02514) were

identified, which will be multiplied for submission to the State Scientific and Technical Expertise.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Продуктивність селекційних номерів пажитниці багаторічної (*Lolium perenne* L.) в розсаднику конкурсного сортопробування

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Анотація. Підвищення ефективності вирощування пажитниці багаторічної можливе за рахунок поліпшення селекційної роботи та чіткої організації насінництва, адже визначальну роль при цьому відіграє сорт. Метою дослідження була оцінка перспективного матеріалу пажитниці багаторічної за ознаками продуктивності при сінокісному та пасовищному способах використання та виділення кращих номерів для передачі на науково-технічну експертизу. Дослідження проведено протягом 2022-2024 рр. в Передкарпатському відділі наукових досліджень Інституту сільськогосподарства Карпатського регіону НААН (Дрогобицький район Львівської області). Підбір селекційного матеріалу та фенологічні спостереження проводили згідно з «Методологією селекції багаторічних бобових і злакових трав у Передкарпатті» (2015) та «Формування та збереження генетичного різноманіття кормових і газонних трав у Передкарпатті» (2015). У конкурсному сортопробуванні при сінокісному способі використання було виділено за висотою рослин 2 номери пажитниці багаторічної (PFZ 02082; PFZ 02514); за облиствленістю 4 (PFZ 02082; PFZ 02136; PFZ 02320; PFZ 02514), за врожайністю зеленої маси 2 номери (PFZ 02082; PFZ 02514), за врожайністю сухої речовини 2 номери (PFZ 02082; PFZ 02514). При пасовищному способі використання за висотою рослин кращими були номери PFZ 02082 та PFZ 02514, за облиственістю PFZ 02082; PFZ 02081 та PFZ 02514, за врожайністю зеленої маси номери PFZ 02082; PFZ 02081; PFZ 02514, за врожайністю сухої речовини PFZ 02082; PFZ 02081; PFZ 02514. Найбільший показник врожайності насіння забезпечили номери PFZ 02082; PFZ 02136; PFZ 02081; PFZ 02514 (0,41; 0,39; 0,37 та 0,35 т/га). Виділено перспективні номери PFZ 02082 та PFZ 02514, сінокісно-пасовищного напрямку використання, які після розмноження будуть передані на Державну науково-технічну експертизу

Ключові слова: пажитниця багаторічна; селекція; зелена маса; суха речовина; насіння



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Regulation of soybean water regime under tillage and fertilisation for sustainable agriculture

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Abstract. Reduced soil moisture due to climate change can reduce crop yields, which negatively impacts food security and food system resilience. Therefore, the study aimed to determine the effect of tillage and fertilisation systems on the accumulation of available soil moisture and soybean yields. The research was conducted on chernozem podsolised medium loamy soil in 2019-2022. The research methods used

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were field, laboratory, comparative and correlation. Soybeans were grown in a 4-field crop rotation according to the generally accepted technology for the conditions of the study region. The dynamics of available moisture reserves in 0-20, 0-40 and 0-100 cm soil layers during the growing season under the influence of tillage and fertilisation systems were presented. The reserves of productive moisture in the soil and the yield of soybean grain under the influence of mouldboard, flat-cut, chisel, disc and differentiated tillage on mineral and organo-mineral nutrition backgrounds were determined. The maximum reserves of available moisture in the root layer of the soil (0-20 cm) on mineral and organic-mineral fertiliser backgrounds were observed in the phase of full germination and flowering of soybeans under chisel tillage, and the minimum under mouldboard tillage. The highest soybean grain yields were obtained with mineral and organo-mineral fertilisation and chisel tillage. In the flowering phase of soybeans under the influence of soil tillage systems, a medium correlation ($r=0.66$) was found between grain yield and available moisture reserves in the 0-40 cm soil layer under mineral fertilisation and a weak correlation ($r=0.36$) under organic-mineral fertilisation. The fertiliser system had the greatest impact on soybean grain yields (62.5%). The study results can be used in informed decision-making on sustainable soil management and conservation to promote global food security and mitigate climate change

Keywords: moisture reserves; soil layer; grain yield; correlation; share of influence

INTRODUCTION

The world is currently experiencing a shortage of dietary protein, which is set to continue in the coming decades. The issue of reduced dietary protein intake is exacerbated by “hidden hunger” due to a deficiency of vitamins, and macro- and microelements in diets. The traditional way to increase the resources of food and feed plant protein is to grow pulses. Soybeans have the highest protein content in grain, especially lysine. One of the reserves for increasing soybean production is the optimisation of the crop’s cultivation technology, using soil cultivation and fertilisation systems. This is particularly relevant in the context of climate change and moisture deficit. Therefore, the development and research of soil cultivation methods that improve the water regime, rational use of available moisture by plants and prevent unproductive losses due to evaporation are promising and should be further refined.

Soybeans (*Glycine max*) are of great economic and social importance due to their high oil content (18%), high-quality protein (up to 40%), positive role in soil fertility, high productivity and profitability (Korobko et al., 2024). Soybeans are a source of polysaccharides, dietary fibre, phytosterols, lecithins, saponins and phytochemicals (isoflavones). Due to its valuable properties, the consumption of soy products in the human diet improves health and reduces the incidence of debilitating diseases, including hyperglycaemia, hypertension, dyslipidaemia, obesity, inflammation, cancer, etc. According to R. Modgil et al. (2021), soybeans are also used to produce paper, plastics, pharmaceuticals, inks, paints, varnishes, pesticides, cosmetics, and biodiesel. Soybeans are demanding weather conditions during the growing season. The limiting factor for high soybean yields is providing plants with moisture, especially during flowering, bean formation and grain filling. According to Q. Li et al. (2023), grain losses can range from 45 to 70% due to drought. In the context of climate change, it is necessary to minimise the stressful

impact of drought on plants and protect the genetic potential of the crop.

One of the main technological operations in agricultural production that directly affects the growth and development of crops is ploughing (Kyrylyuk et al., 2019a). According to M. Xiao et al. (2023), deep ploughing is necessary in fields to provide deep-rooted plants with additional nutrients. Equally important aspects of tillage are crop residue management and increasing the even distribution of moisture, increasing the supply of nutrients to crops and reducing pests and weeds (Lavrenko et al., 2021). However, despite the potential benefits, current conventional tillage methods have substantial drawbacks, including increased soil degradation due to increased erosion rates, disruption of soil structure, compaction and destruction of topsoil, loss of moisture and organic matter, and reduced soil biodiversity (Hrytsiuk et al., 2020).

J. Somasundaram et al. (2020), and F. Jiang et al. (2024) determined that conservation tillage is important in arid regions with a lack of precipitation to preserve moisture and improve agricultural productivity. The study established that zero tillage with straw mulching and zero tillage with stubble leaving increases the moisture content of the soil. Z. Peng et al. (2019), and Z. Ren et al. (2024) highlighted the positive effects of conservation tillage, namely reducing soil erosion, preserving soil structure, increasing soil organic carbon content, improving soil fertility and moisture retention. All these benefits contribute to sustainable agricultural development and mitigate environmental degradation. According to E. Šarauskis et al. (2024), optimising tillage depth can improve soil health, conserve resources, and increase the overall sustainability of agriculture. Conservation tillage systems help to preserve soil and soil moisture and increase crop yields. Decomposed crop residues from conservation tillage improve soil structure and soil health.

Excessive use of synthetic fertilisers can cause changes in soil pH, reduce organic matter content and negatively affect beneficial soil microorganisms. Conversely, the use of organic fertilisers has a positive effect on pH, water-holding capacity, nutrient availability and carbon sequestration, and contributes to soil microbial diversity (Maticic *et al.*, 2024). I. Al-Shammary *et al.* (2024) highlighted the positive impact of integrated nutrient management on soil health and crop yields. Smart nutrient management substantially improves nutrient cycling, preventing soil imbalances and acidification. Implementing such practices is essential for sustainable agriculture. Since most soil physical properties, including soil moisture reserves, vary significantly throughout the year, it is necessary to account for these changes during the development of best practices for sustainable soil management and conservation. Rationalisation of tillage practices to replace mouldboard tillage with reduced tillage could be an alternative method to achieve agricultural sustainability and reduce negative impacts on agricultural ecosystems. Therefore, the study aims to determine the peculiarities of the accumulation of available soil moisture and soybean yields under different systems of basic tillage and fertilisation. The objectives of this study were to determine the impact of tillage and fertilisation systems on the amount of available soil moisture in the 0-20 and 0-100 cm soil layers during the germination, flowering and maturation phases of soybean; to compare moisture reserves in the 0-40 cm soil layer during the flowering phase of soybean under the influence of tillage and fertilisation systems; to determine the influence of soil tillage and fertilisation systems on the formation of soybean grain yield; to establish the correlation between soybean yield and moisture reserves in the 0-40 cm soil layer in the flowering phase under mineral and organo-mineral fertilisation and different soil tillage systems.

MATERIALS AND METHODS

The research was conducted in 2019-2022 at the Khmelnytsky State Agricultural Experimental Station on chernozem podsolised medium loamy soil (width 49°45'33", length 27°23'13"). The soil of the experimental plots was characterised by the following indicators: pHsol – 6.0-6.5, humus content in the topsoil – 2.62-3.12%, total nitrogen – 0.150-0.163%, mobile phosphorus – 12.5-19.61 mg per 1 kg of dry soil, potassium – 65.0-72.0 mg per 1 kg of dry soil. The experimental design includes factors:

- Factor A – soil cultivation: 1. mouldboard (to a depth of 25-27 cm) – control; 2. Flat-cutting (to a depth of 25-27 cm); 3. Chisel (to a depth of 25-27 cm); 4. Disc (to a depth of 10-12 cm); 5. Differentiated (to a depth of 25-27 cm).

- Factor B – fertilisation system: 1. Mineral – $N_{60}P_{60}K_{60}$; 2. Organic-mineral – $N_{30}P_{30}K_{30}$ + winter wheat straw + N_{10} per 1 tonne of straw.

The area of the plot was 40 m². The arrangement of variants in the experiment was systematic and replicated four times. Soybeans were grown following conventional technology in a short rotation crop rotation after winter wheat. The crop rotation in the 4-field crop rotation was as follows: winter wheat, soybeans, spring barley, and white mustard. The research was conducted according to generally accepted methods. Soil samples for moisture determination were taken three times during the growing season at the following stages of soybean growth and development: germination, flowering and full maturity. Soil moisture was determined by the thermostat-weight method in layers. At each plot, soil samples were taken with a drill in quadruplicate. After collection, soil samples were placed in aluminium boxes and covered with lids. Then they were weighed and dried in a thermostat for 3 hours at 105°C. The boxes were re-weighed after drying. The reserves of productive moisture in the soil were determined using Formula 1:

$$W_{m.c.} = (h \times D_v \times W) / 10, \quad (1)$$

where $W_{m.c.}$ – soil moisture reserves, mm; h – thickness of the soil layer, cm; D_v – bulk density of the soil, g/cm³; W – soil moisture content, %; 10 – conversion factor in mm.

To convert the reserves of productive moisture in mm/ha, the resulting figure was divided by 10. The soybean yield was measured at full ripeness by harvesting from each plot separately and weighing the grain. The mathematical processing of the research results was carried out by the method of analysis of variance using Excel and Statistica 6.0. The agrometeorological conditions during the years of the study differed significantly from the average long-term parameters in terms of precipitation, temperature and distribution during the growing season. The standards of the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1979) were applied.

RESULTS

In the soybean phase, when mineral fertilisers ($N_{60}P_{60}K_{60}$) were applied, the most moisture (36.8 mm) was contained in the root layer of soil 0-20 cm, which exceeded the control by 2.7 mm (Table 1).

Under chisel and differentiated tillage, the moisture content was not significant (by 0.1 and 0.2 mm) compared to disking. When using an organo-mineral fertilisation system ($N_{30}P_{30}K_{30}$ + winter wheat straw + N_{10} per 1 tonne of straw) in the 0-20 cm soil layer, the maximum moisture content (36.2 mm) was determined at the chisel system, which exceeded the control by 3.0 mm. In the phase of soybean germination, the highest moisture content (212.3 mm) was recorded in the 0-100 soil layer under the differentiated tillage system, which will be used in the future for the growth and development of the crop. In this variant, the height of soybean plants at the stage of completion of

budding – beginning of flowering was 3-8 cm higher than the control and other systems on both backgrounds. With mineral fertilisation in the soybean

flowering phase, a significant advantage in moisture content in the 0-20 cm and 0-100 cm layers was established when chisel tillage was used.

Table 1. Dynamics of productive moisture in soybean crops under tillage and fertilisation, mm (average for 2019-2022)

Soil cultivation (Factor A)	Fertilisation (Factor B)	Vegetation phases					
		growth		blooming		maturation	
		soil layer, cm					
		0-20	0-100	0-20	0-100	0-20	0-100
Mouldboard (control)	M	34.1	210.6	8.7	96.2	13.9	84.1
	OM	33.2	198.0	11.1	100.4	12.3	87.4
Flat cutter	M	34.9	208.4	10.5	84.0	14.3	96.0
	OM	34.7	207.9	9.2	102.8	13.1	82.8
Chiselled	M	36.7	207.1	12.7	107.0	12.2	85.9
	OM	36.2	208.2	12.8	125.9	16.6	107.4
Disc	M	36.8	209.3	9.0	82.4	12.8	83.4
	OM	34.4	206.9	11.1	102.7	12.9	92.8
Differentiated	M	36.6	212.3	10.3	100.7	13.5	82.4
	OM	35.9	212.3	12.4	122.9	14.6	92.8

Note: M – mineral fertiliser system, OM – organic-mineral fertiliser system

Source: compiled by the authors

When using the organo-mineral fertilisation system, a higher moisture content was also observed in these aspects than in the chisel ploughing. Under the differentiated system, a slight decrease in moisture content was recorded from 0.4 mm in the 0-20 cm layer to 3.0 mm in the 0-100 cm layer on the organic-mineral background. The moisture content during this period had the greatest impact on

the formation of the crop yield because the variants with the highest moisture reserve also had the highest soybean grain yield. The lowest moisture content (26.3 mm) in the soil layer 0-40 cm at the flowering stage of soybean plants was found in the case of mouldboard and disk tillage against the background of using an organic-mineral fertiliser system (Table 2).

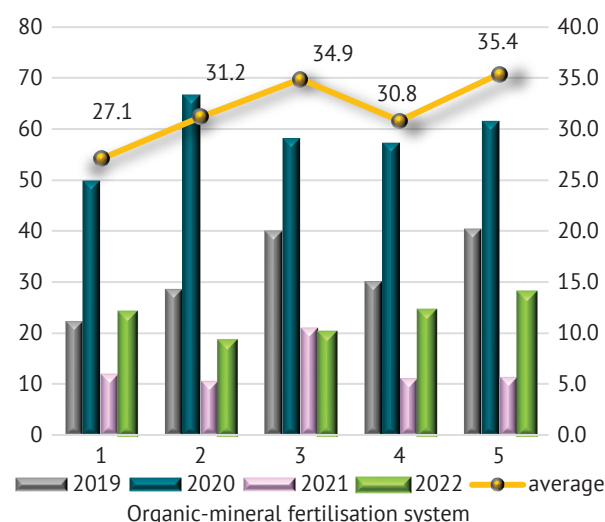
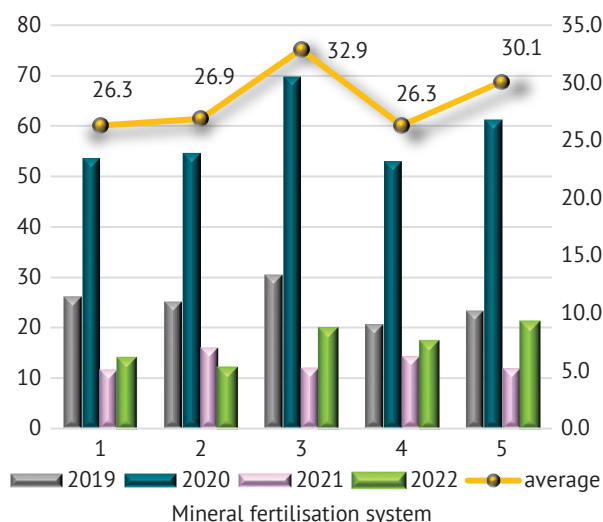


Figure 1. Moisture reserves in the 0-40 cm soil layer under the influence of tillage and fertilisation at the BBCH stage of 61-65 soybean (2019-2022), mm

Note: 1 – Mouldboard tillage (control); 2 – Ploughshare tillage; 3 – Chisel tillage; 4 – Disc tillage; 5 – Differentiated tillage

Source: compiled by the authors

The maximum amount of available moisture (32.9 mm) was recorded in the 0-40 cm soil layer at the BBCH stage of 61-65 soybeans using the mineral

fertilisation system under chisel tillage. With the differentiated tillage system, the amount of moisture was 31.1 mm, which is 25% and 14% higher, respectively,

compared to mouldboard tillage. The use of an organo-mineral fertiliser system ensures the maximum amount of available moisture in the 0-40 cm soil layer under chisel (34.9 mm) and differentiated (35.4 mm) tillage systems. This is 29 and 31% more than in the control. The lowest moisture content (27.1 mm) was found in the case of mouldboard tillage (control). When using the organo-mineral fertilisation system, the content of available soil moisture in the 0-40 cm layer during soybean flowering was 0.8-5.3 mm higher than in the mineral background, depending on the tillage

system. Thus, a higher reserve of available moisture in the 0-40 cm soil layer at the stage of soybean flowering was found in the differentiated and chisel system on both fertilisation patterns. A significant advantage of organic-mineral fertiliser (3-18%) over mineral fertiliser was also recorded, which significantly influenced the formation of crop yields. As a result of statistical analysis, the indicators of the share of influence of the studied factors A (tillage) and B (fertiliser system) on the accumulation of moisture in the 0-40 cm soil layer at the BBCH stage of 61-65 soybean were established (Fig. 2).

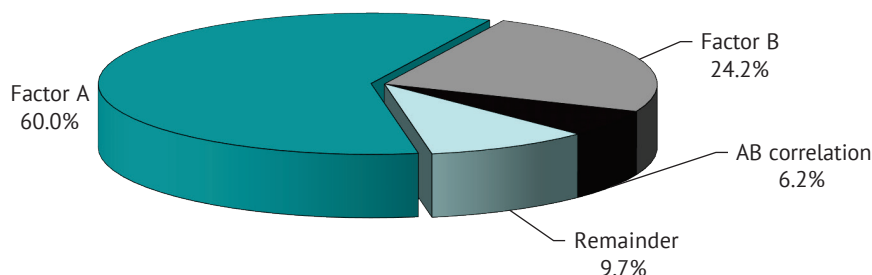


Figure 2. The share of influence of the studied factors on the amount of moisture in the 0-40 cm soil layer at the stage BBCH 61-65 of soybean

Note: HIP_{05} factor A = 0.811 mm, HIP_{05} factor B = 0.207 mm)

Source: compiled by the authors

The two-factor variance analysis determined that the share of the influence of tillage systems on moisture reserves is 60.0%, and the share of fertiliser influence on moisture reserves is 24.2%. The effect of each of the factors on moisture reserves is reliable and significant at the level of $P \leq 0.05$. At the same

time, the share of interaction between the studied factors is insignificant and amounts to only 6.2%. With the application of mineral fertilisers ($N_{60}P_{60}K_{60}$), the yield of soybean grain on average over the four years of the experiment varied from 2.25 to 2.49 t/ha (Fig. 3).

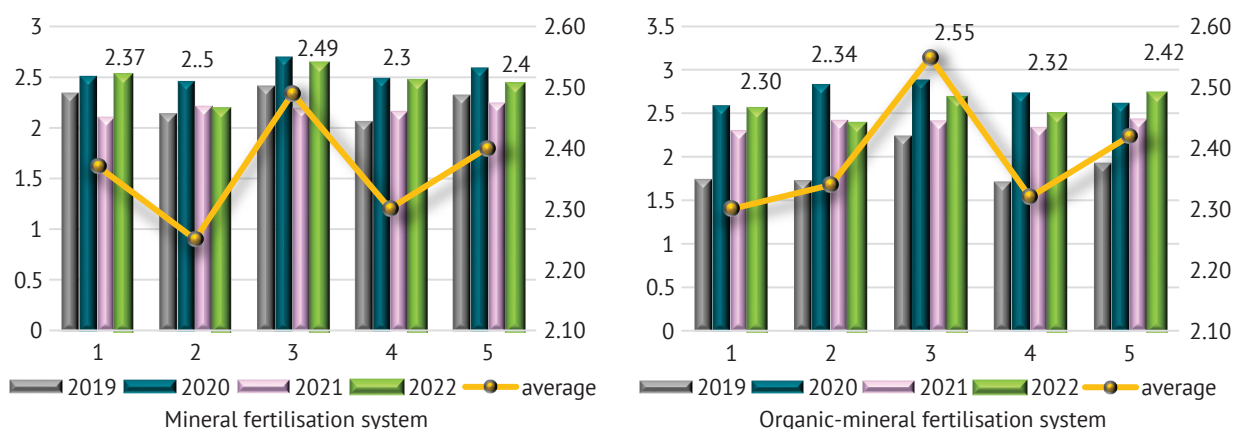


Figure 3. Soybean grain yield under tillage and fertilisation, t/ha

Note: 1 – Mouldboard tillage (control); 2 – Ploughshare tillage; 3 – Chisel tillage; 4 – Disc tillage; 5 – Differentiated tillage

Source: compiled by the authors

With the flat-cutting fertilisation system on the mineral fertilisation background, a 5% decrease in soybean grain yield was observed compared to the control. With the use of mineral nutrition and chisel tillage, the maximum soybean grain yield was recorded – 2.49 t/ha. This is 5% more than in the case of mouldboard

tillage (control). Under the disc and differentiated tillage system on a mineral fertiliser pattern, the grain yield was 2.3-2.4 t/ha. These figures are only 1-3% higher than the soybean grain yield in the control variant. With the use of the organo-mineral nutrition system, the average soybean grain yield was

2.3-2.55 t/ha over the four years of the experiment. The highest soybean grain yield was formed under the influence of the organic-mineral nutrition system and chisel tillage. This is 10.9% higher compared to mouldboard tillage. The use of organo-mineral fertiliser and differentiated tillage increases soybean grain yield by 5.3% compared to the control.

The organic-mineral fertiliser background exceeded the mineral fertiliser background by 1-4% for all tillage methods, except for mouldboard tillage. When using the organo-mineral system and mouldboard

tillage, a slight decrease in soybean grain yield (3%) was observed compared to mineral fertilisation. The moisture content during this period had the greatest influence on the formation of soybean grain yield because the variants with the highest moisture reserve recorded the highest soybean yield. As a result of correlation analysis, the dependence of soybean grain yield on moisture reserves in the 0-40 cm soil layer at the BBCH 61-65 stage was established with the use of mineral fertiliser and different soil tillage (Fig. 4).

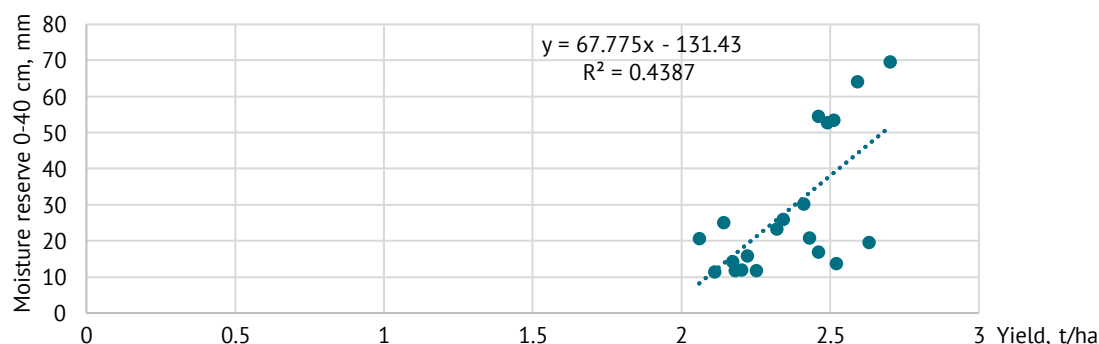


Figure 4. Correlation between soybean yield and moisture reserves in the 0-40 cm soil layer in the flowering phase on a mineral fertiliser background under different tillage systems (2019-2022)

Source: compiled by the authors

The regression model of the dependence of the indicator (Y) – soybean grain yield on moisture reserves in the 0-40 cm soil layer at the BBCH 61-65 stage under different tillage systems on a mineral fertiliser background is as follows (2):

$$Y = 67.775x - 131.43, \quad (2)$$

where Y – soybean grain yield, t/ha; x – moisture reserves in the 0-40 cm soil layer, mm.

Thus, it is possible to note a strong connection between soybean grain yield and moisture reserves in the 0-40 cm layer in the flowering phase on the mineral

background of fertilisation. The analysis of the correlation between soybean yield over the years of research and moisture reserves in the 0-40 cm soil layer determined in the flowering phase under different tillage and mineral nutrition confirms that there is a positive correlation between these indicators of medium strength with $r = 0.66$ ($P \leq 0.05$). The significance level did not exceed the 5% level, which confirms statistical reliability. The dependence of soybean grain yield on moisture reserves in the 0-40 cm soil layer at the BBCH 61-65 stage under different tillage systems on the organic-mineral fertiliser background is shown in Figure 5.

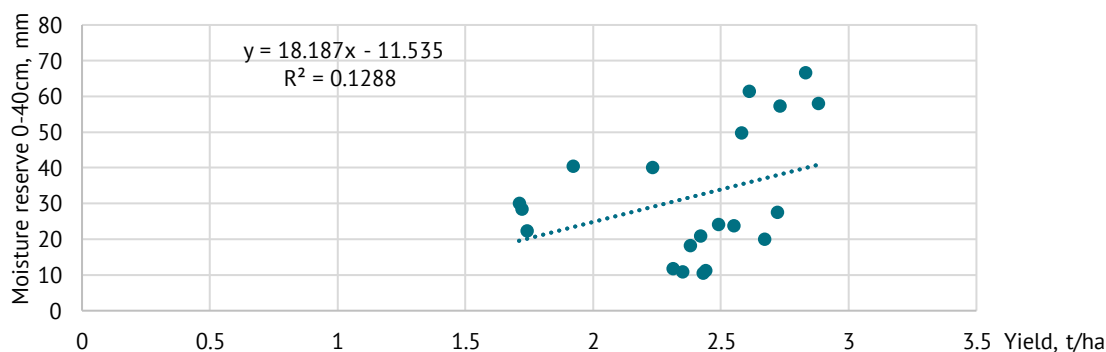


Figure 5. Correlation dependence of soybean yield on moisture reserves in the soil layer 0-40 cm in the flowering phase under different tillage systems and organic-mineral fertilisation (2019-2022)

Source: compiled by the authors

The regression model of the dependence of soybean grain yield (Y) on the amount of moisture in the 0-40 cm soil layer in the flowering phase under the influence of different soil tillage on the organic-mineral fertiliser background is as follows (2):

$$Y = 18.187x - 11.535, \quad (3)$$

where Y – soybean grain yield, t/ha; x – moisture reserves in the 0-40 cm soil layer, mm.

The correlation coefficient between soybean yield and moisture reserves in the 0-40 cm soil layer under

different tillage systems and organic-mineral fertilisation is 0.36. This indicates a weak positive correlation between these indicators. The insignificant relationship between yield and moisture level in the tilth layer over the years can be explained by the uneven distribution of precipitation during the growing season. Dry periods during the formation of soybean grain were followed by rainy periods, sometimes with storms. In the analysis of variance of the effect of tillage and fertiliser systems on soybean grain yield, the significance and reliability of the studied factors were determined (Fig. 6).

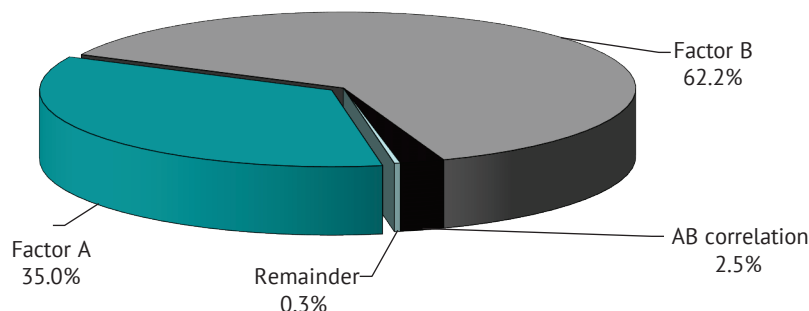


Figure 6. The share of influence of the studied factors on soybean yield in 2019-2022, %

Note: (HIP_{05} (factor A) = 0.009 t/ha, HIP_{05} (factor B) = 0.007 t/ha)

Source: compiled by the authors

Fertilisation (factor B) had a dominant influence on the formation of soybean grain yield with a share of 62.2% ($P \leq 0.05$). The influence of the soil tillage system (factor A) on the formation of soybean grain yield was 35.0%, i.e. almost half. Thus, factor A – fertilisation – has a significant impact on soybean yield among the factors studied. At the same time, the share of interaction between the factors is insignificant and amounted to only 2.5%. The understanding of this interaction is important for sustainable soybean production and facilitating adaptation to climate change. The use of the latest technologies will help to address the new challenges of modern agriculture.

DISCUSSION

As a result of warming, there are droughts, floods, uneven rainfall, etc. All these stressors can pose a threat to global food security. Insufficient soil moisture to satisfy the needs of crops causes drought stress. Reduced soil moisture due to climate change can reduce crop yields, which negatively affects food security and food system resilience (Kyrylyuk *et al.*, 2019b; Leal Filho *et al.*, 2023). Insufficient soil moisture during the growing season limits the growth and development of plants and reduces their yield potential. Therefore, one of the key challenges for sustainable agriculture is to regulate the soil water regime (Dymyrov & Sabluk, 2022).

The yield of soybean grain also depends on meeting its needs for abiotic factors, including heat and moisture supply not only during certain periods of its growth and

development but also throughout the growing season. A. de Almeida *et al.* (2024) and J. Daneshian *et al.* (2024) also noted that the lack of soil moisture is the main cause of abiotic stress, which leads to significant losses in soybean yields in many regions of the world. In this study, the highest amount of available moisture in the 0-20 cm soil layer in the soybean germination phase was found under mineral and organo-mineral fertilisation systems in chisel tillage and the lowest – under mouldboard tillage. The results of the research are consistent with the data by V. Hurtovenko and O. Tsiuk (2023) regarding the impact of tillage on the water regime of typical black soil. The maximum reserves of available moisture in the 0-30 cm and 0-100 cm soil layers during the period of sunflower plant germination were observed under no-till tillage. The lowest reserves of available moisture were observed under the mouldboard multi-depth system of basic tillage.

The current findings correlate with those of M. Furman *et al.* (2021). The study determined that under reduced tillage systems with the use of straw as fertiliser, a significant increase in the amount of productive moisture (by 22%) in the one-metre soil layer in the germination phase of corn, spring barley and winter wheat was observed compared to the mouldboard system. The research of T. Arshad *et al.* (2023) also confirmed the positive impact of conservation tillage on soil moisture reserves. O. Mazur *et al.* (2023) highlighted the dependence of soybean yield not only on the biological characteristics of varieties but also on agroecological

conditions of cultivation. A particularly notable effect of drought stress on the growth and development of soybean plants was found at the stage from flowering to seed filling (R1-R4). This confirms the findings of M. Srebric *et al.* (2020), demonstrating that at the fruiting stages, moisture deficit has a greater impact on soybean yield formation. This is confirmed by the results obtained on the dependence of soybean grain yield on moisture reserves in the soil layer (0-40 cm) during flowering on a mineral fertiliser background under different tillage systems. According to I. Didur *et al.* (2023), sufficient soil moisture can improve soybean growth and increase the number of soybean pods. According to V. Furman *et al.* (2022), high temperature and drought stress negatively affect the photosynthetic capacity of soybean leaves, flower opening and seed formation. This is consistent with the results of this study.

The results of the conducted research established the advantage of organic-mineral fertilisation over mineral fertilisation, which influenced the increase in soybean grain yield. These results correlate with the research of P. Mthiyane *et al.* (2024), which emphasises the role of balanced use of organic fertilisers and minimum amounts of chemical fertilisers to maximise nutrient use efficiency. According to M. Furman *et al.* (2021), the use of by-products (straw) to optimise the mineral fertilisation system increases the yield of spring barley (by 0.32-0.22 t/ha), winter wheat (by 0.45-0.36 t/ha) and corn (by 0.60-0.46 t/ha) compared to mineral fertilisation. S. Dymyrov and V. Sabluk (2022) highlighted the positive effect of mycorrhisation of winter wheat and maize roots by mycorrhizal fungi on the accumulation of productive moisture reserves in the topsoil. An increase in the amount of available moisture in the 0-30 cm soil layer was observed from 5.3 to 47.8% compared to the variant without mycorrhisation. This helps to increase crop yields and address the problems associated with climate change, declining soil fertility and food security. Addressing these challenges is key to achieving the United Nations Sustainable Development Goals (SDGs) by 2030, in particular SDG 2 (Zero Hunger) and SDG 13 (Climate Action), which focus on ending hunger, promoting sustainable agriculture, and taking urgent action to mitigate climate change (Sustainable Development Goals, n.d.). These results can be used in optimisation of land use patterns, preventing soil erosion and modelling hydrological processes.

CONCLUSIONS

In the root layer of the soil (0-20 cm), the highest reserves of available moisture (36.6-36.8 mm) in the phase of full germination of soybeans were on the mineral pattern of nutrition under chisel, differentiated

and disc tillage, and the lowest (34.1 mm) – under mouldboard tillage. On the organo-mineral pattern of nutrition, the use of the chisel tillage system provided the highest supply of available moisture in the soil – 36.2 mm. During the flowering period of soybeans, the highest reserves of available soil moisture in the arable layer were observed in chisel tillage using mineral and organic-mineral nutrition systems – 107.0 and 125.9 mm, respectively. During the flowering period of soybeans, the advantage in moisture reserves in the one-metre soil layer on the organic-mineral fertiliser background compared to the mineral background was found to be 4.2-22.2 mm in all tillage systems. The highest reserve of available moisture in the 0-40 cm soil layer was observed with mineral fertiliser in chisel tillage (32.9 mm) and organic-mineral fertiliser in chisel (34.9 mm) and differentiated (35.4) tillage systems. The content of available moisture in the 0-40 cm soil layer during soybean flowering using the organic-mineral fertiliser system was 3-18% higher than in the mineral fertiliser system, depending on soil tillage. The maximum impact on the accumulation of productive moisture reserves in the 0-40 cm soil layer at the stage of soybean flowering was found in soil tillage systems (60.0%). The impact of fertilisation on productive moisture reserves was 24.2%.

The highest soybean yields (2.49 and 2.55 t/ha) were obtained with chisel tillage and mineral and organic-mineral fertilisation, which is 5 and 12% higher than in the control. On the organo-mineral fertiliser pattern, soybean yields under all no-till tillage were 1-4% higher than under mineral fertiliser and control. An average direct correlation ($r = 0.66$) exists between soybean yield and productive moisture reserves in the 0-40 cm soil layer in the flowering phase of soybean under different tillage systems on a mineral fertiliser background. On the organic-mineral fertiliser background, a weak positive correlation ($r = 0.36$) was found between soybean grain yield and moisture reserves in the 0-40 cm soil layer under different tillage systems. Soil tillage systems had a significant impact on soybean grain yield (35.0%). The fertilisation system had the greatest influence on soybean grain yield – 62.5% ($P \leq 0.05$). Further research may focus on assessing the physical and chemical parameters of soils under the influence of different tillage and fertilisation systems in the context of climate change.

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None.

CONFLICT OF INTEREST

None.

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Регулювання водного режиму посівів сої за дії обробітку ґрунту і удобрення для сталого сільського господарства

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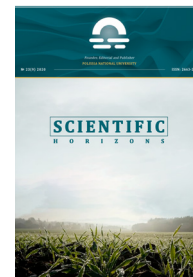
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Анотація. Зниження вологості ґрунту внаслідок зміни клімату може призвести до зменшення урожайності культур, що негативно впливає на продовольчу безпеку і стійкість харчових систем. Тому метою цього дослідження було з'ясувати вплив обробіток ґрунту і систем удобрення на накопичення доступної вологи у ґрунті та урожайність сої. Дослідження були проведені на чорноземному опідзоленому середньо-суглинковому ґрунті протягом 2019-2022. Застосовували методи досліджень – польовий, лабораторний, порівняльно-розрахунковий і кореляційний. Сою вирощували у 4-пільній сівозміні за загальноприйнятою технологією для умов регіону дослідження. Представлено динаміку запасів доступної вологи у 0-20, 0-40 і 0-100 см шарах ґрунті впродовж вегетаційного періоду за дії систем обробітку ґрунту і удобрення. Було визначено запаси продуктивної вологи в ґрунті та урожайність зерна сої за дії полицевого, плоскорізного, чизельного, дискового і диференційованого обробітку ґрунту на мінеральному і органо-мінеральному фонах живлення. Максимальні запаси доступної вологи у кореневмісному шарі ґрунту (0-20 см) на мінеральному і органо-мінеральному фонах удобрення спостерігали у фазі повних сходів і цвітіння сої за чизельного обробітку ґрунту, а мінімальні за полицевого. Найвищі показники урожайності зерна сої отримано за мінерального і органо-мінерального удобрення і чизельного обробітку ґрунту. У фазі цвітіння сої за дії систем обробітку ґрунту між урожайністю зерна і запасами доступної вологи у шарі ґрунту 0-40 см встановлено середню кореляційну залежність ($r = 0,66$) за мінерального удобрення і слабку кореляційну залежність ($r = 0,36$) за органо-мінерального удобрення. На урожайність зерна сої максимальний вплив (62,5 %) виявила система удобрення. Результати цього дослідження можуть бути використані у прийнятті рішень стосовно сталого управління і збереження ґрунтів для сприяння глобальної продовольчої безпеки і пом'якшення змін клімату

Ключові слова: запаси вологи; шар ґрунту; урожайність зерна; кореляція; частка впливу



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Influence of biostimulants on physiological processes, productivity, and quality of pea crop in modern agriculture

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Abstract. The purpose of this study was to investigate the effects of biostimulants on increasing the adaptive mechanisms of pea (*Pisum sativum* L.) under water deficit conditions, with an emphasis on optimising physiological functions, biochemical processes, and morphological development of plants. The experiment was conducted

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under controlled conditions that helped to accurately model the effect of water stress. The study used comprehensive evaluation methods, including physiological parameters (photosynthetic activity, transpiration), biochemical markers (antioxidant enzyme activity, proline level), and morphological parameters (length and weight of the root system). The findings confirmed that treatment with biostimulants significantly increased the adaptive potential of plants. Photosynthetic activity in the treated plants was 82%, which was substantially higher than in the control group, where this level reached only 60%. Transpiration in the treated plants stayed stable, ensuring optimised water balance even under stress. Biochemical analysis showed that the activity of superoxide dismutase and catalase in plants treated with biostimulants increased by 145% compared to control plants, which contributed to a major reduction in oxidative stress. The level of proline, as a key osmotic regulator, was also significantly higher in the treated plants, which maintained the stability of the cellular water balance. Morphological studies revealed that the plants treated with biostimulants had a more developed root system: the length of the roots was 10 cm longer, while the weight was greater than in the control. This helped the plants to use moisture from deeper soil layers, which improved their resistance to drought. As a result, the yield loss in the treated plants was half that of the control group. These findings highlighted the effectiveness of using biostimulants to increase pea resistance to water deficit and maintain productivity. This makes the proposed approach promising for implementation in agricultural technology, especially in arid regions where water deficit is the primary limiting factor for crops

Keywords: plant adaptation; abiotic factors; stress resistance; transpiration; water balance

INTRODUCTION

In the context of global climate change, accompanied by more frequent droughts and unstable water supplies, there is a growing need to develop effective methods for adapting crops to stressful conditions. Pea is a valuable crop for food security due to its rich protein content and ability to fix atmospheric nitrogen, which helps improve soil fertility. However, this crop is quite sensitive to water shortages, which greatly limits its productivity in arid regions. The relevance of this study is driven by the need to optimise pea production under drought conditions, which is a major challenge for the agricultural sector in Ukraine and other countries with comparable climatic conditions. Currently, there are a limited number of studies that comprehensively investigate the effect of biostimulants on the physiological, biochemical, and morphological adaptation mechanisms of pea.

The principal problem is that conventional pea cultivation technologies are not effective enough to reduce the effects of abiotic stresses, specifically water deficit (Drobitko *et al.*, 2024). Existing approaches, such as optimising irrigation or fertilisation regimes, have a limited impact on maintaining the physiological stability of plants under stressful conditions. At the same time, the mechanisms underlying plant adaptation to water stress, such as regulation of transpiration, activation of antioxidant defences, and improvement of osmotic balance, are still understudied in the context of practical applications (Krychkovska *et al.*, 2024).

E. Szpunar-Krok (2022) investigated the effect of foliar application of biostimulants on the physiological response of peas. The researcher found that such preparations help maintain photosynthetic activity, reduce water loss through leaves, and improve yields under conditions of limited water supply. This emphasised the significance of the effects of biostimulants on basic

physiological processes. S.A. Ismaiel *et al.* (2022) focused on the relationship between biostimulants, soil characteristics, and pea yield in the field. In their study, the researchers showed that biostimulants not only increase plant resistance to stress factors but also improve the physical and chemical properties of the soil, creating optimised conditions for growth. A.M. Castiglione *et al.* (2021) highlighted the role of microbial biostimulants in modern agriculture. The researchers noted that such preparations can improve plant resistance to abiotic stresses by stimulating the synthesis of metabolites involved in the regulation of the stress response. A. Kocira *et al.* (2020) analysed changes in biochemical parameters and yield of beans after the application of biostimulants. The researchers demonstrated the positive effects of the preparations on protein content, regulation of nitrogen metabolism, and overall plant yield.

A.F. El Sheikha *et al.* (2022) focused on the effects of biostimulants on the growth and nutritional quality of legumes. The findings indicated an increase in yield and improvement in the nutritional value of beans due to the use of biostimulants, which makes these products a valuable tool for sustainable agriculture. W. Biel *et al.* (2023) investigated the effects of amino acid biostimulants in combination with nitrogen fertilisation and irrigation on the composition of pea seeds. The researchers' findings confirmed the considerable effects of these factors on the energy value of seeds and overall plant productivity. S. Naz *et al.* (2023) investigated the use of seaweed extracts to stimulate pea growth. The researchers showed that such biostimulants increase plant productivity by improving physiological indices such as photosynthetic efficiency and biomass accumulation. F.A. Youssef *et al.* (2024) analysed the effects of combined use of organic and mineral fertilisers

in pea cultivation. The researchers noted that organic biostimulants can compensate for the negative effects of water deficit, ensuring stable growth and yield.

R. Johnson *et al.* (2024) considered biostimulants as a promising tool for increasing crop productivity and resistance to abiotic stress. The researchers focused on the possibilities of integrating biostimulants into sustainable agriculture strategies. L. Nephal *et al.* (2020) applied a metabolomic approach to analysing the effects of biostimulants in their study. The study demonstrated that these preparations activate metabolic pathways that contribute to plant adaptation to stressful conditions such as drought. Despite considerable progress in the study of the effects of biostimulants, there are important aspects that are still understudied. The molecular mechanisms of action require deeper analysis, including the role of signalling pathways and genetic regulation of stress adaptation. The effects of biostimulants under conditions of multi-stress factors, such as a combination of drought and hot temperatures, are also poorly understood.

There is a lack of data on the long-term impact of biostimulants on the productivity and health of agroecosystems. The impact on soil microbiota and the environment requires further investigation, especially in the context of sustainable development. The comparative effectiveness of assorted types of biostimulants, as well as their interaction with other agronomic practices, are still understudied. The purpose of this study was to comprehensively assess the effects of biostimulants on the resistance and productivity of peas under water deficit conditions.

MATERIALS AND METHODS

The study was conducted in 2023 at the Podillia Research Centre of the Podillia State University. For the

experiment, the pea variety "Gambit" was chosen, which is a *Pisum sativum* L. variety with a growing season of 75-85 days (from May to July). This variety is characterised by stable yields and adaptation to moderate arid conditions, which makes it suitable for assessing the effects of biostimulants. Plants were grown in a controlled greenhouse environment, where the temperature was kept constant at 22-24°C during the day and 18-20°C at night, which corresponds to suitable conditions for pea growth. Three main groups were created for the study, differing in terms of water supply and treatment with biostimulants. The control group included plants that were grown without biostimulant treatment at optimised soil moisture, which was maintained at 70% of the field moisture capacity.

Experimental group 1 involved growing plants under the same conditions of optimised moisture (70% field moisture capacity), but with additional treatment with biostimulants. Experimental group 2 included plants under water deficit conditions with reduced soil moisture to 30% of the field moisture capacity. This group was divided into two subgroups: experimental Subgroup 2.1, where plants were treated with a biostimulant, and experimental Subgroup 2.2, where no biostimulants were used. Such a division helped to analyse the effects of both water deficit and biostimulants on plants under differing moisture content conditions, as well as to compare the results with the control values. The Aminovit biostimulant was used in combination with the Algafit preparation, which contains a complex of amino acids that stimulate metabolic processes in plants, with the ability to improve physiological processes and increase plant resistance to stressful conditions (Table 1). The preparation was applied by spraying the leaves in the phase of active plant growth, starting from the third week after sowing.

Table 1. Effect of biostimulants on plants in different conditions

Group/subgroup	Growing conditions	Preparations	Dosage (ml/l)	Treatment frequency
Control (Group 1)	Optimum moisture content (70% MWC)**	Not applied	–	–
Optimised with biostimulants (Group 2)	Optimum moisture content (70% MWC)**	Aminovit + Algafit	Aminovit (2 ml) + Algafit (3 ml) per 1 litre of water	Spraying of leaves, every 10 days, starting from the 3 rd week after sowing
No treatment (Group 2, Subgroup 2.2)	Water deficit (30% MWC)**	Aminovit + Algafit	Aminovit (2.5 ml) + Algafit (3.5 ml) per 1 litre of water	Spraying of leaves, every 7–10 days, starting from the 3 rd week after sowing
With treatment with biostimulants (Group 2, Subgroup 2.1)	Water deficit (30% MWC)**	Not applied	–	

Note: MWC – maximum water capacity

Source: developed by the authors

Containers filled with soil substrate with the addition of perlite were used to grow the plants, which

provided homogeneous conditions for the development of the root system. The soil moisture level was

maintained using an Irritrol Junior Max automated irrigation system (manufacturer: Irritrol Systems), which enabled precise control of the field soil moisture capacity. The temperature in the greenhouse was regulated using a ThermoGrow TG-100 thermostat (manufacturer: ThermoTech). Modern instruments were used to measure physiological parameters. Photosynthetic activity was determined using a LI-6400XT portable gas exchange analyser (manufacturer: LI-COR Biosciences), which measured the intensity of carbon dioxide assimilation. The level of transpiration was determined using a LeafPorometer SC-1 moisture sensor (manufacturer: Decagon Devices), which enabled an accurate assessment of water evaporation through the leaves.

Biochemical parameters were determined by analysing leaf samples. To measure the level of proline, the study employed the method of spectrophotometry with a ninhydrin reaction on a UV-1800 spectrophotometer (manufacturer: Shimadzu). The activity of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), was analysed using specific substrates on the same device. These indicators helped to assess how effectively the plants neutralise oxidative stress caused by water deficit. Morphological parameters were assessed at the end of the experiment. The length of the roots, the weight of the root system, and the general condition of the plants were measured. For this, GX-200 digital scales (manufacturer: A&D Company) and Vernier Caliper IP54 calipers (manufacturer: Mitutoyo) were used. The general condition of the plants was determined on a scale from 1 to 10 points, considering visual signs of stress, such as wilting, discolouration of leaves, or stunting. All the data obtained were processed statistically using Statistica 12 software (manufacturer: StatSoft), which helped to analyse the reliability of the findings. This approach helped to draw conclusions about the effectiveness of biostimulants in increasing pea stress resistance and its ability to adapt to adverse water deficit conditions. 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

The results showed that the use of biostimulants positively affected the ability of plants to adapt to stressful conditions and maintain productivity. In Group 1 (control), where moisture conditions stayed optimum,

the plants showed stable indicators of the number and weight of beans. However, in Subgroup 2.2 (water deficit without biostimulants), which was experiencing a water deficit, a significant decrease in these indicators was observed. In Subgroup 2.2 (water deficit without biostimulants), a sharp decline in productivity was observed: the number of beans decreased by 46% and the average weight of one bean by 40% compared to control plants. This indicated that plant adaptation mechanisms were insufficient to overcome water stress without additional support. In Subgroup 2.1 (water deficit with biostimulants), the number of beans decreased by only 23% compared to optimum moisture conditions, while the average weight of one bean decreased by 16%. This demonstrates the effectiveness of biostimulants in mitigating the adverse impact of stressful conditions, enabling plants to maintain productivity even under adverse conditions. These findings are explained by the activation of adaptive mechanisms that were enhanced by the biostimulant treatment.

Physiological analyses confirmed the positive effects of biostimulants on plant resistance to stressful conditions (Khalid *et al.*, 2024). In plants of Subgroup 2.1 treated with biostimulants, an increase in the activity of antioxidant enzymes such as SOD and CAT, which play a significant role in reducing oxidative stress, was observed. There was also a 1.5-fold increase in the level of proline compared to Subgroup 2.2, which provided better osmoprotection of cells under stressful conditions. Plants treated with biostimulants maintained stable photosynthetic activity even under water deficit conditions. Unlike plants in Subgroup 2.2 with water deficit, which showed a significant decrease in photosynthetic activity, plants in Subgroup 2.1 treated with biostimulants maintained a more efficient gas exchange, which contributed to the maintenance of growth and development. In the control group, under optimum water conditions, photosynthesis rates stayed stable.

Morphological changes also confirmed the effectiveness of the biostimulants. Plants in Subgroup 2.1 treated with biostimulants had a more developed root system, which contributed to better absorption of moisture from deep soil layers. In contrast, the root system of the control plants from Subgroup 2.2 with water deficit was less developed, which limited their ability to withstand droughts. These findings confirmed the effectiveness of biostimulants in increasing plant stress resistance (Table 2).

Table 2. Comparison of plant productivity in control and biostimulant-treated conditions

Experiment conditions	Plant group	Beans, pcs	Average mass of 1 bean, g	Productivity reduction, %
Optimum moisture content	Control (Group 1)	50	0.35	–
	Optimum with biostimulants (Group 2)	52	0.37	–
	No treatment (Group 2, Subgroup 2.2)	27	0.21	46/40
Water deficit	With biostimulant treatment (Group 2, Subgroup 2.1)	40	0.31	23/16

Source: developed by the authors

Quantitative data also show the benefits of using biostimulants. Water deficit conditions significantly affected the yield of Subgroup 2.2 (water deficit without biostimulant treatment), which was not treated with biostimulants, while the control group (Group 1), which was grown under optimum moisture conditions, showed stable yields. In Group 2, a slight improvement in productivity was recorded compared to the control group: the number of beans increased to 52, while the average weight of one bean increased to 0.37 g. This is evidence of the stimulating effects of biostimulants even under optimum moisture conditions. The data analysis revealed that in Subgroup 2.2 (water deficit without biostimulant treatment), under water deficit conditions, the average yield of control plants was only 60% of the level recorded under optimum moisture conditions. In contrast, in Subgroup 2.1, which received biostimulant treatment, the yield was about 82%, suggesting a significant reduction in productivity losses due to the use of biostimulants. The use of biostimulants reduced yield losses by 25% compared to control plants under stressful conditions, demonstrating the effectiveness of these products in maintaining productivity even under water shortages.

The next stage of the study was to investigate the effects of biostimulants on the physiological parameters of peas. The analysis included measurements of photosynthetic activity, transpiration, antioxidant enzyme activity (SOD and CAT), proline levels, and chlorophyll content. The findings revealed that in Subgroup 2.2, the photosynthetic activity under stressful conditions decreased to 60% of the control level under optimum moisture content conditions, while in Subgroup 2.1, this indicator stayed at 82%. The data indicate that the biostimulants helped the plants to maintain the efficiency of photosynthetic processes necessary to support growth and development (Baltazar *et al.*, 2021). The level of transpiration also showed marked differences between the groups. In the control group (Group 1), the level of transpiration was 100%, while in Group 2 (treated) it increased slightly to 102% due to increased water exchange. In the control Subgroup 2.2, transpiration decreased to 50%, indicating a sharp restriction of

the water balance of plants under stress. At the same time, in Subgroup 2.1, where biostimulants were used, transpiration was maintained at 75%, ensuring better water exchange and efficient use of moisture.

The activity of antioxidant enzymes SOD and CAT confirmed the protective effect of biostimulants under stressful conditions. In the control (Group 1), the enzyme activity was taken as 100%, while in Group 2 it increased to 110%, indicating stimulation of the antioxidant system. In Subgroup 2.2, the activity of SOD and CAT decreased to 70%, suggesting increased oxidative stress due to moisture deficiency. At the same time, in Subgroup 2.1, the enzyme activity reached 145%, which provided considerable protection of cells from oxidative damage. This information is based on data confirming that enzymes play a vital role in reducing oxidative stress in response to moisture deficit. The level of proline, a significant amino acid for osmoprotection, also revealed substantial changes. In the control (Group 1), this indicator was 1 unit, while in Group 2 – 1.1 units, which confirmed the insignificant effects of biostimulants at optimum moisture content. In Subgroup 2.2, the level of proline increased to 1.5 units, which was the response of plants to water stress. However, in Subgroup 2.1, the proline level reached 2.5 units, which confirmed the activation of adaptive mechanisms due to the use of biostimulants.

Chlorophyll content is a vital indicator of plant resistance to stressful conditions (Cherven *et al.*, 2024). In the control (Group 1), this indicator was 100%, while in Group 2 it increased to 104%, which demonstrated the stimulating effects of biostimulants on the photosynthetic apparatus. In Subgroup 2.2, the chlorophyll content decreased to 58%, suggesting the inhibition of photosynthetic processes under stressful conditions. In Subgroup 2.1, where biostimulants were used, the chlorophyll content was maintained at 80%, which confirmed the effectiveness of the preparations in preserving the physiological activity of plants (Ostrowski *et al.*, 2020). Maintaining a strong level of chlorophyll was a crucial indicator of plant resistance to drought, as this pigment is a key component of photosynthesis (Table 3).

Table 3. Physiological parameters of control and biostimulant-treated plants under different soil moisture conditions

Indicator	Control group (group 1, optimum moisture content)	Treated group (Group 2, optimum moisture content)	Control Subgroup 2.2 (water deficit)	Treated Subgroup 2.1 (water deficit)
Photosynthetic activity, %	100	105	60	82
Transpiration level, %	100	102	50	75
SOD and CAT activity, %	100	110	70	145
Proline level, relative units	1.0	1.1	1.5	2.5
Chlorophyll content, %	100	104	58	80

Source: developed by the authors

The results of this stage confirmed that biostimulants considerably improve the physiological parameters of plants under water deficit conditions. Plants

treated with biostimulants (Subgroup 2.1) maintained greater levels of photosynthesis and transpiration, supported antioxidant protection, and accumulated more

osmoprotectors such as proline. This enabled them to withstand stress more effectively and maintain physiological stability. The next stage of the study was to investigate the changes in pea adaptation mechanisms that occurred under the influence of biostimulants in conditions of water deficit. This stage was aimed at understanding how these products affect the internal processes of plants that ensure their resistance to drought, and what physiological and biochemical changes contribute to improved adaptation.

To start with, the study analysed osmotic regulation in plants. This process is one of the key mechanisms that enables plants to maintain water balance even under conditions of limited access to moisture. In control plants that were not treated with biostimulants, a considerable disturbance of osmotic balance was observed, which was manifested in a decrease in cell turgor and wilting of leaves. At the same time, Subgroup 2.1 showed significantly better osmotic regulation. This was achieved due to an increase in the accumulation of osmoprotectants, specifically proline and soluble sugars, which act as osmoregulators, stabilising the water balance in cells. After analysing osmotic regulation, the next step was to study morphological changes in plants. The focus was on the development of the root system, as the roots are the key organ that provides plants with access to water from deep soil layers. Under conditions of water deficit, Subgroup 2.2, which was not treated with biostimulants, experienced a decrease in root length and weight. This limited the ability of plants to effectively absorb moisture from the soil, which substantially affected adaptation to stressful conditions. In the control group with optimum moisture conditions, no such changes were observed. At the same time, in Subgroup 2.1, the root system developed more actively. It was longer and had a greater total weight, which indicated a stimulating effect of biostimulants on root growth even under stressful conditions. These data confirmed that the improvement in water uptake due to biostimulant treatment provided plants with sufficient water to support the basic physiological processes necessary for growth and development (Franzoni *et al.*, 2022).

Additionally, changes in leaf structure were analysed. Plants in Subgroup 2.2, which were exposed to water deficit conditions, showed a decrease in leaf area and density, which is a typical adaptive response to drought. In Subgroup 2.1, which was grown under optimum moisture conditions, no such changes were recorded. The leaves retained a larger area and density, which ensured more efficient absorption of sunlight for photosynthesis. This enabled the plants to maintain a greater level of metabolic activity even in conditions of water deficit, based on the data (Chaski & Petropoulos, 2022). One of the key aspects of the study was to examine changes in the antioxidant defence of plants. During drought, reactive oxygen species accumulate in plant cells, which can cause damage to cell membranes, proteins, and DNA. To neutralise these harmful compounds, plants activate antioxidant mechanisms. In plants, the activity of antioxidant enzymes such as SOD and CAT was insufficient to effectively protect against oxidative stress. This led to extensive damage to cellular structures. In plants treated with biostimulants 2.1, the activity of SOD and CAT increased by 1.5-2 times compared to control plants. This reduced the level of oxidative stress and preserved the integrity of cell membranes.

The next step was to study changes in the content of stress hormones, specifically abscisic acid. In the control plants, the concentration of abscisic acid was considerably higher, indicating a strong response to drought. At the same time, in plants treated with biostimulants, the level of abscisic acid was lower, indicating the ability to better adapt to stress without excessive activation of stress mechanisms. This confirmed the regulatory role of biostimulants in mitigating the adverse effects of drought (Ma *et al.*, 2022). The study ended with an assessment of the general condition of the plants. The Subgroup 2.2 showed signs of severe stress, such as wilting, stunting, and reduced productivity. At the same time, in Subgroup 2.1, these signs were less pronounced, and the general condition stayed stable. This indicated that biostimulants considerably increased plant resistance to drought by activating adaptive mechanisms (Table 4).

Table 4. Changes in pea adaptation mechanisms under the influence of biostimulants

Parameter	Control Subgroup 2.2 (water deficit)	Treated Subgroup 2.1 (water deficit)	Changes in the treated group, %
Level of osmoprotectants	100	170	+70
Root length, cm	15	25	+67
SOD and CAT activity, %	100	200	+100
Absciscic acid level	150	100	-33
General condition of plants (score)	3	8	+166

Source: developed by the authors

The findings of this stage revealed that biostimulants activate various adaptive mechanisms of peas, such as improving osmotic regulation, stimulating the

development of the root system, increasing antioxidant protection, and reducing the level of stress hormones. As a result, the plants adapted more effectively to

water shortages, maintaining the stability of physiological processes and general condition. This confirmed the high effectiveness of biostimulants in improving plant resistance to drought conditions. The study demonstrated that the use of biostimulants has a comprehensive positive effect on peas under water deficit conditions. The effect of the preparations was manifested in the improvement of physiological, biochemical, and morphological parameters of plants, which played a key role in adaptation to stressful conditions. The findings showed that the biostimulants activated natural plant defence mechanisms, reducing the effects of stress and maintaining productivity.

One of the key mechanisms activated by biostimulants was the regulation of photosynthetic activity. In plants that did not receive biostimulants, water deficit led to a substantial reduction in photosynthesis due to gas exchange restrictions and chloroplast dysfunction. At the same time, in the treated plants, this process stayed stable, with a decrease in activity of only 18% compared to optimum conditions. This helped to save energy for growth and development even under stressful conditions. Transpiration played a significant role in maintaining the water balance. In the control group, plants reduced transpiration by 50% in an attempt to minimise water loss, but this also negatively affected the photosynthesis. In the plants treated with biostimulants, the level of transpiration decreased by only 25%, which enabled them to maintain the water balance and ensure efficient gas exchange.

One of the key findings of the study was an increase in the antioxidant defence of plants under the influence of biostimulants. During drought, reactive oxygen species accumulate in plants, causing damage to cell membranes, proteins, and DNA. In control plants, the activity of antioxidant enzymes such as SOD and CAT was insufficient to protect cells from oxidative stress. In plants treated with biostimulants, the activity of these enzymes doubled, which ensured the protection of cellular structures and reduced the level of oxidative stress. Osmoregulation, which is a vital component of adaptation to water deficit, was also greatly improved in plants treated with biostimulants. These plants significantly increased the level of proline, an osmoprotectant that helps cells store water and maintain the stability of enzymatic processes. In the control group, the level of proline increased by only 50% compared to optimum conditions, while in the treated plants, this indicator increased by 70%.

Morphological changes were also significant. In the control plants, the development of the root system was limited, which reduced their ability to obtain water from deep soil layers. In contrast, in plants treated with the biostimulants, the length of the roots increased by 67%, which ensured access to water even in drought conditions. The leaves of the treated plants also retained a larger area and density, which contrib-

uted to more efficient photosynthesis. These changes resulted in maintaining pea productivity even under stressful conditions. In the control group, the number of beans decreased by 46%, while in the treated plants this figure decreased by only 23%. The average weight of beans in the control plants decreased by 40%, while in the treated plants – only by 16%. This showed that biostimulants not only improved plant adaptation to drought but also ensured stable productivity.

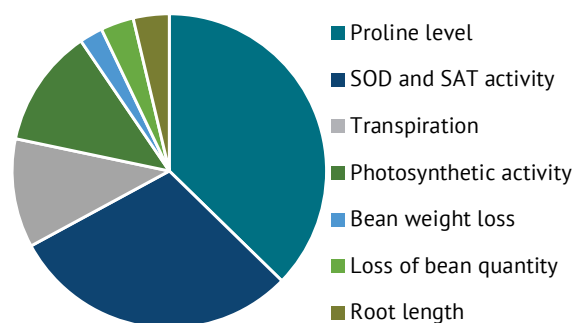


Figure 1. Distribution of key physiological and biochemical parameters that influenced the adaptation of peas to stressful conditions after the use of biostimulants
Source: developed by the authors of this study

The largest contribution belonged to the activity of antioxidant enzymes (SOD and CAT) and proline levels, which are considerably greater than those of the control group. This indicates the significance of these parameters in reducing oxidative stress and maintaining plant water balance (Sattar *et al.*, 2024). Photosynthetic activity and transpiration also account for a considerable share, ensuring the stability of basic physiological processes even under stressful conditions. Root length, although accounting for a smaller share, reflects improvements in morphological characteristics that enable plants to obtain water more efficiently. Losses in the number and weight of beans were the least pronounced, suggesting that the plants treated with biostimulants retain their productivity. This highlights the effectiveness in maintaining vital functions and yields even under water deficit conditions (Iacomassi *et al.*, 2022). The figure demonstrated that biostimulants provide a complex effect by activating interrelated plant adaptive mechanisms.

DISCUSSION

The findings of the study confirmed that the use of biostimulants considerably increases the resistance of peas to water deficit by activating physiological, biochemical, and morphological adaptation mechanisms. Photosynthetic activity, which underlies plant productivity, was substantially reduced in the control group due to gas exchange restrictions. In plants treated with biostimulants, this indicator stayed stable, suggesting the ability to maintain chloroplast function and support

the synthesis of organic matter even under stressful conditions. The biostimulants also provided optimum regulation of transpiration, enabling plants to maintain water balance without substantially limiting gas exchange. Control plants that had a sharp decrease in transpiration showed major difficulties in maintaining the required level of photosynthesis, which adversely affected their growth and development.

Biochemical parameters also confirmed the effectiveness of the biostimulants. The high proline levels in the treated plants protected cells from dehydration, maintaining the stability of physiological processes. The activity of antioxidant enzymes such as SOD and CAT was significantly greater in the treated plants, which reduced oxidative stress and prevented damage to cell membranes. Morphological changes, specifically the development of the root system, suggest that the biostimulants stimulated the formation of longer and more massive roots (Burdina & Priss, 2016; Myronycheva *et al.*, 2017). This helped the plants to obtain moisture from deeper soil layers, ensuring a stable water balance (Yeraliyeva *et al.*, 2017). The findings of the study on the impact of biostimulants on pea adaptation mechanisms under water deficit conditions are consistent with and complement the findings of other researchers. However, in many respects, this study has a deeper level of detail, especially in the assessment of physiological, biochemical, and morphological changes. V. Mazur *et al.* (2022) focused on the technological methods of growing peas in the Right-Bank Forest-Steppe of Ukraine. The researchers confirmed the value of maintaining photosynthetic activity to ensure pea yields. They concluded that optimisation of agronomic conditions, specifically soil moisture, is crucial for plant adaptation. The present study complemented these findings by demonstrating that the use of biostimulants further increases photosynthetic activity even under water deficit conditions, which was not detailed in V. Mazur *et al.* was not considered in detail. M.V. Peresunko (2021) studied the influence of technological methods on the grain productivity of peas. The researcher's experiment revealed that the level of productivity substantially depends on the methods of growing and processing crops. At the same time, the study did not analyse biochemical parameters such as proline levels or antioxidant enzyme activity. This study showed that biostimulants affect not only productivity, but also key internal mechanisms that contribute to stress adaptation. M.P. Saltan (2020) analysed the effects of microfertilisers on maize productivity, which is analogous to the present study in the context of the effects of agrotechnology on plant adaptation. The researcher found that fertiliser application reduces yield losses, but the study did not address the mechanisms underlying this adaptation. The present study took a deeper look at the impact of biostimulants, demonstrating that the effectiveness is conditioned by improved antioxidant defence and osmotic regulation.

In their review of unmanned agricultural machinery, B. Khokhlov *et al.* (2023) focused on innovative methods of increasing the productivity of the agricultural sector. The researchers noted that modern technologies, including automated monitoring systems, helped to optimise crop cultivation by precisely controlling environmental parameters such as soil moisture and plant health. This approach is consistent with the current use of automated soil moisture monitoring systems. However, B. Khokhlov *et al.* focused mainly on technical aspects, while the present study complemented this by analysing the biological mechanisms of pea adaptation to water deficit. Specifically, they demonstrated how biostimulants contribute to the preservation of photosynthetic activity, root development, and antioxidant enzyme activity, which was not considered in the researchers' review. S.O. Butenko (2022) analysed the influence of growth regulators with anti-stress effect on the productivity of white mustard in the northeastern Forest-Steppe of Ukraine. The researcher emphasised that the use of such products can substantially reduce yield losses under stressful conditions. The similarity between the present study and S. Butenko's research lies in the investigation of the effects of biological products on plant adaptation to stressful conditions. However, the present study had a wider range of analysis. It not only assessed plant productivity, but also examined internal mechanisms of adaptation, such as increased proline levels, antioxidant enzyme activity, and transpiration regulation. This helped to better understand how biostimulants affect plant resistance to water deficit. S.V. Didichenko and N.V. Telekalo (2020) considered the technological aspects of increasing crop productivity. The researchers emphasised that optimisation of agronomic practices, including the choice of fertilisers and cultivation methods, is crucial for the adaptation of crops to adverse conditions. The findings of these researchers are useful in the context of managing external factors. The present study went beyond general agronomic aspects by detailing the physiological and biochemical mechanisms underlying adaptation. For example, the present study revealed that biostimulants activate plant antioxidant defences by increasing SOD and CAT activity, which was not covered in the researchers' study.

O.O. Tsokalo (2022) focused on the significance of improving cultivation technologies to maintain plant productivity in the face of climate change. The researcher emphasised that adaptation of crop production requires both optimisation of agricultural technologies and introduction of innovative methods. The present findings confirmed the conclusions of O.O. Tsokalo and considerably supplemented them by the analysis of the effects of biostimulants on osmoregulation and antioxidant protection of plants. The data revealed that by increasing the level of proline and CAT and SOD activity, plants can counteract

stress more effectively. A.Y. Romanko (2021) investigated the development of soybean productivity depending on the technological methods of cultivation. The researchers emphasised the significance of applying microfertilisers and growth regulators to maintain yields under adverse conditions. The present study is consistent with these findings but extended them by evaluating concrete physiological and biochemical processes that affect plant adaptation to water deficit. For instance, this study demonstrated that biostimulants promote the development of the root system, which is critical for maintaining water balance under stressful conditions.

The study confirmed that the use of biostimulants provides a comprehensive effect on the resistance of peas to water deficit. The uniqueness of the findings lies in the identification of the relationship between the increase in photosynthetic activity, activation of antioxidant defence, optimisation of transpiration and development of the root system. Comparison with other studies demonstrated that the present study expands the existing knowledge by supplementing it with an analysis of physiological and biochemical mechanisms of adaptation. This emphasised the value of biostimulants as an innovative tool for increasing crop productivity under stressful conditions.

CONCLUSIONS

The study conducted a comprehensive analysis of the effects of biostimulants on the physiological, biochemical, and morphological adaptation mechanisms of pea (*Pisum sativum* L.) under water deficit conditions. Particular attention was paid to the assessment of photosynthetic activity, transpiration, antioxidant enzyme activity, osmotic regulators, and morphological changes in plants. The findings provided a comprehensive understanding of how biostimulant treatment contributes to increased crop resistance and productivity under stressful conditions. The present study also confirmed that biostimulants positively affect the plant physiological processes. Specifically, the photosynthetic activity of the treated plants stayed at 82% of optimum conditions even under water deficit, which indicates the stability of chloroplast functioning and carbon dioxide assimilation processes. This helped the plants to maintain the synthesis of organic compounds and meet the energy needs for growth and development. The level of

transpiration in plants treated with biostimulants was 75%, indicating effective regulation of water evaporation to maintain water balance.

Biochemical parameters revealed that biostimulants activate adaptive mechanisms at the molecular level. The activity of antioxidant enzymes (SOD and CAT) was greater in the treated plants, which helped to reduce the level of oxidative stress caused by the accumulation of reactive oxygen species. The increased level of proline, which increased to 2.5 relative units, contributed to osmoprotection of cells, reducing their dehydration and maintaining turgor. Morphological studies showed that biostimulants considerably improve the development of the root system. The length and weight of the roots of plants treated with biostimulants were greater, which allowed for better absorption of moisture from deep soil layers. This ensured a stable water supply even in conditions of water shortage, which was not achieved in the control subgroup.

Productivity was also significantly greater in plants treated with biostimulants. Losses in the number and weight of beans under stressful conditions were considerably lower than in the control subgroup. The treatment with biostimulants reduced the yield decline, while maintaining stability even under unfavourable conditions. This confirmed that biostimulants not only preserve the vital activity of the crop but also contributed to the stable formation of the yield. The study emphasised the value of an integrated approach that factors in the physiological, biochemical, and morphological aspects of biostimulants. The findings demonstrated that the use of biostimulants has great potential to increase crop resilience to climate change and ensure stable productivity.

At the same time, the experiments were conducted under controlled conditions, which limited the extrapolation of the findings to field conditions with multiple stressors. Further research should focus on long-term field experiments, considering the interaction of biostimulants with other agricultural technologies to create integrated solutions in arid regions.

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CONFLICT OF INTEREST

None.

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Вплив біостимуляторів на фізіологічні процеси, продуктивність та якість врожаю гороху в умовах сучасного землеробства

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Анотація. Метою дослідження було вивчення впливу біостимуляторів на підвищення адаптаційних механізмів гороху (*Pisum sativum* L.) в умовах дефіциту води, з акцентом на оптимізацію фізіологічних функцій, біохімічних процесів та морфологічного розвитку рослин. Експеримент проводився у контрольованих умовах, які дозволяли точно моделювати вплив водного стресу. У рамках дослідження використовувалися комплексні методи оцінки, що включали фізіологічні показники (фотосинтетична активність, транспірація), біохімічні маркери (активність антиоксидантних ферментів, рівень проліну) та морфологічні параметри (довжина і маса кореневої системи). Результати підтвердили, що обробка біостимуляторами значно підвищувала адаптаційний потенціал рослин. Фотосинтетична активність у рослин, які отримували обробку, становила 82 %, що суттєво перевищувало показник контрольної групи, де цей рівень досягав лише 60 %. Транспірація в оброблених рослин залишалася стабільною, забезпечуючи оптимальний водний баланс навіть за умов стресу. Біохімічний аналіз показав, що активність супероксиддисмутази і каталази у рослин, оброблених біостимуляторами, зросла на 145 % порівняно з контрольними, що сприяло значному зниженню оксидативного стресу. Рівень проліну, як ключового осмотичного регулятора, також був значно вищим у оброблених рослин, що підтримувало стабільність клітинного водного балансу. Морфологічні дослідження виявили, що рослини, оброблені біостимуляторами, мали розвиненішу кореневу систему: довжина коренів була більшою на 10 см, а маса перевищувала контрольні показники. Це дозволяло рослинам використовувати вологу з глибших шарів ґрунту, що покращувало їхню стійкість до посухи. У підсумку втрати врожайності у рослин, які отримували обробку, були вдвічі меншими, ніж у контрольній групі. Отримані результати підкреслюють ефективність використання біостимуляторів для підвищення стійкості гороху до водного дефіциту та збереження продуктивності. Це робить запропонований підхід перспективним для впровадження в агротехнології, особливо у посушливих регіонах, де дефіцит вологи є основним обмежувальним фактором для сільськогосподарських культур

Ключові слова: адаптація рослин; абіотичні фактори; стресостійкість; транспірація; водний баланс



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Transformation of soil fertility under long-term irrigation with mineralised water

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Abstract. Irrigation with saline water is a challenging yet necessary practice for maintaining sustainable crop production in arid and semi-arid regions, particularly under conditions of climate change and freshwater scarcity. Given the potentially harmful effects of saline water on soil properties, particularly fertility, the primary aim of this study was to determine the extent of impact effects on dark chestnut soils in southern Ukraine, resulting from continuous irrigation of croplands with mineralised water from the Inhulets irrigation system. The Inhulets irrigation water is characterised by a high sodium content (10.49-21.63 me/L), elevated levels of toxic ions (11.83 21.97 me/L), and a high total dissolved solids content (1489-2280 mg/L). The research was conducted between 2003 and 2018 in the Bilozerka District of the Kherson Region. Soil fertility was assessed using the following indicators: humus content and nitrogen, phosphorus, and potassium concentrations. Changes in soil traits were evaluated for statistical significance at $P < 0.05$ using the Mann-Kendall trend test and cumulative sums of change methods. The findings revealed a statistically significant trend towards a decline in soil humus content (-0.73%; Z-statistic: 2.0381). Conversely, slight but statistically insignificant increasing trends were observed in nitrogen, phosphorus, and potassium concentrations (+9, +2, and +65 mg/kg, respectively; Z-statistics: 0.6794, 0, and 0, respectively). Notably, positive changes were primarily observed after 2013, whereas the earlier period was predominantly characterised by declining soil fertility across all studied indicators. The study's outcomes hold significant practical value as they highlight the long-term effects of continuous irrigation with mineralised water on soil fertility. These findings provide crucial insights into land reclamation and agrochemical measures necessary to mitigate soil degradation and ensure sustainable agricultural practices in vulnerable agroecosystems

Keywords: humus; land reclamation; nitrogen; phosphorus; potassium; salinity

INTRODUCTION

Current challenges in agriculture are predominantly associated with food security, which is increasingly under threat due to global population growth, degradation of natural resources, environmental pollution, climate change, irrational anthropogenic activities, and, notably, the intensification of military conflicts. Ensuring an adequate supply of food, both in terms of quantity and quality, to meet the growing nutritional demands has become an increasingly complex task each successive year. Irresponsible human activities and inefficient utilisation of natural resources – such as soil, water, biological organisms, and meteorological resources inputs – have not only significantly worsened global ecological conditions but also caused a severe decline in agricultural land productivity (Wolski *et al.*, 2020). Coupled with climate change and its associated impacts, including unstable weather patterns and an increased frequency of extreme meteorological events – such as prolonged droughts, hailstorms, flooding, and rapid, unpredictable changes in air temperature – this situation has undermined the sustainability of agroecosystems. Consequently, food insecurity has worsened in the most vulnerable regions, such as sub-Saharan Africa, southern Africa, and South Asia, noted by as K. Ouko and M. Odiwuor (2023). Furthermore, the COVID-19 pandemic exacerbated malnutrition in these areas, intensifying food insecurity, particularly in Asia and Africa, as

observed by G. Rasul (2021). To counteract the adverse effects of climate change and meet food demands, it is imperative to develop and implement effective measures for conserving natural resources in agriculture. Soil, as it is the primary environment and fundamental resource for agricultural production, should be prioritised. Moreover, soil quality plays a critical role in enhancing climate resilience, as emphasised by L. Qiao *et al.* (2022). H. El-Ramady *et al.* (2022) highlighted the crucial role of soil fertility in ensuring human well-being. It is evident that soil preserving soil is not only vital for agroecological sustainability but also essential for socio-economic stability. The success or failure of soil preservation efforts will be pivotal in determining the survival of future generations.

Soil conservation is a multifaceted process involving various soil management practices, such as rational tillage, fertilisation, optimal crop rotations, amelioration techniques, and soil microbiota preservation methods. Among the key components of healthy soil are its ameliorative conditions and nutrient status, which are shaped by numerous biological and anthropogenic factors. One significant determinant is the quality of irrigation water, which plays a pivotal role in influencing soil properties. M. Licata *et al.* (2022) confirmed that irrigation can significantly affect soil properties, but its impact depends on the irrigation method,

the quality of the water, the initial soil properties, and the soil's susceptibility to external influences. Irrigation influences soil structure, water permeability, physical and chemical properties, ameliorative conditions, and overall fertility.

Irrigation with saline water, characterised by high levels of total dissolved solids and elevated sodium content, is particularly detrimental to both plants and soils. Risks of secondary salinisation and sodification are further exacerbated by improper tillage and fertilisation practices. The accumulation of toxic salts degrades soil chemical properties and structure, impairs nutrient uptake by plants, and reduces crop yields. When sodium accumulates in the soil, the structure deteriorates further, leading to soil crusting, reduced water infiltration, diminished porosity, and inadequate aeration. V. Phogat *et al.* (2020) and B. Hailu and H. Mehari (2021) warned that these adverse processes hinder the growth and development of agricultural crops and, if not properly managed, can render soils unsuitable for agricultural use. P. Lykhovyd (2021) emphasised that southern Ukraine, located in a semi-arid climatic zone, relies heavily on irrigation for the overall crop production and sustainable development of agriculture. However, a significant portion of land in the region is irrigated with water of suboptimal quality. L. Hranovska *et al.* (2022) noted that the Inhulets irrigated land array, continuously supplied with water from the Inhulets irrigation system, is affected by high levels of total dissolved solids and sodium. In light of the potential hazards of soil degradation and fertility loss, this study aimed to investigate the long-term impacts of irrigation and agricultural activities on soil fertility indicators in the Inhulets irrigated land array.

MATERIALS AND METHODS

The study was conducted between 2003 and 2018 through in-field soil surveys on the Inhulets irrigated land array in the Bilozerka District of Kherson Region, southern Ukraine. Data collected after this period are incomplete due to disruptions caused by the COVID-19 pandemic and military activities in the region. The climate of the study area is characterised as semi-arid, with a deficient natural water balance (Lykhovyd, 2021). The soil in the region is classified as typical dark-chestnut, medium-loamy soil. The Inhulets irrigation system provides water to farms in the Kherson and Mykolaiv regions of Ukraine. To ensure the safety of plants and soil, the water quality requires substantial improvement, as it is contaminated by waste and effluent discharges from metallurgical enterprises. According to the specifications of the national standard DSTU 2730:2015 (2016), the water is classified as "Limited suitable for irrigation" (second class). Similarly, based on international standards and methodologies for agricultural water quality assessment outlined by the FAO (n.d.) and

DSTU 2730:2015 (2016), the Inhulets irrigation water is deemed to have limitations for irrigation use (Pescod, 1992). High levels of sodium (10.49–21.63 me/L), toxic ions (11.83–21.97 me/L), and total dissolved solids (1489–2280 mg/L) are the primary issues associated with the Inhulets irrigation water. The use of such water for irrigation adversely affects the physical, chemical, and biological properties of the soil, leading to a decline in crop growth, productivity, and yield quality (Lykhovyd & Kozlenko, 2018). Consequently, it is crucial to assess the long-term effects of irrigation with such water on soil properties, particularly fertility indicators such as humus and macronutrient content.

Humus content in soil was determined using the Turin method (DSTU 7855:2015, 2016). Nitrogen content was assessed indirectly through the soil nitrification activity method developed by Kravkov (DSTU 7538:2014, 2015). The content of mobile phosphorus and exchangeable potassium was measured using the Machygin method (DSTU 4114-2002, 2003). The presence or absence of statistically significant trends in soil humus and macronutrient content was evaluated using the MannKendall trend test at $P < 0.05$ (Hamed, 2008). Additionally, the cumulative sums of change (CUSUM) algorithm were applied to identify trends and determine points of change in the time series of soil properties (Sabir *et al.*, 2024). All calculations were performed using a self-developed script in Python 3, with the VS Code IDE as the development environment.

RESULTS AND DISCUSSION

Humus is a dynamic and complex mixture of organic compounds formed through the decomposition and humification of plant and animal residues. It constitutes the primary component of overall soil organic matter and is a critical factor in soil fertility, formation, contributing to carbon storage and influencing soil buffer capacity, structure, and microbiota (Gerke, 2022). Irrigation significantly alters the processes of humus formation and related dynamics. The quantitative humus content exhibits zonal characteristics, determined by soil genesis factors such as soil formation type, granulometric composition, vegetation type, and agrotechnological practices (Baliuk *et al.*, 2020). This applies to the dark-chestnut irrigated soils of the Inhulets irrigated land array, as confirmed by the results of the soil survey (Table 1). The findings from the CUSUM analysis indicate a trend towards dehumification (Fig. 1). Long-term irrigation of highly mineralised water has resulted in an overall decline in humus content in the surveyed soils, averaging a reduction of 0.046% per year. The Mann-Kendall trend test confirmed a statistically significant trend of decreasing humus content, with a Z-statistic of 2.0381. Between 2003 and 2018, the weighted average humus content in the land array fell by 0.73%.

Table 1. Weighted average indicators of humus content in irrigated soils in the Bilozerka District of Kherson Region (Inhulets irrigated land array, 2003-2018)

Year	Humus, %	Gradation	± to previous
2003	2.43	Moderate	-
2008	2.34	Moderate	-0.09
2013	2.16	Moderate	-0.18
2018	1.70	Low	-0.46

Source: compiled by the authors

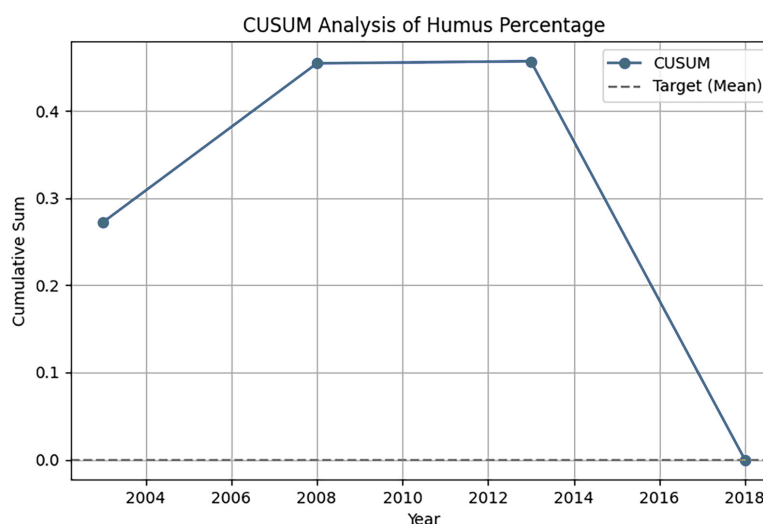


Figure 1. CUSUM plot for humus content in irrigated soils in the Bilozerka District of Kherson Region (Inhulets irrigated land array, 2003-2018)

Source: compiled by the authors

Continuous irrigation with highly mineralised water has led to a decline in soil areas with high or moderately high humus content, resulting in their reclassification into categories of moderate and low humus content (Table 2). The spatial variability of

soil properties is primarily characterised by non-stationary (atypical) patterns in their distribution across irrigated agricultural landscapes. This variability is largely influenced by agricultural practices and soil biodiversity levels.

Table 2. Comparative assessment of irrigated and non-irrigated lands in the Bilozerka District of Kherson Region (Inhulets irrigated land array), by humus content, %

Land array	Area, ha	Area ordered by the humus content												Weighted average indicator, %	
		Very low < 1.1%	Low				Moderate		Moderately high		High		Very high > 5.0%		
		1.1-2.0%		2.1-3.0%		3.1-4.0%		4.1-5.0%							
		ha	%	ha	%	ha	%	ha	%	ha	%	ha	%		
2013 year															
Total areas	98,226.6	368.8	0.4	58,538.8	59.6	37,239.2	37.9	2,079.8	2.1	-	-	-	-	2.06	
Non-irrigated arable land	83,368.5	-	-	50,559.3	51.5	31,153.5	31.7	1,655.7	1.7	-	-	-	-	2.06	
Irrigated arable land	11,109.5	-	-	5,102.2	5.2* 45.9**	5,689.9	5.8 51.2	317.5	0.3 2.9	-	-	-	-	2.16	
2018 year															
Total areas	96,853.5	3,294.2	3.4	61,220.2	63.2	27,746.7	28.6	4,430.8	4.6	12.9	0.0	148.8	0.2	1.97	
Non-irrigated arable land	88,401.9	2,321.7	2.4	55,341.8	57.1	26,189.3	27.0	4,387.5	4.5	12.9	0.0	148.8	0.2	2.00	
Irrigated arable land	6,206.4	592.6	0.6 9.6	4,412.6	4.6 71.01	1,190.4	1.2 19.2	10.8	0.0 0.2	-	-	-	-	1.70	

Note: * – share of the total surveyed land; ** – share of the surveyed irrigated land

Source: compiled by the authors

The characteristics of the soil cover determine the initial humus content, which, during economic activity, undergoes dynamic changes influenced by the intensity and level of agriculture within land plots. Under conditions of continuous irrigation with highly mineralised waters from the Inhulets irrigation system, the humus content is, on average, 0.3% lower than in non-irrigated lands. This reduction results from the intensity and technological aspects of irrigated land reclamation, including poor water quality, irrigation rates, and crop rotation practices.

Dehumification of soils under continuous irrigation with saline water can be attributed to the increased mineralisation of organic matter due to intensive cultivation, imbalance in production and soil-forming processes, inadequate incorporation of crop residues and organic fertilisers into the arable layer, a rise in fallow crops, a decline in the share of perennial grasses, and the absence of field crop rotations. Prolonged unilateral application of mineral fertilisers particularly physiologically acidic forms, insufficient use of plant residues as green manure, and burning of stubble and crop residues, exacerbate this issue. These factors contribute to erosion, including irrigation-induced soil erosion and deflation. Changes in humus content also occur due to long-term irrigation practices (Vozhehova, 2021).

Another important constituent of soil health and fertility is the content of plant-available micronutrients

such as nitrogen, phosphorus, and potassium. Nitrogen plays a crucial role in enhancing crop yields. It is a component of proteins, which constitute the cytoplasm and cell nucleus, as well as amino acids, nucleic acids, chlorophyll, alkaloids, phosphatides, vitamins, enzymes, and other biologically active substances essential for normal plant growth and development (Toor *et al.*, 2021). The primary sources of nitrogen for plants include organic and mineral fertilisers, soil organic matter, biological nitrogen fixation, and nitrogen introduced through atmospheric precipitation. The nitrogen nutrition regime of plants in the soils of southern Ukraine is a critical characteristic of soil fertility, with nitrogen often being the most limiting nutrient. The ammonium nitrogen content in soils serves as an indicator of soil management practices and is an indirect measure of soil fertility. Additionally, higher soil nitrifying activity is regarded as a sign of fertile soil (Gamajunova *et al.*, 2021).

Under continuous irrigation, the dark-chestnut soils of the Inhulets irrigated land array exhibit an increase in the content of available nitrogen over time. CUSUM analysis indicates a trend towards nitrogen content increment in the examined soils following its sharp decline before 2013 (Fig. 2). However, this trend is statistically insignificant, according to the Mann-Kendall trend test, with a Zstatistic of 0.6794 (Table 3).

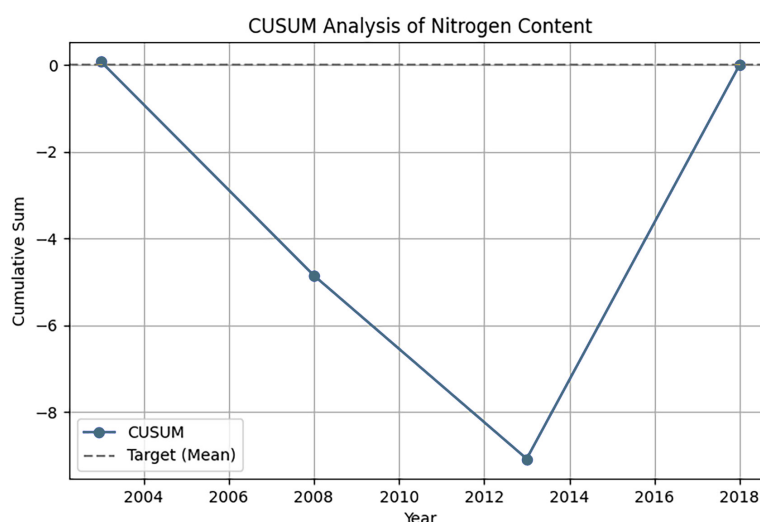


Figure 2. CUSUM plot for nitrogen content in irrigated soils in the Bilozerka District of Kherson Region (Inhulets irrigated land array, 2003-2018)

Source: compiled by the authors

Table 3. Weighted average indicators of nitrogen content in irrigated soils in the Bilozerka District of Kherson Region (Inhulets irrigated land array, 2003-2018)

Year	Nitrogen, mg/kg	Gradation	± to previous
2003	27.0	Moderately high	
2008	22.0	Moderately high	-5.0
2013	22.7	Moderately high	+0.7
2018	36.0	High	+13.3

Source: compiled by the authors

The nitrogen content in the irrigated soils, which corresponds to quality gradations ranging from moderate to high (8.1-30.0 mg/kg), accounts for 37.0% of the agricultural land area. The largest share (59.2%)

consists of areas with high nitrogen content (Table 4). The weighted average nitrogen content under continuous irrigation is higher than that of non-irrigated lands.

Table 4. Comparative assessment of irrigated and non-irrigated lands in the Bilozerka District of Kherson Region (Inhulets irrigated land array), by nitrogen content, mg/kg

Land array	Area, ha	Areas of soils ordered by nitrogen content												Weighted average indicator, mg/kg
		Very low		Low		Moderate		Moderately high		High		Very high		
		< 5.0		5.1-8.0		8.1-15.0		15.1-30.0		30.1-60.0		> 60.0		
		ha	%	ha	%	ha	%	ha	%	ha	%	ha	%	
2013 year														
Total areas	98,226.6	4,195.8	4.3	11,139.6	11.3	38,021.8	38.7	29,760.0	30.3	14,324.5	14.6	785.0	0.8	18.0
Non-irrigated arable land	83,368.5	2,997.7	3.6	10,468.4	12.6	32,510.2	39.0	26,145.2	31.4	10,847.5	13.0	399.6	0.5	17.4
Irrigated arable land	11,109.5 100	110.8	1.0	396.5	3.6	4,739.0	42.7	2,549.0	22.9	2,936.7	26.4	377.5	3.4	22.7
2018 year														
Total areas	96,853.5	305.4	0.3	581.6	0.6	9,229.3	9.5	40,260.6	41.6	41,523.5	42.9	4,953.2	5.1	31.7
Non-irrigated arable land	88,401.9	86.9	0.1	24.0	0.0	8,511.7	9.6	37,552.4	42.5	37,480.5	42.4	4,746.5	5.4	31.8
Irrigated arable land	6,206.4	34.0	0.5	0.0	0.0	158.3	2.6	2,132.9	34.4	3,674.5	59.2	206.7	3.3	36.0

Source: compiled by the authors

Phosphorus is one of the most important elements in plant nutrition. After organic matter and nitrogen, phosphorus is often the most deficient element in crop cultivation. It is a component of nucleoproteins, sugar phosphates, phosphatides, and other compounds, actively participating in metabolic processes of protein synthesis, determining cellular energy dynamics, and influencing plant growth (Toor *et al.*, 2021). A substantial proportion of available soil phosphorus is stored

in soil organic matter. When organic matter is depleted due to intensive tillage, erosion, and unsustainable agricultural practices, phosphorus deficiency becomes a pressing issue. No statistically significant trend was observed in soil phosphorus dynamics under continuous irrigation with mineralised water; the Z-statistic from the Mann-Kendall trend test equals zero (Table 5). However, a slight upward trend was evident in the CUSUM plot after 2013 (Fig. 3).

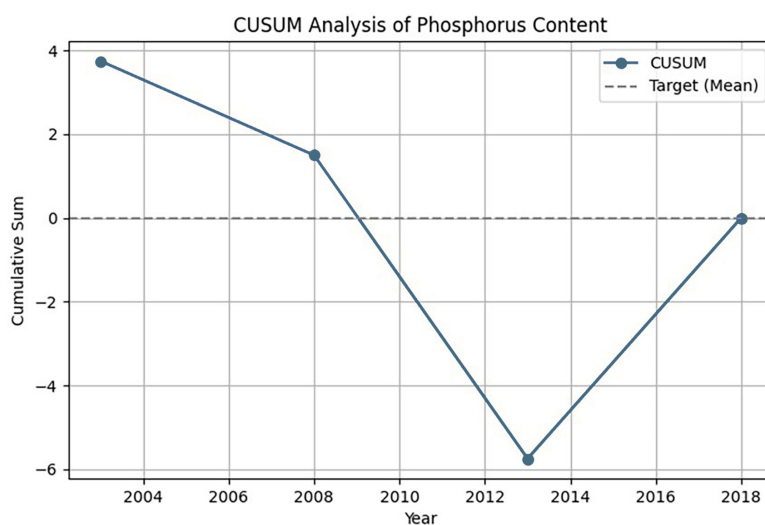


Figure 3. CUSUM plot for phosphorus content in irrigated soils in the Bilozerka District of Kherson Region (Inhulets irrigated land array, 2003-2018)

Source: compiled by the authors

Table 5. Weighted average indicators of phosphorus content in irrigated soils in the Bilozerka District of Kherson Region (Inhulets irrigated land array, 2003-2018)

Year	Phosphorus, mg/kg	Gradation	± to previous
2003	62.0	Very high	-
2008	56.0	High	-6.0
2013	51.0	High	-5.0
2018	64.0	Very high	+13.0

Source: compiled by the authors

Very high phosphorus content (> 60.0 mg/kg) characterises 40.6% of the irrigated land area. High phosphorus content (46-60 mg/kg) accounts for 15.48% of the area, while moderately high phosphorus levels (31-45 mg/kg) represent 20.89% of

the surveyed area (Table 6). The weighted average content of mobile phosphorus compounds under continuous irrigation with mineralised waters of the Inhulets irrigation system is higher than in non-irrigated lands.

Table 6. Comparative assessment of irrigated and non-irrigated lands in the Bilozerka District of Kherson Region (Inhulets irrigated land array), by the content of mobile phosphorus compounds

Land array	Area, ha	Areas of soils ordered by phosphorus content						Weighted average indicator, mg, kg
		Very low < 11	Low 11-15	Moderate 16-30	Moderately high 31-45	High 46-60	Very high > 60	
		ha	ha	ha	ha	ha	ha	
2013 year								
Total areas	98,226.6	120.6	2,293.1	24,773.6	23,911.7	14,450.1	32,677.5	48
Non-irrigated arable land	83,368.5	63.6	1,999.6	21,664.6	20,856.0	12,256.2	26,528.5	47
Irrigated arable land	11,109.5	<u>57.0</u> 0.51	<u>285.6</u> 2.57	<u>2,211.7</u> 19.91	<u>2,321.3</u> 20.89	<u>1,720.0</u> 15.48	<u>4,514.0</u> 40.63	51
2018 year								
Total areas	96,853.5	0.0	133.1	6,172.1	11,124.8	9,336.3	70,087.2	65
Non-irrigated arable land	88,401.9	0.0	133.1	5,820.2	9,932.0	9,052.9	63,463.7	65
Irrigated arable land	6,206.4	0.0	0.0	351.9	1,153.8	283.4	4,417.3	64

Source: compiled by the authors

The most effective measure for replenishing the mobile phosphorus content in irrigated soil is the application of phosphorus-containing mineral fertilisers. Phosphorus in mineral fertilisers, under certain environmental conditions, is more mobile and available to plants than that in organic fertilisers. Therefore, rational phosphorus fertilisation is critical for maintaining a positive balance of this essential nutrition in the soils irrigated with mineralised waters. In addition to the direct input of phosphorus through fertilisation, it is possible to increase the mobile forms of this element in soil by employing phosphorus-mobilising bacterial strains. These strains not only enhance soil fertility but also improve overall soil health and promote environmental sustainability (El Attar *et al.*, 2022).

Potassium plays an essential role in the life cycle of crops. It indirectly participates in nitrogen metabolism, influences the accumulation of amino acids and energy processes, and regulates respiration. The presence of various forms of potassium in soils is associated with primary and secondary minerals, as well as the characteristics of their transformations.

Potassium is the most mobile element, migrating both into plants and within the soil. The total volume of potassium in the soils of southern Ukraine is typically much higher than that of nitrogen or phosphorus. The richer the soil is in clay and organic matter, the higher its total potassium content. The total potassium content, along with its mobile forms (water-soluble, exchangeable, and non-exchangeable), determines the potassium regime of the soil. The primary source of potassium available to plants is exchangeable potassium, which is stored in the soil adsorption complex (Toor *et al.*, 2021). The content of exchangeable potassium in irrigated soils is strongly influenced by natural zonal features. Different soil types are characterised by varying amounts of exchangeable potassium. More potassium is typically found in heavy clay and loamy soils, whereas sandy and sandy loamy soils are characterised by significantly lower potassium content (Breus & Skok, 2021).

Despite a notable increase in microelement content in 2018 (Table 7), the Mann-Kendall test did not identify any significant trend in the content of

exchangeable potassium (Z-statistic is zero). Figure 4 illustrates a steep increase in potassium content in the investigated soils after 2013, similar to trends

previously observed for nitrogen and phosphorus. This dynamic change suggests that a significant event in soil amelioration occurred in 2013.

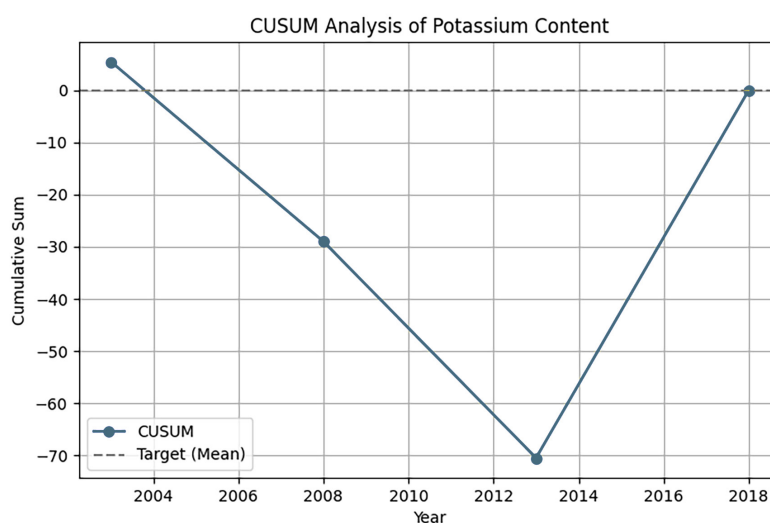


Figure 4. CUSUM plot for potassium content in irrigated soils in the Bilozerka District of Kherson Region (Inhulets irrigated land array, 2003-2018)

Source: compiled by the authors

Table 7. Weighted average indicators of content of exchangeable potassium compounds in irrigated soils in the Bilozerka District of Kherson Region (Inhulets irrigated land array, 2003-2018)

Year	Potassium, mg/kg	Gradation	± to previous
2003	410	Very high	
2008	370	High	-40.0
2013	363	High	-7.0
2018	475	Very high	+112.0

Source: compiled by the authors

The content of exchangeable potassium in soils (Table 8), categorised by gradations, shows that very high content potassium levels (>400.0 mg/kg) characterise 68.1% of the irrigated land area. High potassium content (301-400 mg/kg) accounts for 23.05% of

the area, while moderately high levels (201-300 mg/kg) – represent 8.93% of the surveyed area. Overall, irrigation with Inhulets water did not significantly affect the content of exchangeable potassium in the examined soils.

Table 8. Comparative assessment of irrigated and non-irrigated lands in the Bilozerka District of Kherson Region (Inhulets irrigated land array), by the content of exchangeable potassium

Land array	Area, ha	Areas of soils ordered by potassium content						Weighted average indicator, mg/kg
		Very low < 51	Low 51-100	Moderate 101-200	Moderately high 201-300	High 301-400	Very high > 400	
		ha	ha	ha	ha	ha	ha	
2013 year								
Total areas	98,226.6	0.0	0.0	8,686.8	30,052.9	31,231.2	28,255.8	364
Non-irrigated arable land	83,368.5	0.0	0.0	7,321.9	25,545.5	26,240.3	24,260.7	365
Irrigated arable land	11,109.5	0.0	0.0	406.0	3,593.0	4,130.8	2,979.8	363
2018 year								
Total areas	96,853.5	0.0	6.2	60.3	10,004.3	15,240.8	71,541.9	502
Non-irrigated arable land	88,401.9	0.0	6.2	60.3	9,410.9	13,810.0	65,114.5	503
Irrigated arable land	6,206.4	0.0	0.0	0.0	554.4	1,430.8	4,221.2	475
	100	0.0	0.0	0.0	8.93	23.05	68.01	

Source: compiled by the authors

As a result, it should be stated that the Inhulets irrigation water had a significant effect on the dark-chestnut soils of the Belozerkha District in terms of humus content (a negative tendency towards a decrease) and available nitrogen content (a positive trend towards an increase). No statistically significant trend was observed in mobile phosphorus or exchangeable potassium. Therefore, it can be concluded that mineralised water has a strong adverse effect on soil organic matter accumulation but has little to no significant impact on the availability of mineral macronutrients in soils. However, as general soil salinity increases – particularly when combined with sodium accumulation – the availability of macronutrients from the soil decreases, as plants struggle to absorb nutrients under conditions of high osmotic pressure in the soil solution, and unfavourable soil structure.

A. Mohanavelu *et al.* (2021) claim that approximately one-third of the total irrigated land areas globally are subject to the negative impacts of secondary salinisation and sodification. These issues arise as a consequence of irrigation and, in turn, result in the inhibition of agricultural plant growth and development, as well as a reduction in effective soil fertility and the overall sustainability of agricultural ecosystems. The authors suggest that soil salinity and sodicity pose a significant threat to food security and require the development of effective mitigation strategies, particularly for irrigated lands. Among the possible solutions, A. Mohanavelu *et al.* (2021) recommend improved drainage systems, monitoring of irrigation water usage and quality, and the cultivation of salt-tolerant crops with phytoremediation properties.

In a short-term study, A. Turdaliev *et al.* (2022) also observed no significant effects of irrigation with mineralised water on winter wheat yields or soils. However, they acknowledged that the study spanned only a three-year period and noted some degree of sodium accumulation, which is potentially hazardous due to the risk of secondary soil sodification and the deterioration of its physical and chemical properties. Comprehensive studies confirm the harmful effects of saline water irrigation on plants and soils, resulting in drastic reductions in crop yields and deterioration in soil quality (Cheng *et al.*, 2021). Soil salinity has been shown to increase significantly, even under short-term irrigation with water salinity exceeding 4.0 dS/m, and water salinity levels higher than 4.4 dS/m are deemed unsuitable for irrigation. When no alternative water sources are available, mineralised irrigation water must be used cautiously and accompanied by careful soil monitoring to prevent soil salinisation and sodification (Feng *et al.*, 2021). Amelioration measures, such as gypsum application, rational tillage, and crop rotation systems – particularly those which involve crops with phytoremediation capacities such as alfalfa, chickpea, and melilot – along with irrigation water pretreatment,

are crucial for preserving soil health under conditions of saline water irrigation (Kankarla *et al.*, 2020).

According to K. Negacz *et al.* (2022), approximately 17 million km² of global soil cover is subject to the negative effects of salinity, and only 11.76% of these soils show potential for agricultural use. The greatest future potential for saline agriculture is associated with salt-affected soils in non-depleted basins, with or without irrigation, while the least promising soils are found in depleted basins. Saline water irrigation can exacerbate problems associated with salt-affected soils, reducing water and nutrient availability for agricultural plants and decreasing soil fertility. The metaanalysis conducted by M. Cheng *et al.* (2021) revealed that saline water irrigation leads to crop yield losses and reduced water use efficiency, in addition to posing various hazards to soil health. The most salt-tolerant crops identified were cotton, wheat, and grain maize. As a measure to mitigate the adverse impacts of mineralised water on plants and soil, M. Cheng *et al.* (2021) propose the use of drip irrigation. Furthermore, it is worth noting that certain legumes exhibit significant salt tolerance and some desalination potential. Under conditions where the soil is treated with specific root-zone bacteria, such as growth-promoting rhizobacteria, beneficial effects on soils irrigated with mineralised water have been observed, without significant reduction in yield (Hussain *et al.*, 2020; Hafez *et al.*, 2021).

Although saline water irrigation poses environmental hazards and contributes to crop losses, some scientists argue that under conditions of water scarcity – prevalent in global agriculture over recent decades – irrigation with pre-treated mineralised water could provide a viable solution for ensuring food security. However, this approach would necessitate careful ecological monitoring and crop system diversification within the framework of resource-efficient climate-smart agriculture on marginal croplands.

CONCLUSIONS

As a result of a long-term investigation, it was determined that continuous irrigation with mineralised water results in a significant decrease in soil humus content. From 2003 to 2018, humus content in the investigated dark-chestnut soils decreased from 2.43% to 1.70%, equating to 42.94%. Regarding macronutrients, no general adverse effects were identified. However, for individual nutritious, nitrogen content increased by 9.0 mg/kg (33.33%); phosphorus content increased by 2.0 mg/kg (3.23%); and potassium content increased by 65 mg/kg (15.85%). Nevertheless, the general deterioration of soil structure, along with sodium accumulation and increased osmotic pressure in the soil solution, may reduce the efficiency of nutrient uptake by agricultural plants. Irrigation with saline water should only be conducted if no alternative options are available and must be accompanied by thorough

monitoring of soil health and the implementation of precautionary amelioration measures. These measures should include gypsum application, rational tillage practices, and crop rotation systems. Further studies are required to identify and monitor the long-term effects of continuous irrigation with mineralised water from the Inhulets irrigation system on the soils of the irrigated land array. This research should encompass not only dark-chestnut soils but also other soil types, present in the specified land array. Additionally, beyond fertility parameters, factors such as biological

activity, soil microbiota composition, and the physical, chemical, and mechanical properties of soils must be assessed to provide the most comprehensive evaluation of land conditions and their dynamic changes resulting from irrigation.

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CONFLICT OF INTEREST

None.

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Трансформація родючості ґрунтів під впливом тривалого зрошення мінералізованою водою

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Анотація. Зрошення водами з високим вмістом солей є небезпечною необхідністю для сталого виробництва сільськогосподарських культур у посушливих та напівпосушливих регіонах в умовах зміни клімату та дефіциту прісної води. Враховуючи потенційний шкідливий вплив мінералізованих вод на стан ґрунтів, особливо на їх родючість, основною метою цього дослідження було встановити масштаби негативного впливу на темно-каштанові ґрунти Півдня України, який проявився внаслідок тривалого зрошення орних земель мінералізованою водою з Інгулецької зрошувальної системи. Інгулецька зрошувальна вода характеризується високим вмістом натрію (10,49-21,63 мг-екв./л), великою кількістю токсичних іонів (11,83-21,97 мг-екв./л) та високим загальним вмістом розчинних солей (1489-2280 мг/л). Дослідження проводили впродовж 2003-2018 років у Білозерському районі Херсонської області. Родючість ґрунту оцінювали за такими показниками: вміст гумусу, азоту, фосфору та калію. Достовірність змін ґрунтових ознак визначали при $P < 0,05$ за допомогою тренд-тесту Манна-Кендалла та методу кумулятивних сум змін. В результаті було встановлено, що існує статистично значуща тенденція до зменшення вмісту гумусу в ґрунті ($-0,73\%$; Z-статистика 2,0381); в той же час, для азоту, фосфору та калію спостерігаються незначні, але достовірні тенденції до збільшення (+9, +2, +65 мг/кг відповідно; Z-статистика 0,6794, 0, і 0 відповідно). Варто зазначити, що основна позитивна динаміка припадає на період після 2013 року, оскільки попередній період характеризується переважно зниженням родючості ґрунтів за всіма досліджуваними показниками. Результати дослідження мають високу практичну цінність і важливість, оскільки окреслюють наслідки довготривалої трансформації родючості ґрунтів під впливом безперервного зрошення мінералізованою водою, надають цінну інформацію щодо меліоративних та агрохімічних заходів, які слід враховувати в таких умовах сільськогосподарського землекористування для запобігання деградації ґрунтів у вразливих агроєкосистемах

Ключові слова: гумус; меліорація ґрунту; азот; фосфор; калій; засолення



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Biochemical composition and pharmacological potential of species of the genus *Arenaria* L.

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Abstract. The study aimed to determine the dependence of the content of phenolic compounds, flavonoids and antioxidant activity of *Arenaria* L. species on soil and climatic conditions of the Nakhchivan Autonomous Republic. For this purpose, morphological analysis, high-performance liquid chromatography and spectrophotometry were used, and the data obtained were statistically analysed. The main results showed that the samples from the chernozem soils of the Sharur district had the highest content of phenolic compounds (42.5 ± 3.2 mg/g dry weight) and flavonoids (11.8 ± 1.1 mg/g dry weight), as well as high antioxidant activity, in particular, the IC₅₀ was 23.3 ± 1.8 µg/ml, and the antioxidant capacity of antioxidant equivalent capacity of trolox (AEAC) reached 4.2 ± 0.3 mmol TE/g. Samples from mountainous areas, such as vicinity of Mount Garagush and Chalkhangala villages in Kangarli district, showed the lowest levels: the content of phenolic compounds was 24.5 ± 2.6 mg/g, flavonoids – 6.2 ± 0.5 mg/g, IC₅₀ was 43.8 ± 3.1 µg/ml, and AEAC – 2.2 ± 0.2 mmol TE/g. A clear correlation was determined between the content of biologically active compounds and the chemical composition of the soil, particularly a high content of nitrogen ($0.25 \pm 0.02\%$) and phosphorus (220 ± 15 mg/kg) contributed to the maximum accumulation of these compounds. The practical significance of the study is determined by recommendations for optimal conditions for the cultivation of *Arenaria* L. to obtain plants with a high content of antioxidants. The findings highlight the importance of adapting agronomic approaches to specific environmental conditions to maximise the pharmacological potential of these plants

Keywords: phenolic compounds; flavonoids; antioxidant activity; soil and climatic conditions; bioactive substances; biodiversity

INTRODUCTION

The genus *Arenaria* L. includes plant species that are widely distributed in different climatic zones and have a rich chemical composition. Nevertheless, their biochemical potential and pharmacological properties

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were not sufficiently studied. The study of these plants is relevant due to the possibility of identifying new biologically active compounds that can be used in pharmaceuticals, cosmetics and the food industry. The genus *Arenaria* L., which belongs to the *Caryophyllaceae* family, is represented by approximately 300 species, many of which are narrowly endemic. Species of this genus are widely distributed around the world, but their concentration is highest in mountainous and alpine regions, making them important for biodiversity and conservation. In Southwest Asia, the genus is highly endemic. For instance, *Arenaria kandavanensis*, found in northern Iran, is a highly endemic species restricted to the Kandovan Mountains. This species is included in the section *Eremogone* and is being studied to clarify its taxonomic status and develop keys to identify related species (Lazkov & Sennikov, 2020).

The species *A. graminea* C.A. Mey, which is included in the flora of the Nakhchivan Autonomous Republic, was included in the list of subendemics of the Republic of Azerbaijan. Another example is *Arenaria bulica*, a rare species rediscovered in the Zagros Mountains after 139 years. This discovery highlighted the importance of systematically revisiting specimens and updating data on endemic species. Two other species, *A. minutissima* and *A. semiromica*, were found to be synonyms of *A. bulica*. In addition, a distribution map of this species was created, showing its limited geographical distribution (Pahlevani & Falatoury, 2024). In the Mediterranean, *Arenaria* L. *balearica* is also an endemic species, distributed on islands such as Mallorca, Corsica, and Sardinia. Studies of this species have shown that due to climate change, the habitat suitable for its existence may disappear by 2070, which threatens its conservation (Bobo-Pinilla *et al.*, 2020).

As for the works of other authors, the study by G. Kozłowski *et al.* (2022) analysed the genome size of species of the *Arenaria ciliata* complex, particularly the endemic subspecies *A. ciliata* subsp. *bernensis* from the Swiss Alps. The scientists used flow cytometry and analysed 16 populations comprising 77 individuals. They confirmed a very high level of ploidy in this subspecies ($2n = 20x = 200$), which significantly distinguishes it from other taxa in the group. The conclusions point to the need to recognise this subspecies as a separate species due to its ecological adaptation and threat from climate change. Gaps relate to the lack of information on the molecular mechanisms of adaptation to different habitats. Y. Cui *et al.* (2020) investigated the therapeutic potential of *Arenaria* L. *kansuensis* in pulmonary fibrosis. The authors used a mouse model of fibrosis and determined that *A. kansuensis* extract significantly reduced inflammation, lung tissue damage and oxidative stress. These effects were found to be mediated through activation of the Nrf2 pathway and inhibition of the NF- κ B/TGF- β 1/Smad2/3 pathway. Gaps include a lack of research on long-term effects and potential side effects.

C. Doğan and N. Çakır (2019) analysed the pollen morphology of 16 species of *Arenaria* L. in Turkey. Light and scanning electron microscopy were used to study the pollen structure, in particular the size, number of pores and surface ornamentation. The pollen was found to be periporate and its surface was micro-echinate. However, the ecological aspects of these morphological features remain poorly understood. M.Z. Ferreira *et al.* (2019) revised the taxonomy of *Andryala*. The study determined that some species, including *A. arenaria* L. var. *ficalhoana*, and *Arenaria* L. are synonyms of other taxa. The authors typified several names for the first time and significantly expanded the information on the distribution of the species. A need for further clarification of the ecological relationships of this species remains. Researchers, such as M. Tiburtini *et al.* (2022), addressed the taxonomic analysis of *A. arenaria* subspecies in Italy using an integrative approach. The issue was the uncertainty about the taxonomic status of subspecies, especially those considered endemic to the Apennines. Through the analysis of morphology, genetic data and ecological niches, the study determined that *A. arenaria* subsp. *marginata* is the only endemic subspecies for this region, while other records refer to *A. arenaria* subsp. *praecox*. The study refined the geographical distribution of the subspecies, but more ecological research is needed to understand the habitats of *A. arenaria* subsp. *marginata* and its potential vulnerability to environmental change.

L. Parsons and B.H. Becker (2021) analysed the impact of *Ammophila Arenaria* L. invasion on soil chemistry in the coastal dunes of California. Significant changes in soil chemical properties were found, including high carbon content, which slows down the decomposition of organic matter. The study showed that mechanical removal is more effective than chemical removal, but additional measures are needed to restore the soil. Thus, many studies investigated different species of *Arenaria* L., however, the biochemical composition and pharmacological potential are understudied, which determines the research relevance. The study aimed to establish the dependence of the biochemical composition (phenolic compounds, flavonoids, trace elements) and antioxidant activity of *Arenaria* L. species on the peculiarities of soil and climatic conditions.

MATERIALS AND METHODS

The plant material was collected from various areas of the Nakhchivan Autonomous Republic June to July 2024, which corresponds to the phase of active growth of the studied species – budding and the beginning of flowering. The choice of this period is determined by the maximum concentration of biologically active substances in this phase. Samples of *Arenaria* L. (*A. serpyllifolia*, *A. leptoclados*, *A. rotundifolia*, *A. graminea*, *A. gypsophiloides*, *A. steveniana*, *A. dianthoides*, *A. macrantha*)

were collected in six districts of the Nakhchivan Autonomous Republic (Azerbaijan):

1. Sharur district, characterised by black soil with a high humus content (more than 5%), a moderately continental climate with an average annual temperature of 14°C and precipitation of 300-400 mm per year, moderate soil moisture, and access to water provided by the Araks River and irrigation systems;

2. Sadarak district, similar to Sharur district in terms of soil type (black soil) and climatic conditions (moderately continental, average annual temperature 14.5°C, precipitation 350-450 mm per year), moderate soil moisture, and access to water provided by the Araks River and irrigation systems;

3. Ordubad district with predominantly sandy loam soils with lower humus content (3-4%), more continental climate with higher daily temperature differences (average annual temperature 13°C, precipitation 250-350 mm per year), moderately low soil moisture and limited access to water;

4. Dzhulfinsk district with sandy loam soils, continental climate (average annual temperature 12.5°C, precipitation 200-300 mm per year), low soil moisture and limited access to water;

5. Shakhbuz district, characterised by podzolic soils with low humus content (1-2%), a sharply continental climate with low temperatures (average annual temperature 8-9°C), low precipitation (150-200 mm per year), low soil moisture and limited access to water;

6. Kangarly region with podzolic soils, a sharply continental climate (average annual temperature of 7-8°C, precipitation of 100-150 mm per year), very low soil moisture and very limited access to water.

For each species, samples were collected on a targeted basis, considering their ecological preferences and distribution within each district. The Sharur and Sadarak districts, located on the plain, are characterised by a predominance of black soil, favourable temperature conditions and moderate rainfall. The Ordubad and Dzhulfinsky districts, located in the foothills, are characterised by sandy loam soils, higher temperature fluctuations and lower rainfall than in the plains. The mountainous Shahbuz and Kangarli districts, with predominantly podzolic soils, are characterised by a harsh climate with low temperatures, high solar radiation and a specific hydrological regime. In each district, the collection was carried out in three plots located at a distance of at least 1 km from each other. The collected plants were recorded using the standard herbarium method for further identification and morphological analysis.

The collected plants were dried in the shade at room temperature (20-25°C) for two weeks. The dried samples were ground to a homogeneous powder using an electric grinder (IKA MF 10 basic, IKA-Werke, Germany). The extraction was carried out by the maceration method. A weight of crushed plant material (1 g)

was poured into 10 ml of 70% ethanol. The extraction was carried out in the dark at room temperature for 24 hours with periodic shaking. The resulting extract was filtered through a paper filter (Whatman No. 1) and used for further analyses (International Organization for Standardization, 2021).

The content of phenolic compounds and flavonoids in *Arenaria* L. extracts was determined by high-performance liquid chromatography using an Agilent 1260 Infinity II chromatograph (Germany). The separation was performed on a Zorbax Eclipse XDB-C18 column (4.6×150 mm, 5 µm, Germany) at 25°C. A gradient system consisting of solution A (0.1% formic acid in water) and solution B (acetonitrile) was used as the mobile phase. The gradient mode of elution was as follows: 0-5 min – 5% B; 5-20 min – 5-95% B; 20-25 min – 95% B; 25-30 min – 95-5% B; 30-35 min – 5% B. The flow rate was 1 ml/min. Detection was performed at a wavelength of 280 nm for phenolic compounds and 360 nm for flavonoids. For the identification and quantification of compounds, standard samples of rutin, quercetin, gallic acid, ferulic acid, chlorogenic acid, kaempferol, apigenin, and luteolin (Sigma-Aldrich) were used. Calibration curves were constructed for each standard compound in the concentration range of 10-100 µg/ml. The amount of phenolic compounds and flavonoids in the extracts was calculated by the method of an external standard (International Organization for Standardization, 2020).

Quantitative determination of phenolic compounds was conducted by the Folin-Cocalteu spectrophotometric method. An extract of 0.5 ml was mixed with 2.5 ml of Folin-Cocalteu reagent (diluted with distilled water in a ratio of 1:10) and 2 ml of 7.5% sodium carbonate solution. The mixture was incubated in the dark at room temperature for 30 minutes. The optical density was measured at 760 nm using a spectrophotometer. The content of phenolic compounds was calculated according to the calibration curve constructed using gallic acid as a standard. The content of flavonoids was determined by the spectrophotometric method using aluminium chloride. 1 ml of the extract was mixed with 1 ml of 2% aluminium chloride solution in methanol. The mixture was incubated in the dark at room temperature for 10 minutes. The optical density was measured at 415 nm using a spectrophotometer. The flavonoid content was calculated from the calibration curve constructed using quercetin as a standard.

The antioxidant activity of *Arenaria* L. extracts was determined spectrophotometrically using the 1,1-diphenyl-2-picrylhydrazil (DPPH) test. First, a solution of DPPH in methanol (chemically pure) with a concentration of 0.1 mm was prepared. Next, solutions of the studied extracts in methanol with a concentration of 25 to 100 µg/ml were prepared. To measure the antioxidant activity, 1 ml of DPPH solution was mixed with 1 ml of extract solution (experimental sample).

The mixture was incubated in the dark at room temperature for 30 minutes. After incubation, the optical density of the solutions was measured at 517 nm using a spectrophotometer. The control sample was prepared similarly, but 1 ml of pure methanol was used instead of the extract solution. The percentage of DPPH radical inhibition was calculated by the formula (1):

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100, \quad (1)$$

where A_{control} – optical density of the control sample; A_{sample} – optical density of the sample with the extract.

A quantitative assessment of antioxidant activity was performed by determining the IC₅₀ using Statistica software. The antioxidant activity of the extracts was compared with that of trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich) at the same concentrations to determine its percentage of inhibition. Additionally, antioxidant equivalent capacity of trolox (AEAC) was determined by comparing it with a standard solution of trolox (concentration from 25 to 100 µg/ml) measured under the same conditions. The results were expressed as mmol trolox equivalents per gram of dry weight of the extract (mmol TE/g).

Before chemical analysis, soil samples were air-dried, sieved through a 2 mm mesh sieve and crushed. Total nitrogen (N) was determined by the Kjeldahl method using a mineralisation and distillation apparatus (International Organization for Standardization, 1995a). Available phosphorus (P) was determined by the spectrophotometric method using the molybdate-vanadate reagent after extraction from the soil with Olsen's solution (International Organization for Standardization, 1994a). Exchangeable potassium (K) was determined by flame photometry after extraction from the soil with ammonium acetate solution (International Organization for Standardization, 1994b). The content of exchangeable calcium (Ca) and magnesium (Mg) was determined by the method of complexometric titration after extraction from the soil with ammonium acetate solution (International Organization for Standardization, 1995b). The content of trace elements (Fe, Mn, Zn, Cu) was determined by atomic absorption spectroscopy after acid extraction from the soil (International Organization for Standardization, 1998). All analyses were performed in triplicate.

Statistical data processing was performed using Statistica software (version 14). To assess the relationship between the content of biologically active substances (phenolic compounds and flavonoids) in *Arenaria* L. and the chemical composition of soils (content of macro- and microelements), correlation analysis was used to calculate the Pearson correlation coefficient (r). To compare biochemical parameters (content of phenolic compounds, flavonoids and antioxidant activity) between plants from different regions, a one-factor

analysis of variance was used. In case of statistically significant differences between the groups based on the results of analysis of variance, Tukey's post-hoc test was used to determine which groups differed from each other. The level of statistical significance for all analyses was $p < 0.05$. All data are presented as mean $\pm$ standard deviation.

RESULTS AND DISCUSSION

Biochemical characteristics of taxa of the genus *Arenaria* L. in the systematic context. Species of this genus are characterised by a wide range of morphological features, including herbaceous plants with both perennial and annual growth cycles. They are adapted to a variety of environmental conditions, such as alpine meadows, rocky landscapes, dry steppes and even coastal regions. Due to its adaptive properties, *Arenaria* L. demonstrates a high level of ecological diversity. These species are distributed in different climatic and geographical conditions around the world, including Europe, Asia, North Africa and America. Genetic research conducted by A. Pahlevani and A. Falatoury (2024), in the case of *Arenaria bulica*, their ability to survive in extreme conditions, in the highlands of Iran, was confirmed. However, not all researchers have come to similar conclusions, in particular, the study conducted by A.P. Jejesky de Oliveira and A. Toledo Lourenço (2019) analysed the morphological characteristics of *Allagoptera arenaria* in continental and island populations. The authors determined that the morphological differences between these populations are not significant and do not indicate significant adaptive responses to the isolation of the island environment. This conclusion challenges the universality of the hypothesis of high adaptability of species in different environments, which may contradict the claims of ecological plasticity of the genus *Arenaria* L.

Plants of the genus *Arenaria* L. are predominantly small herbaceous species that can be annual, biennial or perennial. They are characterised by small linear or lanceolate leaves that minimise moisture loss, which is an important adaptation to dry conditions. The flowers, usually white or light pink, have five petals and are arranged in umbellate or rarely cymose inflorescences. The root system is often taproot or superficial, which allows the plants to absorb moisture efficiently even in dry conditions.

The European Alps are one of the main centres of diversity of this genus, as noted by D. Iamónico (2022). For instance, *Arenaria* L. *digyna* demonstrated population stability and genetic plasticity, adapting to the harsh conditions of high mountain areas. These species are key to the formation of alpine ecosystems, and their study defined the impact of climate change on biodiversity. Southwest Asia, in particular Turkey and Iran, are significant centres of biodiversity for *Arenaria* L. However, some studies, such as M. Śliwa *et al.* (2019),

which examined the adaptive mechanisms of the desert-dwelling *Salix arenaria* species, reflect somewhat different values. The results showed that although the plant adapts to arid conditions through the efficient use of water, its photochemical apparatus and physiological processes remain limited. This indicates that not all species demonstrate universal adaptation to extreme conditions, which may challenge similar findings for *Arenaria* L. species. Moreover, Y. Wang *et al.* (2021) highlighted the role of *Arenaria* L. as a key species of alpine ecosystems on the Tibetan Plateau. At the same time, the study emphasised that their adaptation to soil and climate change may be less effective than expected, which calls into question their sustainability in the context of global change.

As noted by D. Ganbarov and S. Babayeva (2022), *Arenaria kandavanensis*, an endemic species found in the Alborzu Mountains, demonstrates a narrow ecological specialisation. These species are important for understanding adaptation mechanisms to harsh environments. The Nakhchivan Autonomous Republic is a unique region for the study of the genus *Arenaria* L. Its isolated geographical location favours the formation of

endemic species adapted to the specific conditions of the region, in particular dry slopes and steppe zones. *Arenaria* L. species are substantial in stabilising ecosystems. The diversity of the *Arenaria* L. genus is not only ecologically important but also evolutionarily important. The high level of polyploidy and genetic diversity allows these species to survive even in unfavourable conditions. Their study determined the evolutionary mechanisms of adaptation and genetic diversity. The genus *Arenaria* L. is highly relevant for the determination of evolutionary processes, ecological adaptation and biodiversity conservation. Regions such as the Nakhchivan Autonomous Republic are central to preserving this wealth and serve as a natural laboratory for studying the impact of climate change on ecosystems. Following E. Novruzova (2024), the representatives of the genus *Arenaria* L. in the flora of Nakhchivan Autonomous Republic are characterised by various biochemical properties, including phenolic compounds, saponins and flavonoids, which can vary depending on the species and habitats. Table 1 shows the classification scheme of the studied species of the genus *Arenaria* L. with their subgenera and sections.

Table 1. Taxonomic composition of the studied species of the genus *Arenaria* L. in the flora of the Nakhchivan Autonomous Republic

Type	Subgenus	Section
<i>Arenaria</i> L. <i>serpyllifolia</i> L.	<i>Arenaria</i> L.	<i>Arenaria</i> L.
<i>A. leptoclados</i> (Rchb.) Guss.	<i>Arenaria</i> L.	<i>Arenaria</i> L.
<i>A. rotundifolia</i> Bieb.	<i>Arenaria</i> L.	<i>Rotundifolia</i> McNeill
<i>A. graminea</i> C.A. Mey.	Eremogone	Eremogone
<i>A. gypsophiloides</i> L.	Eremogone	Eremogone
<i>A. steveniana</i> Boiss.	Eremogone	Eremogone
<i>A. dianthoides</i> Smith	Eremogone	<i>Glomeriflorae</i> F.N. Williams
<i>A. macrantha</i> Schischk. (<i>A. szovitsii</i> auct. non Boiss.)	Eremogone	<i>Rigidae</i> McNeill

Source: compiled by the author

The study of bioactive compounds in the genus *Arenaria* L., which includes *Arenaria* L. *serpyllifolia*, *A. leptoclados*, *A. rotundifolia*, *A. graminea*, *A. gypsophiloides*, *A. steveniana*, *A. dianthoides*, *A. macrantha*, demonstrates a significant diversity of phenolic compounds, flavonoids, saponins, terpenoids and alkaloids. These compounds play a key role in the pharmacological properties of plants, including antioxidant, anti-inflammatory and antimicrobial activity. Phenolic compounds are important secondary metabolites responsible for the antioxidant properties of many species (Zubtsova *et al.*, 2019). A significant amount of phenolic acids and flavonoid derivatives was found in representatives of the genus *Arenaria* L. For instance, *A. serpyllifolia* and *A. graminea* contain high levels of phenolic acids (25-30 mg/g dry weight), which contribute to the neutralisation of free radicals. Other species, such as *A. rotundifolia*, are

noted for the presence of complex polyphenols (up to 20 mg/g), which have anti-inflammatory effects (Cui *et al.*, 2019). Flavonoids, including quercetin and kaempferol, are usually present at the level of 18-22 mg/g, often found in representatives of the genus *Arenaria* L. In *A. gypsophiloides* and *A. dianthoides*, glycoside forms of these compounds demonstrated antimicrobial and antioxidant activity. They are also involved in the regulation of oxidative processes in the body, which helps prevent chronic diseases. These properties confirm the therapeutic potential of these species in traditional medicine (Dong *et al.*, 2023).

Saponins, which are present in significant amounts in *A. macrantha* and *A. graminea*, constitute about 10-15% of dry weight and have haemolytic activity, the ability to lower cholesterol, and antibacterial properties. Their surface-active nature affects the cell membranes

of bacteria, highlighting their importance in pharmacology (Ashour *et al.*, 2019). It was found such bioactive components as β -caryophyllene and other monoterpenes in terpenoids from representatives of the genus *Arenaria* L. For instance, *A. steveniana* and *A. rotundifolia* contain significant concentrations of terpenoids (up to 10-14 mg/g), which are responsible for their antimicrobial activity. Terpenoids are also involved in the modulation of the immune response, increasing its effectiveness. Alkaloids, such as β -carboline derivatives, were identified in *A. dianthoides* and *A. gypsophiloides*, present at the level of 7-9 mg/g. They demonstrate cytotoxic effects against several cancer cell lines, as well as the ability to immunomodulate. Alkaloids are promising components for the development of anticancer drugs, which emphasises the importance of further study of these compounds (Ceesay *et al.*, 2019). Thus, representatives of the genus *Arenaria* L. contain a wide range of bioactive compounds, among which phenolic compounds, flavonoids, saponins, terpenoids and alkaloids are distinguished (Ganbarov *et al.*, 2024).

Bioactive compounds, such as phenolic compounds, flavonoids, saponins, terpenoids and alkaloids, found in different species of *Arenaria* L., demonstrate a pronounced dependence on their taxonomic affiliation, in particular subgenera and sections. The chemical composition of these plants reflects both evolutionary and ecological adaptations. The *Arenaria* L. *serpyllifolia* and *A. leptoclados* are characterised by a high content of phenolic compounds and flavonoids. For instance, flavonoids such as quercetin were found in *A. serpyllifolia* and were noted for their ability to inhibit carboxyesterases, which is significant for protecting the body from toxins.

Phenolic acids in these species provide antioxidant activity and protection against oxidative stress, which is typical for environments with high ultraviolet radiation. *Arenaria* L. *rotundifolia* has an increased content of terpenoids (10-14 mg/g), in particular β -caryophyllene, which ensures adaptation to high mountain conditions. These compounds protect plants from ultraviolet radiation and regulate water balance. Species such as *Arenaria* *graminea*, *A. gypsophiloides*, and *A. steveniana* demonstrate high levels of saponins and terpenoids, which are beneficial in reducing moisture loss and protecting against pathogens in arid and gypsum soils. *A. gypsophiloides* has a high concentration of saponins, which contribute to survival in low humidity conditions (Mursaliyeva *et al.*, 2023). *Arenaria* *macrantha* is characterised by the synthesis of a large number of saponins and terpenoids (8-10 mg/g), which ensures its adaptation to mountain ecosystems with high-temperature differences. Saponins act as biological surfactants, providing resistance to drought. However, seasonal changes significantly affect the concentration of bioactive metabolites, in particular antioxidant compounds. J. Chen *et al.* (2020) showed that such changes may limit the stability of the pharmacological activity of *Arenaria* L. species.

The metabolic composition of *Arenaria* L. species indicates the presence of secondary metabolites that serve as markers for taxonomic groups. For instance, Z. Liu *et al.* (2021), analysed *Arenaria kansuensis* and identified β -carboline alkaloids, flavonoids and terpenoids that can be used as chemical markers to determine taxonomic affiliation. Differences between closely related species are largely related to their taxonomic affiliation. For instance, *Arenaria kansuensis* is dominated by β -carboline alkaloids, while other species are dominated by polyhydroxylated terpenoids or saponins (Nguyen *et al.*, 2021). In addition, the distribution of major flavonoids and phenolic acids depends on both species and environmental conditions (Barba de la Rosa *et al.*, 2019). Environmental conditions, such as climate and soil, significantly affect the metabolic profile of species. In the Qinghai-Tibet Plateau, it was found that the content of flavonoids and β -carboline alkaloids varies depending on the region and season of collection, with the highest concentrations recorded in summer. Similarly, high levels of phenolic acids and terpenoids were found in extracts of *Navicula Arenaria* L. obtained under extreme conditions, indicating adaptation to the environment (Alallaf *et al.*, 2021). These data indicate a complex dependence of the metabolic profile on environmental and taxonomic factors, which opens up opportunities for the use of these metabolites in systematics and pharmacology.

A high concentration of phenolic compounds and flavonoids is key to antioxidant activity. For instance, in the study by A. Eren *et al.* (2023), the presence of biologically active substances in related species was found to play a significant role in neutralising free radicals, thereby reducing oxidative stress and potentially reducing the risk of chronic diseases. The antimicrobial and antifungal properties of flavonoids and terpenoids have also been confirmed by research. Metabolites of some species have shown activity against pathogenic fungi such as *Candida albicans* and *Trichophyton* sp., indicating that these plants can be used in the treatment of fungal infections (Skliar *et al.*, 2020). The anti-inflammatory activity of saponins and flavonoids has also been demonstrated through their ability to modulate oxidative stress and inhibit the activity of enzymes associated with inflammation, such as 5-lipoxygenase (Hachlafi *et al.*, 2023). The conclusions of these studies confirm that bioactive metabolites of *Arenaria* L. species have significant therapeutic potential, especially in reducing oxidative stress, and inflammation and fighting microbial infections.

The systematic position of species determines the range of their therapeutic applications in traditional and modern medicine. Species of the subgenus *Arenaria* L. are mainly used to treat diseases associated with oxidative stress due to their antioxidant effect (Toiu *et al.*, 2019). The subgenus *Eremogone* is promising for the development of antimicrobial and antifungal drugs due

to the high level of saponins and terpenoids activity against pathogens such as *Candida albicans* and *Trichophyton* sp. These species can also be useful in the fight against chronic inflammatory diseases, as they can inhibit the activity of enzymes involved in the inflammatory process. In general, the biochemical composition of species of the genus *Arenaria* L. determines their wide therapeutic potential, hence these plants can be used for the treatment and prevention of diseases in traditional and modern medicine. The systematic position of species determines the dominance of certain metabolites that provide specific pharmacological activity, which is important for the development of targeted therapeutic strategies (Barua *et al.*, 2023).

S. Mawalagedera *et al.* (2019) emphasise that evolutionarily related species often show a similar profile of bioactive metabolites, in particular secondary compounds such as flavonoids, terpenoids, and alkaloids. These compounds are the basis for use in the treatment of inflammation, antioxidant therapy and antifungal infections. *Rotundifolia* species, for example, due to their high content of terpenoids and saponins, have significant potential for use in the modern treatment of inflammatory and immune disorders. Studies of bioactive metabolites, such as antioxidants and immunomodulators, show that evolutionarily related species often demonstrate a similar metabolic profile. For instance, flavonoids can modulate natural killer cells, offering potential in the treatment of cancer and viral diseases. Metabolomic studies have greatly simplified the identification of new bioactive compounds within certain taxa. Y. Chen *et al.* (2021) have developed a classification methodology for identifying structurally similar compounds in traditional medicines. The methodology can be used to identify new metabolites, such as antioxidants and immunomodulators, based on previously known bioactivity profiles.

Similar approaches were proposed by K. Liu *et al.* (2020), describing the creation of reference materials for the harmonisation of large-scale metabolomics data. This methodology is relevant for the standardisation

of raw materials, as it ensures consistency in the quantification of metabolites between different research centres. K. Kulkarni *et al.* (2019) highlighted the main parameters for the standardisation of herbal medicines, including the use of chromatographic and spectroscopic techniques to determine the quality and authenticity of medicinal raw materials, which can be used to address the chemical variability caused by environmental factors and optimise the standardisation process for medical use. The pharmacological value of species of the genus *Arenaria* L. reveals prospects for the targeted search for new biologically active metabolites. For instance, A. Mohiuddin (2019) demonstrated how seasonal and environmental variations affect the chemical composition of plants, which can be used to search for compounds with potentially new therapeutic properties, including antioxidant and antimicrobial effects. The potential of *Arenaria* L. species in pharmacology is enhanced by the development of new approaches to standardisation and targeted search for bioactive metabolites, this allows for the creation of drugs with predictable quality and bioactivity, increasing the efficiency of their use in traditional and modern medicine.

The influence of soil and climatic conditions of the Nakhchivan Autonomous Republic on the biochemical composition of *Arenaria* L. The specificity of the geological structure and climatic conditions of the studied region creates a variety of soil types, which can be used to investigate their influence on the biochemical parameters of plants. To study the influence of soil and climatic conditions on the biochemical composition of *Arenaria* L., six districts of the Nakhchivan Autonomous Republic were selected, covering different landscapes and ecological zones. The diversity of soil and climatic conditions in these areas creates a natural gradient of environmental factors, which can be used to comprehensively assess their impact on the biochemical composition of *Arenaria* L. The results of the biochemical composition analysis presented in Table 2 can be used to compare the content of phenolic compounds and flavonoids in plants from different locations.

Table 2. Content of phenolic compounds and flavonoids in *Arenaria* L. from different locations

District	Soil type	Climatic conditions	Phenolic compounds (mg/g dry weight)	Flavonoids (mg/g dry weight)
Sharursky	Chernozem	Temperate continental, plain	42.5 ± 3.2	11.8 ± 1.1
Sadaraksky	Chernozem	Temperate continental, plain	40.8 ± 2.9	11.2 ± 0.9
Ordubadsky	Sandy loam	Continental, foothill	31.5 ± 2.5	8.5 ± 0.8
Dzhulfinsky	Sandy loam	Continental, foothill	29.7 ± 2.8	7.9 ± 0.7
Shakhbuzsky	Podzolic	Sharp continental, mountainous	26.2 ± 3.1	6.8 ± 0.6
Kangarlinsky	Podzolic	Sharp continental, mountainous	24.5 ± 2.6	6.2 ± 0.5

Note: data are presented as mean ± standard deviation (n = 5)

Source: compiled by the author

The influence of soil and climatic conditions on the biochemical composition of *Arenaria* L. was studied in six districts of the Nakhchivan Autonomous Republic,

covering different soil types and climatic zones. The analysis of the data obtained confirms the hypothesis that plants grown on soils with a high content of

organic matter and in temperate regions have a higher content of biologically active compounds and a stronger antioxidant potential. Plants from the black earth soils of the Sharur district, characterised by a temperate continental climate, have the highest content of phenolic compounds (42.5 ± 3.2 mg/g dry weight) and flavonoids (11.8 ± 1.1 mg/g dry weight). The Sadarak district, also with chernozem soils and a temperate continental climate, demonstrates similarly high levels: 40.8 ± 2.9 mg/g and 11.2 ± 0.9 mg/g for phenolic compounds and flavonoids, respectively. Such high values are associated with the optimal combination of soil fertility, which provides plants with the necessary macro- and microelements, and favourable climatic conditions of the plains, which promotes active metabolism and accumulation of secondary metabolites. In the foothill areas (Ordubad and Dzhulfinsky) with sandy loam soils and a more continental climate characterised by higher temperature fluctuations and lower rainfall, there is a decrease in the content of biologically active substances. In the Ordubad district, the content of phenolic compounds is 31.5 ± 2.5 mg/g, flavonoids – 8.5 ± 0.8 mg/g, and in the Dzhulfa district – 29.7 ± 2.8 mg/g and 7.9 ± 0.7 mg/g, respectively. The decrease in the content of biologically active compounds is probably due to the lower content of nutrients in sandy loam soils and the influence of a more continental climate. The lowest values are characteristic of plants from mountainous areas (Shakhbuz and Kangarli) with podzolic soils and a sharply continental climate: 26.2 ± 3.1 mg/g and 6.8 ± 0.6 mg/g in the Shakhbuz region, and 24.5 ± 2.6 mg/g and 6.2 ± 0.5 mg/g in the Kangarli region for phenolic compounds and flavonoids, respectively. The harsh climate of the highlands with low temperatures, high solar radiation and a specific hydrological regime, combined with the low fertility of podzolic soils, creates limiting conditions for the accumulation of phenolic compounds and flavonoids in *Arenaria* L.

The results of the study of phenolic compounds and flavonoids in *Arenaria* L. samples by high-performance liquid chromatography identified and quantified several biologically active substances. The study determined that the qualitative composition of phenolic compounds varies depending on the place of plant growth. In the samples from the black earth soils of the Sharur and Sadarak districts, which are characterised by high humus and nutrient content, high levels of compounds such as rutin, quercetin, gallic acid, ferulic acid and chlorogenic acid were found. The dominant flavonoids in these samples were kaempferol, apigenin and luteolin, which could be related to optimal conditions for plant growth and development. In plants from the foothill regions (Ordubad and Dzhulfa) growing on less fertile sandy loam soils under conditions of higher insolation and temperature fluctuations, a slight decrease in the concentration of hydroxycinnamic acids such as ferulic and caffeic acids was observed, while the relative

amount of flavonols such as quercetin and kaempferol remained at a fairly high level, probably as an adaptation mechanism to increased UV radiation. The most significant differences in the composition of phenolic compounds were found in samples from mountainous areas (Shakhbuz and Kangarli). Under conditions of low temperatures, high solar radiation, and podzolic soils, a decrease in the content of most of the identified phenolic acids and flavonoids was observed, except for an increased concentration of flavones such as luteolin and apigenin and coumarin derivatives, which may play a protective role under stress.

The antioxidant activity of the extracts, assessed by the DPPH test, also correlates with soil and climatic conditions. The highest activity, characterised by a low IC₅₀ value (23.3 ± 1.8 µg/ml), high AEAC value (4.2 ± 0.3 mmol TE/g) and a high percentage of inhibition ($75.2 \pm 3.5\%$), was observed in samples from the Sharur district. High levels of antioxidant activity (IC₅₀ = 25.1 ± 2.2 µg/ml, AEAC = 3.9 ± 0.4 mmol TE/g, % inhibition = $72.1 \pm 4.1\%$) were also characteristic of plants from Sadarak district. In the samples from Ordubad and Dzhulfinsky districts, the antioxidant activity was moderate (IC₅₀ = 31.7 ± 2.5 and 34.2 ± 2.9 µg/ml, respectively, AEAC = 3.1 ± 0.2 and 2.9 ± 0.3 mmol TE/g, % inhibition = 64.5 ± 3.2 and $61.2 \pm 3.8\%$), and the lowest – in samples from the Shakhbuz (IC₅₀ = 40.5 ± 3.5 µg/ml, AEAC = 2.4 ± 0.2 mmol TE/g, % inhibition = $53.7 \pm 2.9\%$) and Kangarli (IC₅₀ = 43.8 ± 3.1 µg/ml, AEAC = 2.2 ± 0.2 mmol TE/g, % inhibition = $49.5 \pm 2.5\%$) districts.

The chemical analysis of the soils (Table 4) demonstrated that the black soils of the Sharur and Sadarak districts had the highest levels of nitrogen (0.25% and 0.22% respectively), phosphorus (220 and 200 mg/kg), potassium (350 and 320 mg/kg) and other macro- and microelements. Soils in the Ordubad and Dzhulfinsk districts had moderate levels of the main macroelements, while the Shakhbuz and Kangarli districts had the lowest levels of nutrient supply. Correlation analysis confirmed the link between the content of biologically active substances in plants and the chemical composition of soils. For instance, in the Sharur district, the correlation coefficient between the content of phenolic compounds and nitrogen in the soil was 0.88 ($p < 0.01$).

Comparative analysis of the morphological characteristics of *Arenaria* L. from different regions revealed the variability of traits associated with soil and climatic conditions. Plants from lowland areas characterised by favourable conditions of flat terrain with fertile black soil had more developed vegetative organs. The leaves had a larger leaf blade of intense green colour, and the stems were thicker and less branched. Plants from the foothill regions (Ordubad and Dzhulfinsky) growing on less fertile sandy loam soils in a more contrasting climate showed a tendency to reduce leaf size and increase the degree of leaf pubescence, which can be considered

as an adaptation to reduced moisture supply and increased solar radiation intensity. The most pronounced morphological changes were observed in mountainous areas (Shakhbuz and Kangarly) with podzolic soils and harsh climates. Plants of *Arenaria* L. from these

locations were characterised by smaller leaves with dense pubescence, shortened internodes and more compact size in general, which is a typical adaptation to the extreme conditions of the highlands. Data on antioxidant activity are presented in Table 3.

Table 3. Antioxidant activity of *Arenaria* L. extracts from different regions of the Nakhchivan Autonomous Republic

District	IC ₅₀ (µg/ml)	AEAC (mmol TE/g)	% inhibition (at 100 µg/ml)
Sharursky	23.3 ± 1.8	4.2 ± 0.3	75.2 ± 3.5
Sadaraksky	25.1 ± 2.2	3.9 ± 0.4	72.1 ± 4.1
Ordubadsky	31.7 ± 2.5	3.1 ± 0.2	64.5 ± 3.2
Dzhulfinsky	34.2 ± 2.9	2.9 ± 0.3	61.2 ± 3.8
Shakhbuzsky	40.5 ± 3.5	2.4 ± 0.2	53.7 ± 2.9
Kangarlinsky	43.8 ± 3.1	2.2 ± 0.2	49.5 ± 2.5

Note: data presented as mean ± standard deviation (n=3); IC₅₀ – inhibitory concentration of 50%

Source: compiled by the author

As can be seen from Table 3, the antioxidant activity of *Arenaria* L. extracts has a clear dependence on the area of collection, which correlates with the data on the content of phenolic compounds and flavonoids presented in Table 2. Extracts from the Sharur and Sadarak districts, which are characterised by chernozem soils and a temperate continental climate, show the highest antioxidant activity. Thus, extracts from the Sharur district demonstrated the lowest IC₅₀ value (23.3 ± 1.8 µg/ml), indicating high efficiency of antioxidant protection, the highest AEAC value (4.2 ± 0.3 mmol TE/g), which demonstrates a significant antioxidant capacity equivalent to a high concentration of trolox, and a high percentage of DPPH inhibition at a standard concentration of 100 µg/ml (75.2 ± 3.5%), which confirms the expressed antiradical activity. The extracts from the Sadarak region also showed high antioxidant activity with IC₅₀ = 25.1 ± 2.2 µg/ml, AEAC = 3.9 ± 0.4 mmol TE/g and 72.1 ± 4.1% DPPH inhibition. According to Table 2, plants from these districts also contain higher amounts of phenolic compounds and flavonoids (42.5 ± 3.2 mg/g and 11.8 ± 1.1 mg/g for Sharur district, 40.8 ± 2.9 mg/g and 11.2 ± 0.9 mg/g for Sadarak district), which explains their high antioxidant activity, as these compounds are known for their antiradical properties. In the samples from Ordubad (IC₅₀ = 31.7 ± 2.5 µg/ml, AEAC = 3.1 ± 0.2 mmol TE/g, % inhibition = 64.5 ± 3.2%) and Dzhulfinsky (IC₅₀ = 34.2 ± 2.9 µg/ml, AEAC = 2.9 ± 0.3 mmol TE/g, % inhibition = 61.2 ± 3.8%) of the regions collected on sandy loam soils in a more continental climate, a decrease in antioxidant activity is observed. The increase in IC₅₀ values indicates the need for a higher concentration of the extract to achieve 50% DPPH inhibition, which indicates a moderate decrease in antiradical activity. Accordingly, the decrease in AEAC values and percentage of DPPH inhibition confirms this trend.

The data in Table 2 show that plants from these districts contain a lower amount of phenolic compounds and flavonoids (31.5 ± 2.5 mg/g and 8.5 ± 0.8 mg/g for

Ordubad district, 29.7 ± 2.8 mg/g and 7.9 ± 0.7 mg/g for Dzhulfinsky district), which may be the reason for the less pronounced antioxidant activity. The lowest antioxidant activity was recorded for extracts from Shakhbuz (IC₅₀ = 40.5 ± 3.5 µg/ml, AEAC = 2.4 ± 0.2 mmol TE/g, % inhibition = 53.7 ± 2.9%) and Kangarlin (IC₅₀ = 43.8 ± 3.1 µg/ml, AEAC = 2.2 ± 0.2 mmol TE/g, % inhibition = 49.5 ± 2.5%) areas characterised by podzolic soils and harsh highland climate. High IC₅₀ values indicate low free radical scavenging efficiency, while low AEAC and DPPH inhibition percentages indicate a limited antioxidant capacity of these extracts. According to Table 2, plants from these districts contain the lowest amount of phenolic compounds and flavonoids (26.2 ± 3.1 mg/g and 6.8 ± 0.6 mg/g for the Shakhbuz district, 24.5 ± 2.6 mg/g and 6.2 ± 0.5 mg/g for Kangarli district), which explains their low antioxidant activity. Thus, soil and climatic conditions determine both the quantitative content of phenolic compounds and flavonoids and the antioxidant activity of *Arenaria* L.

The chemical composition of soils is an important aspect in the study of the impact of soil and climatic conditions on the biochemical composition and pharmacological potential of plants. Soil is the main source of macro- and microelements necessary for plant growth and development, as well as for the synthesis of biologically active substances. Different content of elements in the soil can affect metabolic processes in plants and, accordingly, the accumulation of phenolic compounds, flavonoids and other substances that determine their antioxidant activity. The study of the chemical composition of soils established a correlation between the content of elements in the soil and the biochemical parameters of plants, which contributes to a deeper understanding of the mechanisms of environmental impact on the pharmacological potential of medicinal plants. The results of the chemical analysis of soils from different regions of the Nakhchivan Autonomous Republic, from which *Arenaria* L. samples were collected, are presented in Table 4.

Table 4. Content of macro- and microelements in soils of different districts of Nakhchivan Autonomous Republic

District	Sharursky	Sadaraksky	Ordubadsky	Dzhulfinsky	Shakhbuzsky	Kangarlinsky
N (%) (nitrogen)	0.25 ± 0.02	0.22 ± 0.03	0.18 ± 0.02	0.15 ± 0.02	0.1 ± 0.01	0.08 ± 0.01
P (mg/kg) (phosphorus)	220 ± 15	200 ± 12	150 ± 10	130 ± 8	80 ± 5	70 ± 4
K (mg/kg) (potassium)	350 ± 20	320 ± 18	280 ± 15	250 ± 12	180 ± 10	150 ± 8
Ca (mg/kg) (calcium)	2,500 ± 150	2,300 ± 130	1,800 ± 100	1,600 ± 90	1,200 ± 70	1,000 ± 60
Mg (mg/kg) (magnesium)	350 ± 25	320 ± 22	250 ± 15	220 ± 12	150 ± 10	120 ± 8
Fe (mg/kg) (iron)	45 ± 3	40 ± 2	35 ± 2	30 ± 2	20 ± 1	18 ± 1
Mn (mg/kg) (manganese)	8.5 ± 0.5	7.8 ± 0.4	6.5 ± 0.3	5.8 ± 0.3	4.2 ± 0.2	3.5 ± 0.2
Zn (mg/kg) (zinc)	1.2 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
Cu (mg/kg) (copper)	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0.1

Note: data are presented as mean ± standard deviation (n = 3)

Source: compiled by the author

Thus, the chemical composition of soils, presented in Table 4, substantially contributes to the formation of the biochemical profile of *Arenaria* L. and determines its pharmacological potential. The chernozems of Sharur and Sadarak districts, rich in humus and nutrients, provide optimal conditions for plant development. The high nitrogen content in these soils stimulates active growth of vegetative mass and biosynthesis of nitrogen-containing compounds, creating the basis for the formation of a complex of biologically active substances. A significant concentration of phosphorus meets the energy needs of the plant organism, supporting intensive metabolism and synthesis of important biomolecules, including phenolic compounds. Sufficient potassium regulates water balance and nutrient transport, optimising physiological processes (Haleeva *et al.*, 2024). High levels of calcium and magnesium ensure the structural integrity of cells, and the activity of enzymes involved in biosynthetic processes. Lastly, the presence of essential trace elements such as iron, manganese, zinc and copper activate various enzyme systems, including those that catalyse the synthesis of phenolic compounds and flavonoids, which determine the antioxidant potential of plants.

The sandy loam soils of the Ordubad and Dzhulfa districts, although containing the necessary elements, demonstrate their moderately reduced concentration. This creates certain limitations for the full disclosure of the biosynthetic potential of *Arenaria* L., which is reflected in a less pronounced accumulation of biologically active substances. In turn, the podzolic soils of the Shahbuz and Kangarlı districts, which are poor in organic matter and nutrients, form an unfavourable environment for the development of *Arenaria* L. Deficiency of nitrogen, phosphorus, potassium and other elements limits plant growth, inhibits biosynthetic processes and reduces the production of phenolic compounds and flavonoids. Low concentrations of trace elements impair the functioning of enzyme systems, which affects the biochemical composition of plants.

Thus, the study of the biochemical composition of representatives of the genus *Arenaria* L., in particular *Arenaria* L. *serpyllifolia* L., *A. leptoclados* (Rchb.) Guss.,

A. rotundifolia Bieb., *A. graminea* C.A. Mey., *A. gypsophiloides* L., *A. steveniana* Boiss., *A. dianthoides* Smith, *A. macrantha* Schischk. (*A. szovitsii* auct. non Boiss.), conducted in six districts of the Nakhchivan Autonomous Republic, demonstrated a close relationship between soil and climatic characteristics and the content of phenolic compounds, flavonoids and antioxidant activity of these plants. Regional differences due to different soil types and climatic conditions caused the formation of a natural gradient of environmental factors that affected biosynthetic processes. The black soils of the lowland Sharur and Sadarak regions with favourable temperature conditions and sufficient moisture supply stimulated the accumulation of a significant number of phenolic compounds and flavonoids and provided high antioxidant activity of the extracts. Plants from the foothill regions of Ordubad and Dzhulfinsky, which grew on sandy loam soils and in a more continental climate with reduced moisture supply and increased temperature fluctuations, were characterised by a moderate decrease in the content of secondary metabolites and a corresponding decrease in antioxidant activity. The most significant decrease in the quantitative and qualitative indicators of biologically active compounds was observed in the highland Shakhbuz and Kangarlı districts with podzolic, nutrient-poor soils and harsh climate, which limited the synthesis of phenolic compounds and flavonoids and reduced the antioxidant potential.

Correlation analysis using Pearson's correlation coefficient (r) in Statistica software revealed a differential influence of soil and climatic factors on the biosynthesis of phenolic compounds and flavonoids in *Arenaria* L. in different regions of Nakhchivan Autonomous Republic. In the Sharur district, with its humus-rich chernozems and temperate continental climate, a strong positive correlation was observed between the content of phenolic compounds and the concentration of nitrogen ($r = 0.88$, $p < 0.01$) and phosphorus ($r = 0.76$, $p < 0.05$) in the soil. A similar trend, although less pronounced, was observed for flavonoids ($r = 0.79$, $p < 0.05$ for N and $r = 0.65$, $p < 0.05$ for P). This indicates that the optimal supply of macronutrients in this area contributes to the accumulation of secondary metabolites.

In the Sadarak district, with similar soil and climatic conditions, positive correlations were also found between plant biochemical parameters and soil N and P content ($r = 0.82$, $p < 0.01$ for phenolic compounds and N, $r = 0.71$, $p < 0.05$ for phenolic compounds and P, $r = 0.75$, $p < 0.05$ for flavonoids and N, $r = 0.62$, $p < 0.05$ for flavonoids and P). In the foothill Ordubad and Dzhulfa districts, the correlation coefficients were slightly lower ($r = 0.65$ - 0.78 , $p < 0.05$), which could be related to the lower nutrient content of sandy loam soils and the influence of a more continental climate with lower rainfall. In the mountainous Shakhbuz and Kangarli districts, with their podzolic soils and harsh climatic conditions, the correlations between the content of phenolic compounds and flavonoids and the concentration of macronutrients were weak and not always statistically significant ($r = 0.55$ - 0.7 , $p < 0.05$ in some cases). Additionally, the study analysed the correlation with climatic parameters such as average annual temperature and precipitation. A negative correlation was found between the content of phenolic compounds and the average annual temperature ($r = -0.75$, $p < 0.05$) and a positive correlation with precipitation ($r = 0.68$, $p < 0.05$) in mountainous areas, indicating the limiting effect of low temperatures and the positive effect of humidity on the biosynthesis of these compounds. Thus, the correlation analysis confirms the gradient of influence of soil and climatic conditions on the biochemical composition of *Arenaria L.*

Recommendations for the cultivation of *Arenaria L.* with a high content of bioactive substances. Optimisation of *Arenaria L.* cultivation conditions for obtaining plants with a high content of bioactive substances, in particular phenolic compounds and flavonoids, which determine its antioxidant potential, is a significant task for the pharmaceutical industry. The results of the study indicate that the key factors affecting the biosynthesis of these compounds are the chemical composition of the soil and climatic conditions. The most favourable conditions for the accumulation of phenolic compounds and flavonoids were found in the Sharur and Sadarak districts of the Nakhchivan Autonomous Republic, which are characterised by the presence of chernozem soils. Black soils, rich in humus, provide *Arenaria L.* plants with essential macronutrients such as nitrogen, phosphorus and potassium. Nitrogen is known to be the main component of amino acids, proteins and nucleic acids, if sufficient amount is provided, stimulates the growth of vegetative mass and overall plant metabolism, creating the preconditions for active biosynthesis of secondary metabolites, including phenolic compounds. Phosphorus is essential for energy metabolism in plants, participating in phosphorylation and adenosine triphosphate formation, which is necessary for energy-dependent biosynthetic processes. Potassium regulates water balance, cell turgor and nutrient transport, providing optimal conditions for the

functioning of metabolic pathways. In addition to macronutrients, trace elements such as iron and manganese play an important role, being cofactors of many enzymes involved in the biosynthesis of phenolic compounds and flavonoids. Black soils provide plants with these trace elements in a bioavailable form.

The moderately continental climate of the Sharur and Sadarak districts with sufficient precipitation and relatively stable temperature conditions also contributes to the accumulation of bioactive compounds in *Arenaria L.* Optimal soil moisture ensures efficient nutrient absorption by the root system, and favourable temperatures support the high activity of enzymes involved in biosynthesis. For the successful cultivation of *Arenaria L.* with a high content of bioactive substances, it is recommended to recreate conditions similar to those observed in Sharur and Sadarak districts. This includes the use of soils with a high humus content and the provision of balanced mineral nutrition for plants, in particular the application of fertilisers enriched with nitrogen, phosphorus and potassium. It is also necessary to monitor the content of microelements and apply them if necessary. Creating an optimal microclimate with controlled temperature and humidity levels will also help to increase the content of biologically active substances in plants. Further research should be aimed at a detailed study of the influence of individual environmental factors on the biosynthesis of phenolic compounds and flavonoids in *Arenaria L.* and the development of specialised agronomic practices to maximise its pharmacological potential.

For the successful commercial cultivation of *Arenaria L.* and the production of raw materials with a high content of biologically active substances, it is necessary to optimise agronomic practices to meet the physiological needs and ecological preferences of the species. Soil condition is crucial in the accumulation of target metabolites. It is recommended to use soils similar in composition to chernozems, characterised by a high humus content (over 5%) and a balanced ratio of macro- and microelements. Humus, as the main source of organic matter, provides plants with the necessary nutrients, and improves soil structure, water capacity and aeration, creating a favourable environment for the development of the root system (Voitovyk *et al.*, 2023). Balanced mineral nutrition, in particular the optimal concentration of nitrogen, phosphorus and potassium, is a key factor for intensive plant growth and efficient biosynthesis of phenolic compounds and flavonoids (Myronova & Bashta, 2023).

Pre-planting soil preparation includes deep ploughing (25-30 cm) or digging to improve aeration and moisture permeability. This promotes the development of a strong root system, ensuring efficient nutrient absorption. The systematic application of organic and mineral fertilisers maintains the optimum level of nutrition during the growing season. Organic fertilisers, such as

compost or humus (specify the dose), improve the physical and chemical properties of the soil and ensure the gradual release of nutrients (Romanchuck *et al.*, 2017). mineral fertilisers (nitrogen, phosphorus and potassium fertilisers, please specify the dose) are used to adjust the nutrient content of the soil. The optimal pH level for *Arenaria* L. is 6.0-7.0. The acidity of the soil is regulated by applying lime or other ameliorants if necessary.

The watering regime should be adapted to the climatic conditions and the stage of plant development. *Arenaria* L. needs regular watering, especially during periods of active growth and in conditions of insufficient rainfall, but excessive soil moisture should be avoided. Sufficient lighting is an important condition for efficient photosynthesis and accumulation of bioactive compounds. It is recommended to grow *Arenaria* L. in open, well-lit areas. Weed control is carried out by regular weeding or herbicide application (specify specific herbicides and doses, if used). To prevent damage to plants by pests and diseases, preventive treatments are carried out with appropriate preparations (specify specific preparations and doses). It is important to follow the recommendations for the use of pesticides and ensure environmental safety. Harvesting is conducted in the flowering phase (June-July) when the concentration of biologically active substances reaches its maximum. The collected raw materials must be dried quickly in a shaded, well-ventilated place or dryers at a temperature not exceeding 40°C to prevent degradation of the target compounds. The dried raw materials are stored in a dry, dark place in hermetically sealed containers to preserve their quality and pharmacological activity. Adherence to these recommendations will provide a high yield of *Arenaria* L. with an optimal content of biologically active substances suitable for use in the pharmaceutical industry.

CONCLUSIONS

The study of the biochemical composition and pharmacological potential of representatives of the genus *Arenaria* L. revealed several important aspects of the influence of soil and climatic conditions on the content of biologically active substances and the antioxidant activity of these plants. The study determined that the species grown on the chernozems of the Sharur district were characterised by the highest content of phenolic

compounds (42.5 ± 3.2 mg/g dry weight) and flavonoids (11.8 ± 1.1 mg/g dry weight), while plants from the podzolic soils of the Kangarly district had the lowest values (24.5 ± 2.6 mg/g and 6.2 ± 0.5 mg/g, respectively). The results confirmed that optimal soil and climatic conditions, in particular high humus and nutrient content, contributed to the accumulation of secondary metabolites.

The antioxidant activity of the extracts also demonstrated a dependence on soil type and climatic conditions. The lowest IC₅₀ value (23.3 ± 1.8 µg/ml), indicating the highest antioxidant efficiency, was recorded for plants from the Sharur district, while in the Kangarli district, the IC₅₀ reached 43.8 ± 3.1 µg/ml. The high antioxidant capacity of extracts from chernozem soils (4.2 ± 0.3 mmol TE/g) confirmed the significant potential of these plants for the pharmaceutical industry. Samples from other areas showed a decrease in antioxidant activity as soil and climatic conditions deteriorated, which was due to a deficiency of macro- and microelements in the soil. In addition, the morphological analysis of plants revealed adaptive features such as a larger leaf blade in species with favourable soil conditions and dense pubescence in extreme highland conditions.

The results of the study highlighted the significant pharmacological potential of *Arenaria* L. for use as a source of natural antioxidants. The highest levels of antioxidant activity and bioactive substance content were observed in plants growing on black soil with favourable climatic conditions, which emphasised the importance of cultivating these species in such conditions to obtain high-quality medicinal raw materials. The study also showed that *Arenaria* L. species can be used to create biologically active supplements and pharmaceuticals with high antioxidant efficiency. The limitation of this work was the lack of analysis of long-term changes in the antioxidant activity of plants. Further research could address the genetic and molecular mechanisms that regulate the biosynthesis of bioactive compounds under different environmental conditions.

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CONFLICT OF INTEREST

None.

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Біохімічний склад та фармакологічний потенціал видів роду *Arenaria* L.

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Анотація. Метою роботи було визначення залежності вмісту фенольних сполук, флавоноїдів та антиоксидантної активності видів роду *Arenaria* L. від ґрунтово-кліматичних умов Нахічеванської Автономної Республіки. Для цього використовували морфологічний аналіз, високоефективну рідинну хроматографію та спектрофотометрію, а отримані дані піддавали статистичній обробці. Основні результати показали, що зразки з чорноземних ґрунтів Шарурського району мали найвищий вміст фенольних сполук ($42,5 \pm 3,2$ мг/г сухої маси) і флавоноїдів ($11,8 \pm 1,1$ мг/г сухої маси), а також високу антиоксидантну активність, зокрема, ІС₅₀ становила $23,3 \pm 1,8$ мкг/мл, а антиоксидантна ємність антиоксидантного еквіваленту тролоксу (АЕАС) досягала $4,2 \pm 0,3$ ммоль ТЕ/г. Зразки з гірських районів, таких як околиці сіл Гарагуш та Чалхангала Кангарлінського району, показали найнижчі показники: вміст фенольних сполук становив $24,5 \pm 2,6$ мг/г, флавоноїдів – $6,2 \pm 0,5$ мг/г, ІС₅₀ – $43,8 \pm 3,1$ мкг/мл, а АЕАС – $2,2 \pm 0,2$ ммоль ТЕ/г. Встановлено чітку кореляцію між вмістом біологічно активних сполук та хімічним складом ґрунту, зокрема високий вміст азоту ($0,25 \pm 0,02$ %) та фосфору (220 ± 15 мг/кг) сприяв максимальному накопиченню цих сполук. Практичне значення роботи визначається рекомендаціями щодо оптимальних умов культивування *Arenaria* L. для отримання рослин з високим вмістом антиоксидантів. Отримані результати підкреслюють важливість адаптації агрономічних підходів до конкретних умов навколишнього середовища для максимальної реалізації фармакологічного потенціалу цих рослин

Ключові слова: фенольні сполуки; флавоноїди; антиоксидантна активність; ґрунтово-кліматичні умови; біологічно активні речовини; біорізноманіття



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Soil microbiomes as component of pedosphere biodiversity and factor in formation of crop yields

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Abstract. The study aimed to evaluate the mechanisms of interaction between soil microbiomes and leading crops to optimise yields and product quality in agricultural production. A comprehensive analysis of the physical and chemical properties of soils (chernozems, grey forest and podzolic soils) and the composition of the soil microbiome, including the number of nitrogen-fixing bacteria *Rhizobium* and

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Azotobacter, bacteria *Bacillus* spp, representatives of the genus *Streptomyces*, and fungi *Glomus* spp. The highest yields of *Kalbex* wheat (50 c/ha), Rhodes corn (80 c/ha), and *Kingstone* soybeans (30 c/ha) were recorded when these crops were grown on black soils. Grey forest soils and podzolic soils showed lower productivity and crop quality. The number of nitrogen-fixing bacteria *Rhizobium* and *Azotobacter* in chernozems reached 6 million colony-forming units (CFU) per gram of soil, while in podzolic soils it was the lowest – 3 million CFU per gram of soil. The mycorrhizal fungi *Glomus* spp. were also most abundant in black soil, with 8 million spores per gram of soil. The study examined the impact of the soil microbiome on the yield of selected crops. A correlation analysis of the microbiome and yields was conducted. The impact of the soil microbiome on crop quality was analysed. The results confirmed that chernozems provide the best conditions for growing crops due to their high biological activity and optimal physical and chemical properties, making them an ideal choice for agronomic practice

Keywords: biodiversity; metagenomics; agroecosystems; pedosphere; agro-industrial complex; biogeochemistry; crop production

INTRODUCTION

Currently, there is a growing interest in the role of soil microbiomes in shaping crop yields. Soil microbiomes are central in maintaining plant vigour, improving nutrient availability and overall ecological balance. However, despite significant advances in this area, there are many gaps in understanding the specific impact of different microbiome components on crop yields and in applying this knowledge in practice. The study of soil microbiomes is relevant not only for understanding basic biological processes but also for developing new agronomic strategies that can significantly improve agricultural efficiency. Therefore, it is necessary to conduct a study that will integrate knowledge about soil microbiomes and yields into a single system.

Modern scientific research confirms the importance of soil microbiomes. W. Anthony *et al.* (2024) highlighted the importance of filling the gaps in soil metagenomics to improve understanding of microbiome functions and their impact on the soil ecosystem. This information is important for an accurate assessment of how microbiomes affect fertility and, consequently, crop yields. In turn, S. Banerjee and M. van der Heijden (2022) considered the concept of “one health” and its importance for soil microbiomes, emphasising their impact on the overall health of ecosystems and on plant health and yield. The study of global soil carbon management as a key aspect for understanding and maintaining the largest terrestrial carbon reserve was carried out by J. Scharlemann *et al.* (2023). This study demonstrated how managing soil carbon can affect microbiomes and their ability to maintain soil fertility. R. Wu *et al.* (2021) studied the effect of moisture on the activity of viruses in soil, which can indirectly affect microbiomes and their functional properties. This shows that changes in environmental conditions can modify soil microbiomes and thus affect plant productivity.

F. Bastida *et al.* (2021) and R. Riley *et al.* (2021) conducted research showing that microbiome diversity and functional abilities have a significant impact on soil health and, consequently, on yields. The results also show that a deeper understanding of microbiomes at

different levels can help to develop new strategies to improve the productivity of agricultural systems. Different approaches to metagenomic data collection for the creation of biome-specific gene catalogues were studied by L. Delgado and A. Andersson (2022). The authors compared methods for collecting and analysing metagenomic data, which allow for more accurate and detailed profiles of microbiomes for specific environmental environments. Y. Zhou *et al.* (2024) found that root microbiomes adapted to local conditions can enhance plant growth and development, which is important for optimising yields.

M. Hartmann and J. Six (2023) examined the relationship between soil structure and microbiome functions in agroecosystems, analysing how soil structure affects the microbiome and how microbiomes can in turn modify soil structure, which is important for improving agronomic practices and increasing productivity. J. Jansson *et al.* (2023) discussed soil microbiome engineering as a strategy to increase the resilience of agroecosystems in a changing environment and proposed different approaches to modifying microbiomes to improve their functionality and resilience to environmental change.

The interaction between plants and their microbiomes, including the processes of formation of microbiome communities and their impact on plant health, was studied by P. Trivedi *et al.* (2020). They also stressed the importance of these interactions for increasing yields and plant health. J. Guo *et al.* (2021) analysed the main microbiota accompanying seeds, the endosphere and rhizosphere, and how these microbiotas can predict plant functional characteristics in different rice varieties, and found the dominance of deterministic processes in the formation of microbiomes. B. Singh *et al.* (2020) studied the role of crop microbiomes in sustainable agriculture and highlighted the importance of microbiomes for increasing the productivity and resilience of agricultural systems, as well as for improving soil and plant health. M. Jiang *et al.* (2024) proposed a solution based on home microbial cultures to improve

plant growth on soils with low fertility, demonstrating the effectiveness of using specific microbiomes to increase yields in conditions of limited fertility. J. Hu *et al.* (2021) investigated the effect of probiotic bacterial consorts on plant growth through the influence on the resident rhizosphere microbiomes and found that the introduction of probiotic consorts can positively affect plant health and increase plant growth.

The study aims to identify the leading features of soil microbiome biodiversity in different agricultural environmental conditions of the Sumy region and to assess their impact on crop yields. It is necessary to determine which components of the microbiome are most important for increasing productivity and how they can be effectively used to improve agronomic practices, and it is also advisable to assess the impact of microbiomes on the quality parameters of agricultural products. The answer to this question involves filling a gap in existing knowledge and providing practical recommendations for agronomists and farmers.

MATERIALS AND METHODS

To achieve the research objective, a comprehensive experiment was conducted, which included several key stages. The study was conducted from March to October 2024 on agronomic plots in the Sumy region, which is a region with a variety of soil types and agroclimatic conditions that significantly affect agricultural production. To ensure the comprehensiveness of the study, several locations were selected that differ not only in soil type but also in crops grown, which was used to study in more detail the impact of different agricultural technologies on crop productivity and quality. The study plots were characterised by the following soil types: podzolised chernozems, grey forest soils and sod-podzolic soils. This approach provides objective data and recommendations for improving agronomic practices in the region. Sampling and materials – samples were obtained from a depth of 0-30 cm, which is optimal for studying the root zone of plants. The microorganisms isolated from the soil, including bacteria and fungi, were identified using modern molecular methods.

Equipment and methods – spectrophotometers (Thermo Fisher Scientific, model NanoDrop – United States of America), pH meters (Metrohm 780 pH Meter – USA), and soil texture analysers (Zehntner ZH 2000 – Switzerland) were used to determine the content of basic nutrients and soil humus. For the identification of microorganisms, automatic systems for

deoxyribonucleic acid sequencing (Illumina NovaSeq 6000 – USA), microscopes (Olympus BX53 – Japan), incubators for cultivating microorganisms (Eppendorf ThermoMixer C – Germany), and specialised kits for polymerase chain reaction (Thermo Scientific TaqMan Universal Master Mix – USA) were used. Traditional agronomic methods were used to assess crop yields, including weighing agricultural products and analysing their quality.

To assess the relationship between the characteristics of the soil microbiome and crop yields, Pearson's correlation coefficients were calculated. This statistical method was used to determine the strength and direction of the linear relationship between the two variables. In general, the statistical processing of the research results was carried out using the statistical computer packages STATISTICA and PAST. Study procedure – soil samples were prepared by drying, crushing and dividing into sub-samples for different types of analysis. Appropriate laboratory techniques were used to extract microbial DNA from the soil, including polymerase chain reaction and DNA sequencing to identify microbial species. Wheat, corn and soybean seeds were sown on the prepared plots. During the growing season, the plants were regularly monitored for phenological growth and development, biometric measurements, and quantitative and qualitative yield indicators. The collected data were analysed using statistical methods, which identified correlations between soil microbiome characteristics and crop yields.

Materials used – chemical reagents: standard reagents for determining the physical and chemical properties of soil, such as solutions for analysing the content of macro- and microelements, humus and hydrogen ions; technical equipment: laboratory centrifuges, automatic pipettes, spectrophotometers, incubators and other data collection devices. Thus, the study included a systematic approach to analysing the impact of soil microbiomes on crop yields, which provided new data and recommendations for improving the efficiency of agronomic practices.

RESULTS

The studied podzolic soils proved to be the most fertile, as physicochemical analysis confirmed that they have the highest humus content, which indicates their ability to provide plants with the necessary nutrients (Table 1). In addition, podzolic chernozems demonstrated the best pH values, which is an important factor for optimal plant growth and development.

Table 1. Physical and chemical properties of soils of different types

Soil type	pH (salt, unit)	Humus content (%)	Nitrogen nitrate (N-NO ₃ , mg/kg)	Phosphorus (P ₂ O ₅ , mg/kg)	Potassium (K ₂ O, mg/kg)
Chernozem	6.5	3.2	15	45	180
Grey forest soils	5.8	2.5	10	35	150
Sod-podzolic soils	4.9	1.8	8	25	120

Source: compiled by the authors

The analysis of the soil microbiome revealed significant differences in the composition of microorganisms depending on the soil type in chernozems, the largest share of microbial biomass consists of bacteria, which account for approximately 60% of the

total mass of microorganisms. The remaining 40% is accounted for by fungi, which are also significant in maintaining the soil ecosystem by contributing to the decomposition of organic residues and improving soil structure (Table 2).

Table 2. Composition of the soil microbiome by type

Soil type	Bacteria (% of total biomass)	Mushrooms (% of total biomass)
Chernozem	60	40
Grey forest soils	55	45
Sod-podzolic soils	50	50

Source: compiled by the authors

In grey forest soils, which are characterised by high humidity, a slightly higher percentage of fungi was observed in the microbiome, which may be due to favourable conditions for the development of fungal colonies, as the moist environment promotes their growth and reproduction. Fungi in such soils can perform important ecological functions, in the processes of symbiotic relationships with plants. The sod-podzolic soils, in turn, showed the most balanced distribution between bacteria and fungi, indicating complex ecological interrelationships in their soil-absorbing complex. This balance can indicate the diversity of microbial species and their ability to adapt to specific environmental conditions, which in turn affects the overall productivity of the soil.

The results of the experiment with the detection of fungi and bacteria were as follows: the number of nitrogen-fixing bacteria *Rhizobium* and *Azotobacter* in podzolic black soils reached 6 million colony-forming units (CFU)/g of soil, which indicates a high nitrogen-fixing capacity of these soils to supply nitrogen to plants, which is critical for growth and development. In grey forest soils, the number of nitrogen-fixing bacteria was slightly lower, at 4.5 million CFU/g soil. Although these soils also provide a certain level of nitrogen, their efficiency is slightly lower compared to black soil. The sod-podzolic soils had the lowest number of nitrogen-fixing bacteria, at 3 million CFU/g soil. This may explain the limited availability of nitrogen and, consequently, lower crop yields on these soils.

The number of representatives of *Streptomyces* in podzolic chernozems reached 4 million CFU/g of soil, which contributes to the efficient decomposition of organic matter and the synthesis of beneficial metabolites that have a positive effect on plant health. In grey forest soils, the number of actinomycetes was 3 million CFU/g soil, indicating moderate activity in the decomposition of organic matter. Sod-podzolic soils showed the lowest number of actinomycetes – 2 million CFU/g soil. This may be due to lower soil productivity due to less efficient decomposition of organic materials. The number of phosphate-mobilising bacteria *Bacillus* spp. in the podzolised chernozem was 3 million CFU/g soil. This indicates an intensification of the processes of

formation of mobile phosphorus compounds, which, accordingly, contributes to an increase in the availability of this element to plant organisms. In grey forest soils, the number of phosphate-mobilising bacteria was 2.5 million CFU/g soil, which provides a certain level of phosphorus but is lower than in black soil. Sod-podzolic soils had the lowest number of phosphate-mobilising bacteria – 1.5 million CFU/g soil, which accordingly reduced the level of availability of mobile forms of this element for plants and harmed crop yields. The analysis of the results of studies on the identification of fungi in the soil-absorbing complex indicates the following pattern: 8 million spores of mycorrhizal fungi *Glomus* spp. per gram of soil were found in podzolic chernozems. Mycorrhizal fungi form symbiotic relationships with plant roots, which improves water and nutrient absorption and increases plant resistance to stressful conditions (Myronycheva et al., 2017). Grey forest soils were characterised by an indicator of 5 million mycorrhizal fungal spores per gram of soil, indicating a lower but still significant activity in improving plant growth and development. The number of mycorrhizal fungal spores in sod-podzolic soils was 4 million per gram of soil. Despite the lower number of mycorrhizal fungi, their presence still contributes to the improvement of plant conditions.

Fungi *Trichoderma* spp. was found in podzolic chernozems in the amount of 5 million spores per gram of soil. These fungi actively promote the decomposition of organic residues and increase the biological activity of the soil. Grey forest soils contained 4 million spores of such fungi per gram of soil, which demonstrates their moderate role in decomposition processes. Sod-podzolic soils were characterised by a content of 3 million spores of fungi of this group per gram of soil, which may explain the less efficient decomposition of organic materials. The presence of pathogenic fungi *Fusarium* spp. was recorded in podzolic chernozems in the amount of 2 million spores per gram of soil. Low numbers of pathogens indicate better plant resistance to disease. Grey forest soils showed the presence of 3 million pathogenic fungal spores per gram of soil, indicating a moderate level of infection risk. Sod-podzolic soils contained 4 million pathogenic fungal spores per

gram of soil. Higher numbers of pathogens can cause increased plant stress and lower yields.

The results of the analysis showed that a higher number of nitrogen-fixing bacteria and mycorrhizal fungi in chernozems contributes to higher crop yields. Podzolised chernozems with a high content of phosphate-mobilising bacteria and mycorrhizal fungi provide the highest quality and quantity of crops (Biya-shev *et al.*, 2024). Grey forest soils and sod-podzolic soils show a lower number of beneficial microorganisms, which partly explains their reduced productivity and slightly lower product quality. The high number of pathogenic fungi in sod-podzolic soils can harm plant health and yield (Havryliuk *et al.*, 2024). The study's analysis of wheat, corn and soybean yields showed that soil type has a significant impact on the productivity of these crops. Podzolic black soils showed the highest yields for all three crops. *Kalbex* wheat grown on podzolised black soil yielded 50 centners per hectare, *Rhodes* corn 80 centners, and *Kingstone* soybeans 30 centners. This is due to the high humus content of chernozems and their favourable physical and chemical properties, such as optimal pH and high forage retention capacity. Black soil promotes better development of the root system of plants and provides them with essential nutrients, which in turn leads to high yields

(Pichura *et al.*, 2023; Voitovyk *et al.*, 2023). The quality of grain obtained from plants grown on black soil was also the best. The weight of 1,000 grains of wheat was 38 grams, corn 250 grams, and soybeans 200 grams.

The yields of crops grown on sod-podzolic soils were the lowest. *Kalbex* wheat yielded 40 centners per hectare, *Rhodes* corn 70 centners and *Kingstone* soybeans 25 centners. The low humus content and poor moisture retention in these soils limit crop productivity. Grain quality was also the lowest, with wheat weighing 33 grams per thousand grains, corn 230 grams and soybeans 180 grams. Grey forest soils had intermediate yields. The *Kalbex* wheat yielded 45 centners per hectare, *Rhodes* corn 75 centners, and *Kingstone* soybeans 28 centners. Grain quality was slightly lower than on podzolic black soil but better than on podzolic soils. The correlation analysis conducted as part of the study revealed a significant positive correlation between the number of bacteria in the soil and crop yields (Fig. 1). This result can be explained by the fact that bacteria play an important role in improving soil quality, as they contribute to the synthesis and mineralisation of organic matter and increase the availability of important nutrients such as nitrogen and phosphorus. These processes allow plants to absorb nutrients more efficiently, which in turn leads to higher yields.

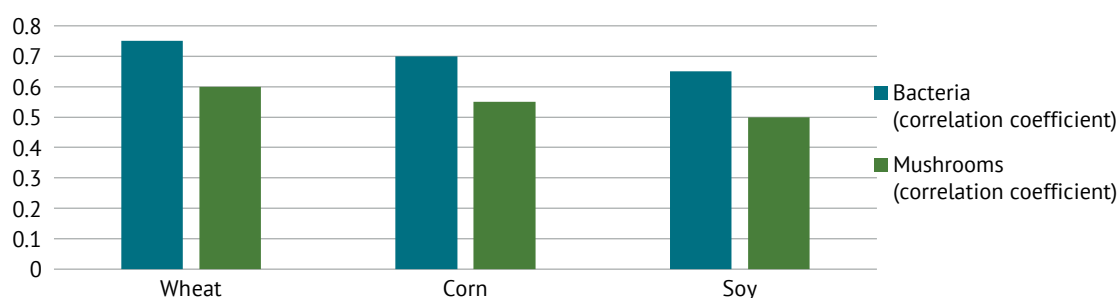


Figure 1. Correlation between soil microbiome and crop yields

Source: compiled by the authors

As for the correlation between the number of fungi in the soil and crop yields, it was less pronounced but still has some positive aspects. Although the impact of fungi on yields is less significant than that of bacteria, their presence can also have a positive impact on the overall health of the soil and plants. Thus, the results of the study emphasise the importance of soil microbiological composition for agronomic productivity. The study also examined the dynamics of changes in the physical and chemical properties of the soil during the growing season of crops. It turned out that podzolised chernozems, which have a high humus content and neutral pH, retain their optimal properties throughout the season. At the beginning of the experiment (in March), the humus content in the podzolic chernozems was 3.2%, and the soil solution reaction index (pH) was 6.5. At the end of the research (October), these indicators

decreased to 3% and 6.4, respectively, which indicates a slight decrease in organic material and a decrease in pH. However, the studied parameters remained within the optimal range.

In March, grey forest soils were characterised by a lower humus content (2.5%) and a close to neutral soil solution pH (5.8 units). At the end of the growing season (October), these figures decreased to 2.3% and 5.7 units, respectively. This indicates a slight decrease in the content of minor acidification of the soil environment, which can affect the efficiency of nutrient uptake by plants. The sod-podzolic soils had the lowest humus content (1.8%) and were characterised by a medium acid reaction of the soil solution (pH 4.9 units). At the end of the season, these indicators decreased to 1.7 and 4.8%, respectively. This indicates a low level of fertility and the need for pH correction to improve conditions for plants.

The study of the quality of the crop showed that black soils provide the best results. For instance, wheat grain grown on these soils contained 12.5% protein and, accordingly, provided the highest evaluation scores. Grain produced on grey forest soils was characterised by lower values: 11.8% protein and slightly lower quality. Wheat grain products produced on sod-podzolic soils had the worst results: 11.2% protein and the lowest grain quality scores. A similar situation was observed concerning the sucrose content in corn grain. Thus, in the products grown on podzolic black soil, the content of this indicator was 6.5% with the highest evaluation scores. The sucrose content in the grain grown on grey forest soils was 6% and in sod-podzolic soils – 5.5%. As for soybean grain, when grown on podzolic black soils, the protein content was 38%, while on grey forest soils, it was 37.5%, and on sod-podzolic soils, it was 37%.

The analysis of the above data showed that different soil types and microbiome composition have a significant impact on crop productivity. Podzolic chernozems, which are characterised by a high humus content and favourable conditions for the development of beneficial microorganisms, show the highest yields. This confirms their high agronomic value and importance for agricultural production. Considering the microbiological aspects of the soil can be a key factor in improving the efficiency of agronomic practices and optimising crop yields in a changing climate.

DISCUSSION

A detailed study focusing on the effect of probiotic bacterial consortia on plant growth was conducted by J. Hu *et al.* (2021). They focused on their impact on the rhizosphere microbiome. Our results confirm that modifying the microbiome by introducing probiotic consortia can significantly improve plant growth. These findings are consistent with the present study, which indicates the importance of microbiological factors in plant development. In addition, Z. Ruan *et al.* (2024) studied the application of microbiome modelling in the context of improving bioremediation, which is an important topic in ecology. The results of their study confirm that appropriate management and modelling of microbiomes can have a positive impact on the functioning of agroecosystems, as well as on the agronomic characteristics of crops. Thus, the integration of microbiological approaches into agronomic practice has the potential to increase agricultural efficiency and preserve the environment.

A detailed analysis of the patterns of microbial community recovery in different environments was carried out by S. Jurburg *et al.* (2024), which includes both natural and anthropogenic ecosystems. This research is relevant for determining how microbiomes influence the resilience of agroecosystems to change, particularly in the face of climate change and intensive land use. The study of microbial communities can provide valuable information on how they interact with plant

species and other organisms, which in turn can contribute to the development of more effective agroecosystem management strategies. L. Philippot *et al.* (2021) addressed the resilience of microbial communities to environmental changes and stressors. The results of this study confirm that soil microbiomes have a significant ability to adapt to environmental changes, which is critical for maintaining ecological balance. These findings highlight the importance of microbial communities in ensuring ecosystem resilience and can serve as a basis for further research in agronomy and ecology.

The effects of priority in the formation of microbiomes, which is a highly important aspect of understanding ecosystem processes, were studied by R. Debray *et al.* (2022). The study determined that the first microbiomes that inhabit the soil can significantly influence the further development and formation of subsequent microbial communities. This observation highlights the importance of initial conditions in shaping soil biodiversity, which can have far-reaching implications for agronomy and ecology. P. Jiang *et al.* (2024) analysed the impact of intercropping on maize growth and nutrient uptake, addressing the relationship between rhizosphere metabolites and microbiomes. This study confirms the results of previous research indicating that agronomic practices, such as organic fertilisation, can significantly alter the structure of the soil microbiome. This, in turn, affects plant health and growth, which is critical for high yields and sustainable agriculture.

A detailed study of the changes in microbial communities that occur during long-term intercropping was conducted by R. Ablimit *et al.* (2022). This study highlights how the long-term use of certain agronomic practices, such as fertilisation, can significantly affect the composition and functioning of the soil microbiome. Changes in microbial communities can have far-reaching consequences for soil fertility, disease resistance and the overall ecological balance in agroecosystems (Khavkhun, 2024). The impact of phosphate fertilisers on the soil microbiome and its metabolic functions was studied by H. Cheng *et al.* (2022). The results of this study confirm the existing findings on the importance of proper fertilisation, as it can significantly affect the health of microbiomes. In particular, the study determined that excessive or insufficient fertiliser application can lead to an imbalance in microbial communities, which in turn can negatively affect soil fertility and the environmental sustainability of agricultural systems.

The factors that influence differences in the distance-decreasing relationships of microbiomes were considered in detail, in the context of environmental conditions and biodiversity, by D. Clark *et al.* (2021). The results of the study confirm that these differences can have a significant impact on the functioning of microbiomes in different environments, which in turn can affect the overall health of ecosystems and their resilience to environmental change. This study highlights

the importance of understanding microbiomes for developing strategies for the conservation and restoration of natural resources. P. Neuberger *et al.* (2024) studied the role of arbuscular mycorrhizae in soils, focusing on their ability to improve nutrient availability to plants. This supports these findings on the importance of mycorrhizae for improving soil and plant health, in the context of increasing soil fertility and reducing the need for chemical fertilisers. P. Neuberger *et al.* emphasise that arbuscular mycorrhizae not only promote plant growth but also play an important role in maintaining ecological balance, making them indispensable components of healthy agroecosystems. The results of the study by Y. Qiao *et al.* (2024) proved that the accumulation and application of organic residues, such as straw, is a factor in enriching soil microbiomes and increasing their biodiversity.

The integration of phenotypes into microbiome networks for the design of synthetic communities, focusing on the potential of microbiome networks to improve agronomic practices, was studied by R. Poudel *et al.* (2023). This study confirms the findings that structured microbiome communities can be used to optimise agronomic processes such as biological pest control and soil fertility, opening new opportunities for sustainable agriculture and organic farming. T. Aizi *et al.* (2023) carried out a detailed analysis of the structure of microbial communities existing in the rhizosphere of *Panax ginseng* and investigated soil properties in larch forests. This study not only adds to existing results but also demonstrates how rhizosphere microbiomes can vary depending on specific soil conditions and temporal changes. The importance of this study is to emphasise the contextual factors that significantly affect the formation and functioning of microbiomes. Moreover, L. Mason *et al.* (2023) studied changes in the composition of microbial communities in the root zone during intercropping. These results confirm previous data indicating that intercropping can significantly affect soil microbiomes. The study by L. Mason *et al.* emphasises the importance of agronomic practices in the formation of sustainable and balanced soil ecosystems, which in turn can contribute to increased crop yields and sustainability.

A detailed study examining the effect of cultivation duration on the aggregation of microbial taxa was conducted by Z. Shi *et al.* (2023). In their work, the authors also emphasise that the duration of agronomic practices can significantly affect the structure and functioning of microbiomes. The results demonstrating this dependence are consistent with previous findings by other researchers, which indicates the importance of long-term use of certain agronomic practices to optimise microbiomes in soil. In turn, V. Pandey *et al.* (2023) conducted a comprehensive analysis of the history and role of microbiomes in plant improvement, focusing on their ability to increase plant resistance to stress and

improve growth performance. This study confirms the findings of previous research that indicates the long-term benefits of using microbiomes to improve plant health. The importance of microbiomes in modern agriculture is becoming increasingly evident, as they are central to ensuring food security and sustainable development of the agricultural sector.

The results of the study are consistent with the findings of other scientists, who confirm that soil microbiomes are an important component of pedospheric biodiversity and a powerful factor in shaping crop yields. The importance of microbiomes in ensuring the sustainability of agroecosystems is critical in the context of climate change and global challenges in agriculture. The results obtained can serve as a basis for further research in microbiome science and agronomy, opening new opportunities for the development of environmentally friendly agricultural technologies and soil management strategies that will contribute to the sustainable development of the agricultural sector.

CONCLUSIONS

The study assessed the state of microbiome biodiversity in different agricultural conditions of the Sumy region and conducted their inventory. A significant influence of soil microbiomes on the yield and quality of crops, in particular, wheat “*Kalbes*”, corn “*Rhodes*” and soybeans “*Kingston*”, was established. The highest yields were recorded when growing crops on podzolic black soil: wheat reached 50 cwt/ha, corn – 80 cwt/ha, soybeans – 30 cwt/ha, which indicates optimal conditions for plant growth and development on this type of soil. Grey forest and sod-podzolic soils showed lower yields, which is due to their lower biological activity and less favourable physical and chemical characteristics. In particular, the number of nitrogen-fixing bacteria *Rhizobium* and *Azotobacter* and mycorrhizal fungi *Glomus* spp. was significantly higher in podzolic chernozems, which had a positive impact on the availability of nutrients for plants. The content of humus and mobile forms of phosphorus was also the highest in these soil types, confirming their high fertility.

The correlation analysis revealed significant positive correlations between the number of bacteria in the soil and crop yields, as bacteria contribute to soil quality, decomposition of organic matter and increased availability of nutrients. The correlation between the number of fungi in the soil and yields was less pronounced, but mycorrhizal fungi are known to have a positive effect on the ability of plants to absorb water and nutrients. The results of the study emphasise the importance of optimal soil microbiological composition for agronomic productivity. However, the study has certain limitations, such as the localised range of soil conditions and crops, which may affect the generalisability of the results. To further improve the results, it is recommended to expand the study to other soil types

and crops, as well as to address seasonal fluctuations and the long-term impact of various agronomic practices on soil microbiomes. This will be used to assess the impact of microbiomes on agroecosystems and develop more effective methods for increasing crop productivity. None.

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CONFLICT OF INTEREST

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Мікробіоми ґрунту як складова біорізноманіття педосфери та чинник формування врожайності сільськогосподарських культур

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Анотація. Метою даного дослідження було оцінювання механізмів взаємодії між мікробіомами ґрунту та провідними культурами для оптимізації врожайності і якості продукції у процесі сільськогосподарського виробництва. У ході дослідження проведено всебічний аналіз фізико-хімічних властивостей ґрунтів (чорноземи, сірі лісові та підзолисті) та склад мікробіому ґрунту, включаючи чисельність азотфіксуючих бактерій *Rhizobium* і *Azotobacter*, бактерій *Bacillus* spp., представників роду *Streptomyces*, грибів *Glomus* spp. Найвищі показники врожайності пшениці сорту *Kalbex* (50 ц/га), кукурудзи Родос (80 ц/га) та сої *Kingstone* (30 ц/га) були зареєстровані при вирощуванні цих культур на чорноземах. Сірі лісові ґрунти і підзолисті ґрунти продемонстрували меншу продуктивність та якість врожаю. Чисельність азотфіксуючих бактерій *Rhizobium* і *Azotobacter* у чорноземах досягала 6 мільйонів колонієутворюючих одиниць (КУО) на грам ґрунту, тоді як у підзолистих ґрунтах вона була найнижчою – 3 мільйони КУО на грам ґрунту. Мікоризні гриби *Glomus* spp. також були найбільш чисельні в чорноземах – 8 мільйонів спор на грам ґрунту. У дослідженні вивчався вплив мікробіому ґрунтів на врожайність обраних культур. Був проведений кореляційний аналіз мікробіому та врожайності. Проаналізовано вплив мікробіому ґрунту на якість врожаю. Отримані результати підтверджують, що чорноземи забезпечують найкращі умови для вирощування культур завдяки високій біологічній активності ґрунту та оптимальним фізико-хімічним властивостям, що робить їх ідеальним вибором для агрономічної практики

Ключові слова: біорізноманіття; метагеноміка; агроєкосистеми; педосфера; агропромисловий комплекс; біогеохімія; рослинництво

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Improving operational efficiency analysis through marketing performance (*Cymbopogon Nardus* L.)

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Abstract. The agricultural sector, particularly citronella oil production, is pivotal to Indonesia's economic growth and export potential. The marketing efficiency of citronella oil in Rokan Hulu Regency, Riau Province was analysed to optimise the contribution of the sector to the economy. A descriptive method alongside qualitative and quantitative approaches, based on statistically analysed primary and secondary data, were used. Five marketing channel strategies were examined, revealing variations in production costs, income generation, and profitability across different channels. The market structure for citronella oil in Rokan Hulu Regency was characterised as oligopsony on both the seller's and buyer's sides, indicating a concentrated market with limited buyers and sellers. Marketing channel I demonstrated the best performance, with the lowest production cost of IDR 477,479.17 per kg, the highest farmer's share of 95.24%, and the best profit-to-cost ratio of 9.35. This channel also had the smallest margin of IDR 8,000 per kg and generated the highest income of IDR 2,400,000 per harvest. In contrast, marketing channel III incurred the highest costs at IDR 1,105,451.26 per kg. Overall, the marketing of citronella oil in Rokan Hulu Regency was found to be efficient, with selling prices remaining relatively profitable for farmers, especially through shorter marketing channels. The efficiency of marketing channels was assessed based on price, with more efficient pricing indicating a better market structure. The study also analysed the marketing functions performed by various institutions, including exchange, physical, and facility functions, which contribute to the overall efficiency of the marketing system. These findings can be utilised by policymakers, agricultural extension services, and farmers to improve marketing strategies and increase the

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efficiency of citronella oil production in the region, ultimately contributing to the development of Indonesia's agricultural export sector and enhancing the economic well-being of citronella farmers in Rokan Hulu Regency

Keywords: citronella oil; farmers' profitability; agricultural sector of Indonesia; agromarketing; marketing channel

INTRODUCTION

The agricultural sector remains a cornerstone of Indonesian economy, supported by the tropical climate, which fosters fertile natural resources. This unique environmental condition enhances agricultural cultivation, making it a more efficient sector. Consequently, the agricultural sector significantly affected Indonesian economic growth, contributing to both domestic food security and export potential. As a mainstay economic sector, Citronella (*Cymbopogon Nardus* L.) is an important essential oil-producing plant with high economic value that can grow well on marginal lands. Indonesia is currently the third largest supplier of citronella oil globally, after China and Taiwan. The average global demand for citronella oil reaches 2,000-2,500 tons per year (Żukowska & Durczyńska, 2024).

The marketing system for citronella in Indonesia faces several challenges, including fluctuating prices, limited market access for farmers, and inefficient supply chains. In the Rokan IV Koto District of Rokan Hulu Regency, citronella has emerged as a significant agricultural commodity, yet farmers often struggle to secure equitable prices and optimise profits due to these inefficiencies. Research by D. Chiarelli *et al.* (2018) and E. Ngoben and M. Muchopa (2023) underscores that the food and plantation sectors in Indonesia are not only meeting domestic demands but also achieving substantial export volumes, motivating farmers to cultivate crops with export potential. The efficiency of marketing agricultural products was analysed in various studies. S. Dimitrijević (2023) highlighted the necessity of enhancing agricultural productivity through improved product quality to achieve higher market prices. Marketing efficiency, as detailed by I. Akite *et al.* (2022), is central in reducing costs and boosting farmers' profits. Nevertheless, traditional agricultural practices and the limited adoption of modern farming technologies frequently obstruct productivity.

Several studies explored marketing performance across diverse agricultural contexts. For instance, Y. Methamontri *et al.* (2022) demonstrated that agricultural marketing efficiency is influenced by socio-economic and institutional dynamics, noting that direct marketing channels, especially for organic rice, can yield higher profits for farmers by bypassing intermediaries. Similarly, F. Wanyoike *et al.* (2023) assessed the sustainability of beef marketing chains, identifying that livestock stock capacity, feed availability, and disease management significantly affect marketing efficiency and farmer income. Despite the breadth of existing research, there is a distinct

lack of studies on citronella marketing performance, particularly in Riau Province and the Rokan IV Koto District. This unique crop, valued for its essential oil properties, should be further analysed in the context of inefficiencies and identification of improvements. M. Mehla *et al.* (2023) argued that aligning product quality with market demands is critical for increasing sales prices and improving farmer income. Additionally, N. Habiyaemye *et al.* (2023) asserted that lowering marketing costs not only enhances profitability but also supports increased sales volumes and overall farmer welfare.

The market potential of citronella is evident, supported by a growing demand for citronella oil, with an annual average demand ranging from 2,000 to 2,500 tonnes (Sarah *et al.*, 2023). To capitalise on this demand, improvement of citronella oil productivity must correlate with market trends. However, as noted by F. Gharsan *et al.* (2022), traditional cultivation practices and the low adoption of advanced agricultural technologies continue to impede productivity. Addressing these challenges necessitates a detailed evaluation of the citronella marketing in Rokan IV Koto District, incorporating insights from other agricultural contexts to develop strategies for enhancing marketing efficiency and farmer profitability.

The study aimed to conduct a thorough analysis of citronella marketing performance in Rokan IV Koto District by examining marketing channels, marketing functions, market structure, and marketing efficiency.

MATERIALS AND METHODS

This research was conducted during 2023 in Rokan Hulu Regency as one of the citronella cultivation centers in Riau Province. The census method was to collect information from 100 farmers and traders of citronella. The choice of research location was determined by purposive sampling of citronella farmers in Rokan hulu regency as the central area for citronella cultivation which produces citronella oil.

Qualitative and quantitative descriptive analysis was employed in data analysis. Qualitative descriptive analysis was conducted by observation of the characteristics of marketing channels, institutions, functions and structure. Quantitative descriptive method was employed to conduct a farming analysis and determine marketing efficiency based on marketing margin approach, farmer's share, and profit and cost ratios.

It was calculated the total cost of farming using a formula:

$$TC = FC + VC, \quad (1)$$

where TC – Total Cost; FC – Fixed Cost; VC – Variable Cost.

The result of dividing total costs by the number of products produced. The average cost of farming was calculated using a formula:

$$AC = \frac{TC}{Q}, \quad (2)$$

where AC – Average Cost; TC – Total Cost; Q – Quantity.

Analysis of farming revenues was calculated using a formula:

$$TR = P \times Q, \quad (3)$$

where TR – Total Revenue; P – Price per unit; Q – Quantity.

Analysis of farming income was calculated using the formula:

$$\pi = TR - TC, \quad (4)$$

where π – Farming income (benefit of farming system); TR – Total Revenue; TC – Total Cost.

Marketing Margin Analysis for the efficiency of citronella oil commodities is therefore as follows:

$$\text{Marketing Margin Ratio} = \frac{\text{Total Marketing Margin}}{\text{Selling Price}}. \quad (5)$$

Farmer's Share analysis is formulated as follows:

$$\text{Farmer's Share} = \frac{\text{Price at Farm Level}}{\text{Final Consumer Price}} \times 100\%. \quad (6)$$

Revenue Cost Ratio is formulated as follows:

$$R/C = \frac{TR}{TC}, \quad (7)$$

where R/C – Revenue-Cost Ratio; TR – Total Revenue; TC – Total Cost.

The following is the formula for the Profit and Cost Ratio (B/C):

$$\text{Profit Ratio} = \frac{\text{Profit}}{\text{Marketing costs}}. \quad (8)$$

This methodological framework was used for a comprehensive analysis of the efficiency of citronella farming and marketing channels, providing actionable insights for improving profitability and sustainability in the agricultural sector.

RESULTS

The study determined that farmers in channel III of table 1 contain fixed cost Rp. 34,135.47 per kg, variable Rp. 1,071,315.79 per kg and total cost 1,105,451.26 per kg. Therefore, the costs incurred were higher, which is proportional to the oil production obtained in this channel. Variable costs are than fixed, as notable from the table with a percentage level of 90.06-97.74%.

Table 1. Average total costs of citronella farmers in Rokan Hulu Regency

Cost breakdown	Cost (Rp/Kg)	Percentage (%)
Channel I		
Fixed Cost	47,479.17	9.94
Variable Costs	430,000.00	90.06
Total	477,479.17	100
Channel II		
Fixed Cost	23,671.01	2.26
Variable Costs	1,025,000.00	97.74
Total	1,048,671.01	100
Channel III		
Fixed Cost	34,135.47	3.09
Variable Costs	1,071,315.79	96.91
Total	1,105,451.26	100
Channel IV		
Fixed Cost	40,327.26	3.70
Variable Costs	1,050,500.00	96.30
Total	1,090,827.26	100
Channel V		
Fixed Cost	48,082.96	5.19
Variable Costs	878,750.00	94.81
Total	926,832.96	100

Source: compiled by the authors

Based on production and sales prices per unit of production, the average revenue from citronella farming per harvest can be obtained. Farmers' income in the research area varies depending on the amount of

citronella oil produced and the selling price prevailing at that time. The highest cost was from IV channel, amounting to Rp. 3,026,800.00, and the smallest revenue in channel I was IDR. 2,400,000.00 (Table 2).

Table 2. Average revenue of citronella farmers in Rokan Hulu Regency

Marketing channel	Oil Production	Retail Price (Rp/Kg)	Revenue
Channel I	15.00	160,000.00	2,400,000.00
Channel II	16.20	160,000.00	2,592,000.00
Channel III	16.63	160,105.26	2,663,473.68
Channel IV	18.90	160,200.00	3,026,800.00
Channel V	17.50	160,500.00	2,809,500.00

Source: compiled by the authors

The highest average income of citronella farming in the research area is channel IV, amounting to Rp. 1,935,972.74, and the lowest revenue is channel II at Rp. 1,543,328.99 (Table 3). Total revenue is greater than total costs incurred. Therefore, all costs incurred in the production process of citronella farming can be covered by farming income. As such,

citronella farming has economic value for farmers in the research area. The average results obtained from respondents' incomes are high enough to be used to meet living needs and support household income. As such, when commodity prices decline, it greatly affects the welfare of farmer households in the research area.

Table 3. Average total income of citronella farmers in Rokan Hulu Regency

Marketing channel	Revenue (Rp/Kg)	Total Cost (Rp/Kg)	Income (Rp/ Harvest)
Channel I	2,400,000.00	477,479.17	1,922,520.83
Channel II	2,592,000.00	1,048,671.01	1,543,328.99
Channel III	2,663,473.68	1,105,451.26	1,558,022.43
Channel IV	3,026,800.00	1,090,827.26	1,935,972.74
Channel V	2,809,500.00	926,832.96	1,882,667.04

Source: compiled by the authors

The calculation results obtained values R/C Ratio among them are as follows: channel I is relatively more feasible as the R/C value is the highest – 5.03, hence every Rp. 1. The farming costs incurred resulted

in a profit of Rp. 4.03. Meanwhile, the lowest value is in channel III, namely 2.41, meaning every Rp. 1, spent will get a profit of Rp. 1.41. Based on established criteria (Table 4).

Table 4. R/C of citronella farmer in Rokan Hulu Regency

Marketing channel	Revenue (Rp/Kg)	Total Cost (Rp/Kg)	Revenue Cost Ratio (R/C)
Channel I	2,400,000.00	477,479.17	5.03
Channel II	2,592,000.00	1,048,671.01	2.47
Channel III	2,663,473.68	1,105,451.26	2.41
Channel IV	3,026,800.00	1,090,827.26	2.77
Channel V	2,809,500.00	926,832.96	3.03

Source: compiled by the authors

The calculation results obtained the highest B/C Ratio value of 4.03. This condition means that every cost incurred is IDR. 1, will get a profit of Rp. 4.03. In channel I and channel III the lowest B/C value is 1.41, meaning the costs incurred are Rp. 1, get a profit of Rp. 1.41.

Thus, it is possible to conclude that citronella farming in Rokan Hulu Regency should be further developed (Table 5). This profit is derived from the farmer's income based on the selling price paid by the farmer and the amount of production produced.

Table 5. B/C of citronella farmer in Rokan Hulu Regency

Marketing channel	Income	Total Cost (Rp/Kg)	Benefit Cost Ratio (B/C)
Channel I	1,922,520.83	477,479.17	4.03
Channel II	1,543,328.99	1,048,671.01	1.47
Channel III	1,558,022.43	1,105,451.26	1.41
Channel IV	1,935,972.74	1,090,827.26	1.77
Channel V	1,882,667.04	926,832.96	2.03

Source: compiled by the authors

Based on the research results, there are 5 marketing channels for citronella oil in Rokan Hulu Regency and several marketing institutions. Differences in marketing channels, including variations in capital owned as well as differences in transportation access were revealed (Patil & Rastog, 2019). The production of citronella oil was 100 respondents per harvest 752 kg. The marketing pattern for citronella oil that is formed is:

- Channel pattern I: Farmers – Village Gathering Traders (PPD) – Consumer;
- Channel pattern II: Farmers – Village Gathering Traders (PPD) – Wholesalers;
- Channel pattern III: Farmers – District Collector Traders (PPK) – Wholesalers;
- IV channel pattern: Farmers – Agents – Wholesalers;
- V channel pattern: Farmers – Village Gathering Traders (PPD) – District Collector Traders (PPK) – Wholesalers

In general, the marketing functions of citronella oil were classified into 3 categories, namely exchange functions, physical functions and facility functions. Based on the results of observations and interviews, most marketing functions are done by marketing institutions. Marketing institutions perform an exchange function in the form of sales activities, physical functions in the form of transportation and storage activities and facil-

ity functions in the form of sorting and grading activities, market information and risk management.

The implementation of the marketing functions of each marketing institution aims to provide added value and facilitating the distribution of citronella oil from farmers to consumers. Marketing institutions have quality standards and different facilities for marketing functions (Walker & Ruekert, 1987; Satola & Milewska, 2022). This process will later influence the value obtained by each marketing institution which is measured based on the price level obtained. On the other hand, the implementation of marketing functions will influence the costs of each marketing institution in the marketing process of citronella oil.

The structure of the marketing market for citronella oil determines the characteristics of marketing institutions that influence the nature of competition and the price of citronella oil on the market. The market structure was analysed using qualitative methods (Rupp *et al.*, 2021). Market structure includes the number of seller and buyer respondents, the nature or heterogeneity of the product, simplicity of market introduction, the level of market information known by seller and buyer respondents and the form of competition. A demonstration of the market structure faced by citronella marketing institutions in Rokan Hulu Regency is provided in Table 6.

Table 6. Market structure faced by marketing institutions in Rokan Hulu Regency

Characteristics				
Seller	Farmer	PPD	PPK	PB
Buyer	PPD, PPK, and Agent	PPK, PB dan Consumer	PB	advanced institutions
Entering and exiting the market	Low	Low	Low	High
Number of sellers/buyers	A lot/ Quite a lot	Quite a lot/ Quite a lot	Little/ Little	Little / Quite a lot
Product properties	Homogeneous	Homogeneous	Differentiation	Differentiation
Market information	Easy	Easy	Easy	Easy
Market Structure				
Seller Side	Oligopsony	Oligopsony	Oligopsony Differentiation	Oligopoly
Buyer Side	Oligopsony	Oligopoly	Oligopoly Differentiation	Oligopsony

Source: compiled by the authors

The marketing margin in this research is calculated based on the five marketing channel schemes for citronella oil. The margin calculation includes the sales price (P_{ji}) minus the purchase price (P_{bi}). The lowest margin

is in channel I, namely IDR. 8,000 kg and has a large margin difference with the other four channels. Analysis of the costs incurred demonstrated that channel one also incurs the smallest costs in comparison to other

channels. This shows that farmers who sell citronella oil to Village Collecting Traders (PPD) and to consumers directly result in smaller margins (Otto *et al.*, 2020).

The results of the farmer's share analysis in this research show that the largest share obtained by farmers is in channel I. The average farmer's share in channel I is 95.24%. Channel I has fewer marketing institutions involved, therefore share received by farmers is greater. The average share in the smallest channel received by farmers is channel V at 84.47%. If the share obtained by farmers in a marketing channel is higher, the marketing channel can be considered more efficient than other channels.

Analysis of the ratio of benefits and costs in this research determined that marketing channel I is 9.35. This condition illustrates that every Rp. 1, marketing costs incurred will provide a profit of Rp. 9.35. The profit and cost ratio in marketing channel II is 2.58, which

means every Rp. 1, the marketing costs incurred will provide a profit of Rp. 2.58. Furthermore, the profit and cost ratio in marketing channel III is 3.97, which means every Rp. 1, the marketing costs incurred will provide a profit of Rp. 3.97. The profit and cost ratio in marketing channel IV is 2.42, which means every Rp. 1, the marketing costs incurred will provide a profit of Rp. 2.42. Lastly, the profit and cost ratio in marketing channel V is 1.23. This means that every Rp. 1, marketing costs incurred only provide a profit of Rp. 1.23. It is possible to conclude that the results of the profit and cost ratio analysis show that marketing channel I is relatively more efficient. This is determined by the largest profit and cost ratio being 9.35. Meanwhile, marketing channel V has the smallest profit to cost ratio of 1.23. The concept of marketing efficiency refers to the price efficiency approach. This approach illustrates that the more efficient the price, the better the level of market structure.

Table 7. Comparison of marketing efficiency values for citronella oil marketing channels in Rokan Hulu Regency

Marketing channel	Total Marketing Costs (Rp/Kg)	Retail Price (Rp/Kg)	Marketing Efficiency (%)
Channel I	1,713.00	168,000.00	0.46
Channel II	6,454.00	185,000.00	3.78
Channel III	4,601.00	185,000.00	2.71
Channel IV	6,681.00	185,000.00	3.92
Channel V	13,865.00	190,000.00	6.96

Source: compiled by the authors

Based on the indicator, an efficient value of <5 percent is efficient. while an efficient value of >5% is defined as inefficient. In Table 4, the most efficient channel is channel I at 0.46%. On the other hand, inefficient marketing channels are channel V at 6.96%. Based on the research results, there are 5 marketing channels for citronella oil in Rokan Hulu Regency. Analysis of the operational efficiency of marketing citronella oil from the five channels is as follows:

In marketing channel I, farmers sell their harvest to PPD (Village Collecting Traders) and then to consumers. The PPD involved in channel I amounted to 3 respondents. The buying and selling process of citronella oil is done at the PPD Warehouse and is based on stock availability according to consumer demand. Consumers contact PPD to buy products. The average amount of citronella oil marketed is only 15/kg or 2%. The sales volume of citronella oil in marketing channel I is small from the total sales. The quantity of citronella oil sales in marketing channel I is dependent on direct consumers. The price received by farmers on this channel is IDR. 160,000/kg, while the price received by consumers is Rp. 168,000/kg.

In marketing channel II, the institutions consist of PPD and Wholesalers (PB). In marketing channel II, the number of PPD consisted of 19 respondents, and PB consisted of 2 respondents. Consumers bought citronella oil on average 162/kg or 21.54% of the total

production of 100 respondents. PPD then sells directly without the intermediary of other marketing institutions to large traders at a price of Rp. 165,000/kg while the price obtained from farmers is Rp. 160,000/kg.

Marketing channel III involves three marketing institutions. Those involved included 41 farmers, 1 sub-district collecting trader (PPK) and 1 large trader. This condition renders marketing channel III the dominant marketing channel in terms of the number of parties involved. The average amount of citronella oil sold on this channel reached 316/kg or 42.02%. The sales volume in marketing channel III is the largest volume in marketing citronella oil in Rokan Hulu Regency. The price received by farmers is IDR 162,000/kg, while the price purchased by wholesalers is IDR 168,000/kg.

In marketing channel IV there are several parties involved, including 26 farmers, 2 agents from the sub-district level and 1 wholesaler in Rokan Hulu district. Prices are determined prices are completely determined by agents and wholesalers. The price obtained by farmers is IDR 160,000/kg. It was recorded that 189 kg or 25.13% were sold by farmers to agents and then resold to wholesalers at a price of IDR 170,000/kg.

Marketing channel V is the channel that mostly involves citronella oil marketing institutions in Rokan Hulu Regency, including farmers, Village Collecting Traders (PPD), District Collecting Traders (PPK), Wholesalers. Farmers sell citronella oil for 9.31% of the total

oil production in Jepara Regency. The price received by farmers from channel V is IDR. 160,000/kg, while the price provided by wholesalers is Rp. 175,000/kg. Berdasarkan efisiensi pemasara biaya operasional, saluran pemasaran I mengeluarkan biaya sebesar Rp 1.333/kg. While marketing channel 2 operational costs were Rp 1,543/kg, marketing channel 3 reaches a cost of 1,543 / kg. Furthermore, marketing costs 4 and 5 are Rp 1,323/kg and Rp 1,143/kg, respectively.

The analysis of citronella oil marketing channels in Ogan Ilir District, Indonesia, identified three marketing channels, which are fewer than the five channels found in this study. However, there are similarities in structure. Their channels involved farmers selling to collecting traders, similar to Channel I where farmers sell to Village Collecting Traders (PPD). The marketing margins in this study (ranging from IDR 8,000 to 15,000/kg) are significantly smaller than in the study by H. Malini *et al.* (2023). Margins range from IDR 30,000 to 250,000/L across three channels. This suggests that the marketing channels in Rokan Hulu Regency is more efficient in terms of price transmission from farmers to consumers.

Regarding the dominance of certain channels, this study found that Channel III, involving sub-district collecting traders (PPK), handled the largest volume (42.02%) of citronella oil. This differs from the findings of Syafruddin *et al.* (2022), T. Rosol *et al.* (2023) who studied citronella oil marketing in Aceh. Authors determined that channels involving district-level traders handled the bulk of the product, suggesting regional variations in marketing structure efficiency. While these findings share some similarities with other studies on essential oil marketing in Indonesia, they also highlight unique aspects of the citronella oil market in Rokan Hulu Regency. The efficiency of shorter channels, the significant role of sub-district level traders, and the relatively stable pricing across channels distinguish this market from those in other regions, emphasising the importance of local context in agricultural marketing studies.

Improving the operational efficiency of marketing in the agricultural sector is a substantial challenge, especially for citronella plants. On the other hand, the marketing potential for the selling price of citronella plants is high once processed into citronella oil. The primary marketing channels used are direct and indirect. Direct marketing channels are used by farmers with substantial production capacities and access to final consumers. This marketing channel has the advantage of high selling prices, resulting in large profits. However, this marketing also suffers from higher marketing costs.

Meanwhile, indirect marketing channels are generally used by farmers who have small capacity and do not have direct access to final consumers. However, this marketing channel has the advantage of lower costs. The weakness that occurs is that farmers cannot gain large profits. The marketing efficiency of citronella oil can be measured using several indicators, such as

marketing margin, farmer's share and profit and cost ratio. The marketing efficiency of agricultural sector commodities was conducted by several experts, including by Kh. Mehrez *et al.* (2023). Research on Saudi coffee commodities was conducted in the Jazan Khawlani area. This research analysed the efficiency of the coffee marketing chain strategy from farmers, producers, traders, retailers, to final consumers. Good presentation of coffee attracts consumer interest, which yields higher profits. However, the longer the marketing chain will reduce the profits received by farmers. The effectiveness of the marketing chain is indirect marketing, but the profits received by farmers are low.

Furthermore, research conducted by Y. Methamontri *et al.* (2022) demonstrated that the marketing efficiency of the agricultural sector is influenced by social, economic and institutional levels. Organic rice can be highly effective in marketing if it is marketed in institutions with direct consumers, institutional members. Farmers will gain higher profits if the marketing chain is consumed directly by consumers. The sustainability of the beef animal marketing chain is influenced by animal stock capacity, the availability of grass as a feed ingredient, and suppressing disease outbreaks. Marketing efficiency occurs when livestock production is high, alongside increased sales. According to F. Wanyoike *et al.* (2023), a high livestock population will increase income, so that production costs can be covered. Based on several studies conducted by experts, the difference in this research lies in the efficiency of the marketing channels used, starting from analysing marketing margins, farmer's share and profit and cost ratios. Therefore, the more efficient the marketing channels used by farmers for citronella oil commodities, the higher the profits they receive.

CONCLUSIONS

Based on the analysis, the marketing of citronella oil in Rokan Hulu Regency is generally efficient, with five distinct marketing channels identified. Among these, marketing channel I demonstrate the best performance and highest efficiency which involves farmers selling directly to Village Collecting Traders (PPD) or consumers, shows the smallest marketing margin of IDR 8,000/kg, the highest farmer's share at 95.24%, and the best profit-to-cost ratio of 9.35.

These metrics indicate that farmers who utilise shorter, more direct marketing routes obtain optimal profits compared to longer channels involving multiple intermediaries. For the efficiency of the citronella oil market in this region is further evidenced by: relatively stable and high selling prices across channels, particularly in shorter routes. A market structure is characterised as oligopsony on both seller's and buyer's sides, indicating a concentrated market with limited buyers and sellers. Varied marketing functions performed by different institutions, including exchange, physical,

and facility functions, which contribute to overall system efficiency.

While longer marketing channels (such as channel V) involve more intermediaries and show lower efficiency, the presence of multiple channels provides farmers with options to suit their production capacity and market access. In conclusion, the citronella oil marketing system in Rokan Hulu Regency demonstrates a balance between efficiency and market access, with shorter channels offering the best returns for farmers. Nonetheless, there remains significant potential for enhancing

the efficiency of longer marketing channels to improve overall market performance and increase farmer profitability. These results provide a foundation for informing policy interventions and strategic initiatives aimed at optimising the citronella oil value chain in the region.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Аналіз підвищення операційної ефективності через маркетингову діяльність (*Cymbopogon Nardus* L.)

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Анотація. Сільськогосподарський сектор, зокрема виробництво олії цитронели, є важливим для економічного зростання та експортного потенціалу Індонезії. Це дослідження було присвячене аналізу ефективності маркетингу олії цитронели в регіоні Рокан Хулу, провінція Ріау, з метою оптимізації внеску цього сектору в економіку. Дослідження використовувало описовий метод із кількісним та якісним підходами, базуючись на первинних і вторинних даних, що аналізувалися за допомогою описової статистики. Було досліджено п'ять стратегій маркетингових каналів, які продемонстрували різницю у витратах виробництва, прибутках та рентабельності. Ринок олії цитронели в регіоні Рокан Хулу було охарактеризовано як олігопсонію як з боку продавців, так і з боку покупців, що вказує на концентрованість ринку з обмеженою кількістю учасників. Найкращі результати показав маркетинговий канал I, з найнижчими виробничими витратами – 477,479,17 IDR/кг, найвищою часткою фермера – 95,24 % і найкращим співвідношенням прибутку до витрат – 9,35. Цей канал також мав найменшу маржу – 8,000 IDR/кг і забезпечував найбільший дохід – 2,400,000 IDR за врожай. Натомість маркетинговий канал III мав найвищі витрати – 1,105,451,26 IDR/кг. Загалом маркетинг олії цитронели в регіоні Рокан Хулу був визнаний ефективним, оскільки ціни продажу залишалися прибутковими для фермерів, особливо через коротші маркетингові канали. Ефективність маркетингових каналів оцінювалася за цінами, причому ефективніша ціна свідчила про кращу структуру ринку. У дослідженні також було проаналізовано маркетингові функції, що виконуються різними установами, включаючи функції обміну, фізичні та допоміжні функції, які сприяють загальній ефективності маркетингової системи. Отримані результати можуть бути використані політиками, службами аграрного консультування та фермерами для вдосконалення маркетингових стратегій і підвищення ефективності виробництва олії цитронели в регіоні, що зрештою сприятиме розвитку експортного сільськогосподарського сектору Індонезії та покращенню економічного добробуту фермерів, які вирощують цитронелу, в регіоні Рокан Хулу.

Ключові слова: олія цитронели; прибутковість фермерів; аграрний сектор Індонезії; агромаркетинг; маркетинговий канал

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The impact of biodiversity on stability of agroecosystems: Practical aspects for farmers

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Abstract. The study aimed to investigate the impact of growing intercrops, such as clover, in sunflower crops on reducing weeds, improving soil properties and increasing the productivity of agroecosystems. The study presents an analysis of the impact of intercrops, in particular clover, on the ecological and agronomic indicators of agroecosystems in sunflower cultivation. The study was conducted in Central Ukraine in the Cherkasy region in 2023. The results of the study indicate that the use of intercrops is an effective means of natural weed control. Thanks to the clover vegetation cover,

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the number of weeds was significantly reduced, which minimised the need for herbicide application. This created favourable conditions for the growth of the main crop without significantly increasing costs. The study determined that intercrops have a positive effect on soil moisture levels, contributing to its preservation during the growing season. This effect was especially noticeable during periods of drought when vegetation covers reduced moisture evaporation. This resulted in improved soil fertility due to an increase in organic matter content. The organic residues of clover that remained after the end of the growing season increased the biological activity of the soil and improved its structure. This contributed to the development of soil microbiota, which ensures better absorption of nutrients. The use of intercrops also positively affected sunflower yields, creating favourable conditions for growth. Improving the physical and chemical properties of the soil has increased the efficiency of resource use, which has resulted in consistently high yields. The results obtained indicate that intercropping is a promising tool for increasing the sustainability of agroecosystems. They also reduce dependence on chemicals, improve soil health and increase the economic efficiency of agriculture

Keywords: biodynamics; water balance; soil processes; sustainable development; weed control

INTRODUCTION

Modern agriculture is facing numerous challenges related to the deterioration of soil quality, rising costs of agrochemicals and the negative impact of climate change on the productivity of agroecosystems. The issues of ensuring the sustainability of agricultural ecosystems, increasing productivity and preserving natural resources are of particular relevance. In this context, the integration of intercrops into agricultural production is a promising solution for increasing agricultural efficiency and reducing the negative impact on the environment. One of the most significant problems in modern agriculture is the excessive use of chemical plant protection products, in particular herbicides (Gurtovenko & Tsyuk, 2024). This causes soil degradation, loss of fertility and a decline in biodiversity. In addition, high competition from weeds remains a major limiting factor for achieving high yields of major crops. The use of intercrops, such as clover, allows for natural control of weeds by creating a dense vegetation cover that limits their access to light and nutrients. Soil moisture retention is another critical factor in ensuring sustainable yields (Drobitko & Alakbarov, 2023). Climate change, including an increase in the frequency of droughts and uneven rainfall distribution, calls for solutions that optimise the water balance of the soil. Intercropping helps to retain moisture by reducing evaporation through plant cover and improves soil structure, increasing water retention capacity (Mokrienko *et al.*, 2024). Increasing the organic matter content of the soil is another important aspect of ensuring long-term fertility. Organic matter acts as a buffer to preserve nutrients, maintain biological activity and improve the physical properties of the soil. Intercrops, leaving behind a significant amount of organic residues, help to replenish humus reserves and create favourable conditions for the development of soil microorganisms (Zymarioieva *et al.*, 2021; Drobitko *et al.*, 2024).

S. Wang (2020) emphasised that agrobiodiversity is a key factor in the stability of agroecosystems. The author noted that the introduction of intercropping

significantly reduced the impact of external stressors such as weeds and droughts by improving ecosystem services. I. Quintero *et al.* (2022) proposed an index for assessing agrobiodiversity, which demonstrates that biodiverse systems are more effective in maintaining ecological functions such as natural weed control and soil fertility. E.G. De la Riva *et al.* (2023) proposed a conceptual model that links functional diversity to human well-being through agroecosystem resilience. The authors emphasised that the introduction of intercropping contributes not only to improved productivity but also to the long-term conservation of ecosystem resources. J.V. Canelas and H.M. Pereira (2022) found that intensive use of land resources negatively affects ecosystem stability, while agrobiodiversity-based strategies significantly increase system resilience.

D. Beillouin *et al.* (2021) analysed the impact of crop diversification and concluded that the introduction of intercropping has a predominantly positive, albeit variable, effect on ecosystem services, including moisture conservation and weed control. At the same time, M. Dardonville *et al.* (2022) demonstrated that biodiversity-based systems demonstrate stability, which is important in the context of climate change. The environmental impacts of agricultural systems were studied by M. Padhiary and R. Kumar (2024), determining that increasing biodiversity through the introduction of intercropping minimises the negative environmental impact of agriculture, including soil degradation. M. Carof *et al.* (2022) emphasised the importance of a long-term approach to implementing biodiversity-based systems to ensure sustainable yield growth. R. Duflot *et al.* (2021) highlighted that farming intensity indirectly reduces yields through negative impacts on agrobiodiversity and key ecological functions. They emphasised the importance of reducing dependence on chemicals through the natural regulatory mechanisms provided by intercropping. E. Narayana and L. Merugu (2021) emphasised the seasonal dependence of ecosystem functions and proved that intercropping contributes to the

conservation of soil biodiversity, which has a positive impact on soil fertility. Research confirms that intercropping reduces dependence on chemicals, reducing herbicide costs and supporting environmental sustainability. They also improve the yields of main crops, providing long-term economic benefits for farmers.

The introduction of such systems can not only be used to adapt to climate change but also to create sustainable agroecosystems that combine environmental and economic efficiency. The study aimed to investigate the effectiveness of using intercrops, in particular clover, in sunflower cultivation, and to determine the practical importance for farmers. The objectives of the study were to determine the impact of intercrops on the number of weeds, moisture levels and soil organic matter content, as well as to assess their impact on sunflower yield and economic efficiency of agroecosystems.

MATERIALS AND METHODS

The study was conducted in 2023 on the experimental fields of the Bohdan Khmelnytsky Cherkasy National University near Cherkasy on experimental plots divided into two categories: a control plot sown exclusively with sunflower and an experimental plot where clover was sown between sunflower rows. For the experiment, a mid-season sunflower variety Yason with high drought tolerance and a red clover variety Poliska 3, known for its ability to improve soil fertility, were used. The experimental plots were selected based on the following soil characteristics: average humus content (3.2-3.5%), light loamy texture, neutral pH (6.5-7) and an agronomic history that included the cultivation of grain crops for the previous three years without the use of perennial grasses. To ensure representative results, the homogeneity of moisture conditions and the level of organic matter in the soil were included. Before the beginning of the experiment, the initial indicators of the plots were analysed. Soil moisture and organic matter levels were measured using standard agrochemical methods. All agrotechnical measures, including soil preparation, sowing, crop care and harvesting, were conducted following generally accepted standards, in particular the recommendations of the Institute of Oilseeds of the National Academy of Sciences of Ukraine. The size of each plot was 0.5 ha, which was used to compare the results under the same growing conditions.

The number of weeds was determined by counting in 1 m² control points evenly spaced across each plot. The counts were made manually at the beginning (May), middle (July) and end of the growing season (August), and the results were averaged for each plot. Weed control efficiency was assessed by comparing the number of weeds in the experimental and control plots. The methodology considered the species structure of weeds, which was used to determine the specifics of clover's impact on the growth of different types of unwanted plants. One-way analysis of

variance (ANOVA) was used to determine statistically significant differences between the groups. The study complied with the ethical standards set out in the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1973) and the Convention on Biological Diversity (1992).

To measure the soil moisture level, 15 tensiometers were used, which were evenly distributed across the plots to ensure the data were representative. Five tensiometers were installed on each experimental and control plot, which was used to obtain average moisture values for each group. The tensiometers were placed in the central part and the corners of the plots, avoiding areas that could be subject to localised moisture anomalies (e.g. areas with poor drainage or slopes). All instruments were installed at the same depth of 20 cm, according to the methodology for calculating soil moisture (Allen *et al.*, 1998). This methodology involves determining the water tension in the soil, which allows for estimating the availability of moisture for plants. The nitrogen content was determined by Kjeldahl's Method (1983), and the number of microorganisms was estimated by serial soil dilutions and colony counts on selective media. This approach made it possible to identify changes that could be related to the activities of the intercrop. To analyse the data, a standard calculation of soil moisture content, defined as the ratio of water mass to the mass of dry soil dried at 105°C to a constant weight, was used. Drought periods were emphasised to assess the effectiveness of intercropping in conserving moisture, which included time series analysis and comparison with control plots.

The yield of the main crops was determined after the end of the growing season. All plants from the plots were harvested mechanically, after which the harvest was weighed. The data were converted into tonnes per hectare (t/ha) for productivity analysis. The methodology also incorporated crop quality, which was assessed according to DSTU 4138-2002 (2004). The results were used to assess the economic efficiency of introducing intercropping and statistical data were processed using Microsoft Excel. To determine the content of organic matter in the soil, laboratory analyses were carried out according to the Turin method (Standard Operating Procedure for..., 2021). Soil samples were collected from each plot before the start of the experiment and after the end of each season. Sampling was conducted at a depth of 0-20 cm at five points of each plot, which ensured the representativeness of the samples. The Tyurin method was used to determine the humus content of the soil based on the oxidation of organic matter with a chromium mixture, followed by titration of the solution. This approach ensured high accuracy and reproducibility of the results.

The practical significance of the study was assessed by comparing the economic costs and benefits of introducing intercropping. The reduction in herbicide costs

was determined based on the average price of chemicals that could be used to control weeds in the control plot. Improvements in soil quality were measured by changes in organic matter content, moisture levels and erosion resistance. The additional income from increased yields was calculated based on the average market price of sunflower, accounting for the cost of sowing clover. The data obtained was used to comprehensively assess the impact of intercropping on the productivity of agroecosystems and prove its effectiveness as a means of increasing soil fertility, reducing the cost of agrochemicals and ensuring the long-term sustainability of agricultural systems. The use of standardised measurement methods and laboratory analysis ensured the accuracy and reliability of the results.

RESULTS

The impact of intercrops on the number of weeds. The study compared the number of weeds in two experimental plots: a control plot sown with sunflower only and a plot with a row crop where clover was sown between sunflower rows. This was done to evaluate the effectiveness of clover in reducing the number of weeds, which are the main competitors of cultivated plants for resources. In the control plot, where no intercropping was used, the average number of weeds was 120 per m². This figure indicated the absence of natural barriers to weed germination. Sunflower, as the main crop, could not effectively restrain growth, as the arrangement of plants with wide row spacing created space for the active spread of weeds. Light, moisture and nutrients were available without significant restrictions. In addition, the provision of sufficient light is confirmed by the results of monitoring microclimat-

ic conditions in areas where the light level remained stable throughout the experiment (Stepovyi, 2024). As a result, weeds competed with sunflowers, potentially reducing yields.

In the clover plot, the number of weeds was significantly reduced. On average, 66 weeds per m² were recorded, which corresponded to a 45% reduction compared to the control plot. This reduction was determined by the influence of clover as an intercrop. Furthermore, the dense vegetation cover reduced the access of light to the soil, which significantly slowed down the germination of weed seeds. In addition, the clover competed with weeds for water and nutrients, reducing the availability of unwanted plants. Additionally, weed biomass was reduced in the clover plot. In the control plot, the average weed biomass was 500 g/m², while in the intercropped plot it dropped to 220 g/m². This indicated not only a decrease in the number of weeds but also a decrease in the viability of weeds, which, even under favourable conditions, could not reach the same weight as in the control plot.

The average weed height in the control plot was 25 cm, while in the clover plot, it was only 15 cm. This once again confirmed the suppressive effect of the intercrop. The clover created a competitive environment in which weeds could not fully develop their growth potential. In addition to the direct effect on weeds, other positive effects were also recorded in the clover plot. The soil moisture content here was 19.6%, while in the control plot, it was 17%. This indicated that the clover vegetation cover slowed down the evaporation of moisture, preserving it in the soil. The moisture retention had a positive effect on the main crop, sunflower, contributing to better growth (Table 1).

Table 1. The impact of intercrops on the number of weeds

Metric	Control plot (sunflower)	Plot with clover (Sunflower + Clover)
Average number of weeds (pcs/m ²)	120	66
Reduction of weeds (%)	-	45%
Average weed height (cm)	25	15
Weed biomass (g/m ²)	500	220
Sunflower yield (t/ha)	2.5	3
Soil moisture (%)	17	19.6
Organic matter content in the soil (%)	3.2	3.8

Source: compiled by the authors

The study demonstrated that the use of intercropping significantly reduces weed pressure, providing more favourable conditions for the main crop. The reduction in weeds, height and biomass contributed to a 20% increase in sunflower yield, which was 3 t/ha in the clover plot compared to 2.5 t/ha in the control plot. The results confirmed that the introduction of intercropping, such as clover, can be an effective tool for ecological weed control and increase the productivity of agroecosystems, which is consistent with the data

(Pavliuk, 2020), which describes in detail the environmental and agronomic benefits of such practices. This not only reduces the number of unwanted plants but also preserves the natural resources of the soil and improves its quality.

The impact of intercrops on soil moisture levels. The study aimed to determine the impact of intercropping on soil moisture levels. This parameter is of key importance for the productivity of agroecosystems, as water availability is a critical factor for the growth of

crops. The study was conducted on two plots: a control plot where only sunflower was grown, and an experimental plot where clover was sown between sunflower rows. Soil moisture was measured at a depth of 20 cm throughout the growing season using soil sensors. In the control plot, the average moisture level was 17%, which was due to the lack of vegetation between rows. This condition contributed to the rapid evaporation of water from the soil surface, especially under conditions of high temperatures and insufficient rainfall during the study period. In the experimental plot with clover row spacing, the average moisture level was significantly higher and amounted to 19.6%, which is 15% more than in the control plot. The clover created a dense cover that acted as a natural mulch. This slowed down the process of water evaporation from the soil surface and contributed to water retention at depth, which is

confirmed by previously established data on the effectiveness of such practices. At the same time, the clover root system improved the soil structure, increasing the ability to retain water.

In the control plot, the soil was less moisture stable, which was noticeable during periods of drought. Under such conditions, moisture evaporation increased significantly, reducing the availability of water for the sunflower root system. This reduced plant growth rates, resistance to stress factors and potential yields. In the area with clover, the moisture stability was higher. Inter-cropping reduced sharp moisture fluctuations, providing a more uniform water supply throughout the growing season. In addition, the improved soil structure contributed to more efficient water absorption during precipitation, which allowed for the accumulation and storage of moisture even under unfavourable conditions (Table 2).

Table 2. *The impact of intercrops on soil moisture levels*

Metric	Control plot (sunflower)	Plot with clover (Sunflower + Clover)
Average soil moisture level (%)	17	19.6
Maximum soil moisture during precipitation (%)	22	24.8
Minimum soil moisture during drought (%)	12.5	16
Deviation of the humidity level during the period (%)	9.5	6.8
Improved moisture retention (%)	-	+15%

Source: *compiled by the authors*

These results demonstrated the importance of intercropping to preserve soil moisture, which is a key factor in regions with low rainfall or frequent droughts. The use of clover improved the water supply of crops, increased their resistance to stressful conditions and ensured stable yields. The results obtained concluded that intercropping is an effective tool for improving soil water regime in modern agriculture. The use of this crop has the potential to spread in different agroclimatic conditions, contributing to the productivity and sustainability of agroecosystems.

The impact of intercrops on yields. The third stage of the research analysed the impact of intercrops on sunflower yields. The observation covered two separate plots under the same agroclimatic conditions: the first, where sunflower was grown without additional crops, and the second, where clover was sown between the rows. This approach was used to assess how the use of a catch crop can affect the productivity of the main crop by regulating ecosystem factors. On the plot where only sunflower was grown, the yield was 2.5 tonnes per hectare. Despite standard agronomic practices, significant competition from weeds and unstable soil moisture harmed plant development. In such conditions, weeds, which amounted to 120 units/m², actively absorbed nutrients and moisture, reducing the availability of these resources for sunflowers. In addition, evaporation of moisture from the soil surface, which was not

regulated by the natural cover, contributed to additional water losses, especially during dry periods. On the plot with clover row spacing, the yield increased to 2.8 t/ha, which was a 12% increase compared to the control plot. Clover effectively acted as a natural regulator of ecosystem processes. It reduced competition from weeds, the number of which was reduced by 45% to 66 plants per square metre. This improved the availability of moisture and nutrients for the sunflower. In addition, the dense cover of clover reduced water evaporation, ensuring a more even water balance in the soil. During periods of drought, the moisture level in this area remained 15% higher than in the area without the intercrop, which significantly reduced plant stress.

Another key positive effect was the improvement of soil properties. In the soil where clover grew, there was a 0.6% increase in organic matter content (3.8% compared to 3.2% in the control plot). This improved the soil structure and created more favourable conditions for the development of the sunflower root system. Yields in the clover plot increased due to more efficient use of available resources. Higher humidity levels and reduced competition from weeds ensured stable plant growth throughout the growing season. This allowed sunflowers to better adapt to changes in weather conditions and reduce the impact of stress factors such as periods of drought or temperature fluctuations (Table 3).

Table 3 . The impact of intercrops on yields

Metric	Control plot (sunflower)	Plot with clover (Sunflower + Clover)
Sunflower yields (t/ha)	2.5	2.8
Yield increase (%)	-	+12%
Number of weeds (pcs/m ²)	120	66
Soil moisture (%)	17	19.6
Organic matter content in the soil (%)	3.2	3.8

Source: compiled by the authors

The study results showed that intercrops such as clover can significantly improve the conditions for sunflower growth by providing a more stable level of soil moisture, reducing weeds and increasing soil fertility. The 12% increase in yields proved the feasibility of using such crops in modern agricultural technologies. The use of clover created a natural balance in the agricultural ecosystem, reducing dependence on chemical weed control methods and maintaining the sustainability of production processes (Cherlinka, 2024).

Organic matter content in the soil. The fourth stage of the study determined the impact of the intercrop, clover, on the organic matter content of the soil during sunflower cultivation. On the control plot, where only sunflower was grown, the organic matter content was 3.2%. This corresponds to the typical figure for intensive farming, where organic matter comes mainly from the residues of the main crop. However, due to the lack of additional organic sources, such as intercrops, humification processes in the soil were limited. There was also a greater vulnerability of the soil to erosion processes, which further reduced the level of organic matter. In the plot where the clover was sown between sunflower rows, the organic matter content increased to 3.8%, which was an 18% increase compared to the control plot. Clover proved to be an effective source of organic matter. The residues after the end of the growing season included both plant biomass and the root system, which ensured the supply of organic material directly to the soil. This created favourable conditions for the

development of soil microorganisms, which intensified the processes of decomposition and humification.

The results also demonstrated that clover had a positive effect on soil structure. Thanks to its developed root system, clover reduced soil compaction, improved aeration and contributed to moisture retention (Romanchuk *et al.*, 2017; Basanets, 2024). In such conditions, organic matter was better integrated into the soil profile, ensuring stability and increasing fertility. The key factor in the growth of organic matter was the total amount of plant residues. In the control plot, the biomass of residues was 450 g/m², while in the clover plot, this figure increased to 600 g/m², which is 33% more. The additional contribution of organic material from the clover provided nutrients to the soil and improved its physical and chemical properties.

The effect of intercropping was also reflected in the increase in the number of active microorganisms in the soil. In the clover plot, the number of microorganisms reached 3.5×10^6 /g soil, which was 67% higher than in the control plot (2.1×10^6 /g soil). This confirmed that clover created favourable conditions for biological activity, which in turn stimulated the processes of humification and improved soil structure (Tkachenko *et al.*, 2021). Another significant indicator was the increase in soil nitrogen content. In the clover plot, this indicator increased to 0.27% compared to 0.21% in the control plot (Table 4). Clover, as a legume, could fix atmospheric nitrogen and accumulate in the soil, which had a positive effect on fertility (Agrostory, 2021).

Table 4. Organic matter content in the soil

Metric	Control plot (sunflower)	Plot with clover (Sunflower + Clover)
Organic matter content (%)	3.2	3.8
Organic matter growth (%)	-	+18%
Biomass of main crop residues (g/m ²)	450	450
Biomass of intercrop residues (g/m ²)	-	150
Total amount of residue (g/m ²)	450	600
Number of active microorganisms (10 ⁶ /g soil)	2.1	3.5
Content of nitrogen in the soil (%)	0.21	0.27

Source: compiled by the authors

The results of the study demonstrated that the use of intercrops, such as clover, significantly affects the accumulation of organic matter in the soil. The 18% increase in organic matter content was achieved both by increasing

the amount of plant residues and by improving the conditions for the development of soil microorganisms. This confirms that intercropping can contribute to sustainable agriculture by promoting long-term soil fertility.

The practical importance of intercropping for farmers. The final stage of the research was to assess the practical importance of introducing intercrops such as clover in sunflower cultivation. In 2023, the results of field experiments showed that intercrops have significant potential to increase agricultural production efficiency by reducing costs, improving soil quality and ensuring the sustainability of agroecosystems. These aspects are important for farmers seeking to optimise costs and ensure long-term economic benefits. The reduction of herbicide costs was one of the key results of the study. The use of clover as an intercrop was used to naturally control the number of weeds, reducing the number by 40-60% depending on the conditions of the year. The dense cover of clover created competition for weeds, limiting access to light and nutrients. This significantly reduced the need for weed control chemicals, which reduced herbicide costs. Based on the data obtained, the savings on chemicals averaged 15-30 USD per hectare, depending on regional conditions. This is especially relevant for farmers in the context of rising prices for agrochemicals and requirements for greener production. Another important practical aspect was the improvement of soil quality. The plots where clover was used as an intercrop showed an 18% increase in soil organic matter content compared to the control plots. This was achieved through the introduction of organic matter after the end of the clover growing season. In addition, the dense cover of the inter-row crop helped reduce the risk of erosion, improved soil structure and preserved its water-holding properties. The increased amount of organic matter in the soil created favourable conditions for the development of soil microorganisms, which maintained the natural fertility of the soil.

The increase in yields was another significant result that has practical implications for farmers. On plots with intercropping, sunflower yields increased by an average of 12% (from 2.5 t/ha to 2.8 t/ha). This was made possible by reducing weed competition, improving moisture availability and enriching the soil with nitrogen, which is fixed by the clover root system. The additional costs of planting the intercropping were minimal compared to the economic benefits gained through increased yields and reduced chemical costs. The results of the study also confirmed that the use of intercropping contributes to the creation of sustainable agroecosystems. Intercropping not only improved soil properties but also maintained the natural balance in the system, reducing the negative impact of intensive agriculture (Resource-saving farming, 2022). Economic benefits were combined with environmental sustainability, making this approach promising for long-term implementation. The creation of sustainable agroecosystems reduced dependence on synthetic inputs such as herbicides and mineral fertilisers and ensured stable yields even in adverse climatic conditions (Fig. 1).

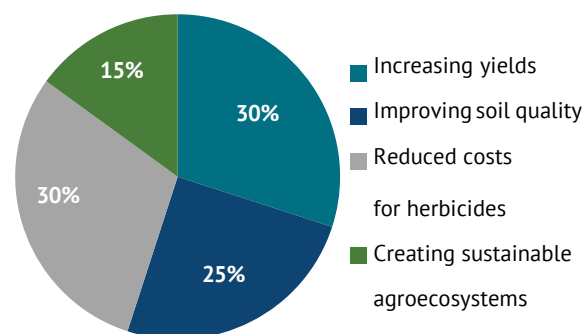


Figure 1. Practical importance of intercropping for farmers

Source: compiled by the authors

Thus, the practical implications of introducing intercropping for farmers included reducing costs, increasing yields and creating sustainable agroecosystems that provide both economic benefits and environmental sustainability. These results can provide a basis for introducing intercropping into agricultural practice as an effective way to improve the productivity and sustainability of agroecosystems.

DISCUSSION

The results of the study confirm the effectiveness of introducing intercrops, in particular clover, in sunflower cultivation. The data obtained indicate a significant improvement in key agroecosystem indicators, such as weeds, soil moisture, soil organic matter and main crop yields. These findings underline that intercropping is a promising solution for improving the environmental and economic efficiency of agricultural production. One of the most important results was the reduction of weeds in the plots with intercropping. This demonstrates that intercropping can provide natural control of unwanted plants, reducing the impact on the main crop. Due to the dense plant cover of the clover, weeds were deprived of the necessary access to light, water and nutrients, which inhibited their development. This reduces the use of herbicides, reducing the cost of agrochemicals and reducing the environmental burden on the agroecosystem.

N. Kovka (2023) emphasises the importance of biodiversity for increasing the resilience of agroecosystems, focusing on ecological intensification as an innovative approach. The study confirms that increasing biodiversity, in particular using intercropping, reduces the negative impact of weeds and improves soil quality. This is consistent with the reported results of a 40-60% reduction in weeds and an 18% increase in organic matter. However, this study complements these data with quantitative indicators of economic benefits, whereas the study by N. Kovka only presents general conclusions. Y.Y. Chupryna (2022) conducted an agroecological assessment of the biodiversity of the genus *Triticum* L. in the agroecosystem of the Eastern Forest-Steppe of

Ukraine. The results demonstrated that population biodiversity ensures crop resilience to biotic and abiotic factors. This study similarly addressed agroecosystem resilience, although emphasising the practical aspects of reducing herbicide costs and increasing yields of major crops. The practical orientation distinguishes this study, rendering it more applicable to farmers.

I.I. Mostoviyak (2020) addressed the integrated plant protection system as a basis for the formation of balanced agroecosystems. The authors emphasise the importance of reducing dependence on chemical crop protection products. This aspect is fully consistent with these results, which show that the introduction of intercropping can reduce herbicide costs by 20-30%. In addition, this study complements the findings of I.I. Mostoviyak with data on the impact of intercrops on soil moisture and organic matter. V.A. Karpenko (2023) studied soil biomonitoring in garden agroecosystems in the Dnipro region, focusing on the importance of organic matter for improving soil properties. The study confirms that an increase in organic matter improves soil structure and water-holding capacity. These results of an 18% increase in organic matter are consistent with these findings. However, this study has a broader context, covering additional aspects such as yield and cost reduction.

L.I. Moklyachuk *et al.* (2020) addressed the need to adapt agriculture to climate change through the introduction of environmentally friendly farming practices, among which a special place is occupied by improving the water-holding properties of the soil. The authors emphasise that increasing the soil's ability to retain moisture is a key factor in ensuring the resilience of agricultural ecosystems, especially in the face of frequent droughts caused by climate change. This study confirms similar findings, demonstrating the effectiveness of intercrops such as clover in increasing soil moisture by 15-20%. However, in contrast to a study by L.I. Moklyachuk *et al.*, this study provides a more detailed quantitative analysis of this effect over the entire growing season. In particular, the study includes an analysis of the moisture dynamics at different stages of the main crop growth, which allows for a more accurate assessment of the effectiveness of the implemented measures during critical periods of plant development.

D.O. Makarov and O.A. Romanashenko (2021) emphasised the importance of environmentally friendly agricultural technologies that reduce dependence on chemicals. This aspect correlates with these results, which reported a 20-30% reduction in herbicide costs due to natural weed control through intercropping. At the same time, this study has the additional advantage of increasing yields by 12%, which demonstrates not only the environmental but also the economic feasibility of introducing intercropping. E.O. Synychenko (2024) emphasised the importance of integrating environmental and economic aspects into sustainable land use

systems, highlighting the need to implement practices that ensure both productivity and environmental sustainability. These results support these findings, demonstrating that the use of intercrops such as clover can increase sunflower yields by 12%, reduce herbicide costs by 20-30% and improve soil health. Compared to the study by E.O. Synychenko, this study complements the economic evaluation of the results with specific quantitative data on costs and benefits to farmers.

O.L. Klyachenko *et al.* (2022) addressed the principles and importance of biodiversity in detail, emphasising its key role as the basis for the stability of agroecosystems. The authors emphasised that biodiversity contributes to the resilience of agroecosystems to external stresses by maintaining ecosystem services such as water conservation, weed control, soil fertility and microclimate improvement. They also highlighted the importance of integrating natural mechanisms into modern agriculture to minimise reliance on chemical inputs. The results of this study confirm the main conclusions of O.L. Klyachenko *et al.* but with additional practical aspects. In particular, the study determined that the use of inter-row crops, such as clover, can reduce the number of weeds by 40-60% and increase the level of soil moisture by 15-20%. These indicators are a strong argument in favour of a biological approach to managing agroecosystems.

K.S. Zakharenko (2023) analysed in detail the bioindication of the ecological state of garden agroecosystems, focusing on the key role of improving soil characteristics to ensure the sustainability of ecosystems. The author emphasised that an increase in organic matter content is the main factor that not only improves soil structure but also increases its fertility, promotes moisture retention and creates favourable conditions for the development of microbial diversity. This, in turn, positively affected the productivity of agroecosystems and their resilience to stress factors.

The results fully correlate with Zakharenko's findings, showing that intercropping with clover increased soil organic matter by 18%. This increase not only improved soil structure but also significantly increased its water-holding capacity, reducing the need for additional agricultural practices such as irrigation. Overall, the results of this study confirm the findings of other authors on the importance of biodiversity and sustainable land use but extend these insights with specific field and economic data. This provides a deeper understanding of the effectiveness of intercropping and its role in the formation of balanced agroecosystems, which allows for more informed recommendations for widespread implementation. Thus, the data obtained underline the significant potential of intercrops in forming sustainable agroecosystems and creating conditions for balanced land use. They can be useful for farmers, agronomists and scientists interested in implementing innovative environmental practices in agriculture.

CONCLUSIONS

The study included a comprehensive assessment of the impact of intercrops, in particular clover, on key agroecosystem indicators in sunflower cultivation in Central Ukraine. The study included an analysis of weeds, soil moisture levels, organic matter content, and the impact of intercrops on the yield of the main crop. The results demonstrate the effectiveness of intercropping in increasing agricultural sustainability and optimising the economic component. The study showed that the use of intercrops contributes to a significant reduction in the number of weeds. Thanks to the dense vegetation cover of clover, which limits the access of weeds to light, water and nutrients, natural weed control is possible without the intensive use of chemicals. This opens the prospect of reducing the cost of herbicides and reducing the negative environmental impact on the soil.

Improved soil moisture levels in areas with intercropping confirmed the ability to reduce moisture loss by slowing evaporation. Vegetation cover also contributed to the stability of the water regime in drought conditions, which provided favourable conditions for sunflower growth. This aspect is particularly important in the context of climate change, which is increasingly affecting the agricultural sector. The increase in soil organic matter content in the experimental plots demonstrated the significant contribution of intercrops to improving soil fertility. The organic mass left by clover after the end of the growing season activated soil processes, in particular humification, and contributed to the development of soil biota. These results

confirm that intercropping is an effective tool for restoring degraded soils and maintaining long-term productivity. The study also showed that the use of intercrops helps to increase sunflower yields. This was achieved by reducing weed competition, improving moisture and enriching the soil with nitrogen. As a result, the introduction of intercropping provided not only environmental but also economic benefits, demonstrating the potential to optimise costs and increase profitability. In general, the study confirmed that intercropping is crucial for creating sustainable agroecosystems. They increase the productivity of main crops, reduce the cost of agrochemicals, improve soil quality and create environmentally friendly conditions for farming.

The results obtained can be used as a basis for developing practical recommendations for the introduction of intercropping into agricultural practice to increase the sustainability and efficiency of agroecosystems. A limitation of the study is the focus on one crop (sunflower) and the specific climatic conditions of the Cherkasy region, which may make it difficult to adapt the results to other regions and crops. Further research should cover different types of intercrops, other agro-climatic zones and a longer observation period to study long-term effects.

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CONFLICT OF INTEREST

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Вплив біорізноманіття на стабільність агроєкосистем: практичні аспекти для фермерів

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Анотація. Метою роботи було дослідити вплив вирощування міжрядних культур, таких як конюшина, у посівах соняшника на зменшення кількості бур'янів, покращення властивостей ґрунту та підвищення продуктивності агроєкосистем. У статті представлено дослідження впливу міжрядних культур, зокрема конюшини, на екологічні та агрономічні показники агроєкосистем при вирощуванні соняшника. Дослідження було проведено в умовах Центральної України в Черкаській області в 2023 році. Результати дослідження вказують на те, що використання міжрядних культур є ефективним засобом природного контролю бур'янів. Завдяки рослинному покриву конюшини вдалося суттєво знизити кількість бур'янів, що мінімізувало потребу в застосуванні гербіцидів. Це створило сприятливі умови для росту основної культури без значного збільшення затрат. Встановлено, що міжрядні культури позитивно впливають на рівень вологості ґрунту, сприяючи збереженню під час вегетаційного періоду. Особливо помітним цей ефект був у періоди посухи, коли рослинний покрив зменшував випаровування вологи. Результатом стало покращення родючості ґрунту за рахунок збільшення вмісту органічної речовини. Органічні залишки конюшини, що залишалися після завершення вегетаційного періоду, підвищували біологічну активність ґрунту та покращували структуру. Це сприяло розвитку ґрунтової мікробіоти, яка забезпечує краще засвоєння поживних речовин. Використання міжрядних культур також позитивно вплинуло на врожайність соняшника, створивши сприятливі умови для росту. Поліпшення фізичних і хімічних властивостей ґрунту дозволило підвищити ефективність використання ресурсів, що дало змогу отримати стабільно високі врожаї. Отримані результати свідчать про те, що міжрядні культури є перспективним інструментом для підвищення стійкості агроєкосистем. Та сприяють зменшенню залежності від хімічних засобів, покращують стан ґрунту та підвищують економічну ефективність сільського господарства

Ключові слова: біодинаміка; водний баланс; ґрунтові процеси; сталий розвиток; контроль бур'янів



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Comprehensive analysis of shadow economy countermeasures and taxation of hidden income in the agricultural sector of the Republic of Azerbaijan

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Abstract. The study aimed to identify the main causes of the shadow economy in the agricultural sector of Azerbaijan, in particular the factors contributing to informal employment, and to assess the effectiveness of measures aimed at reducing it. The study conducted a comprehensive analysis of countermeasures to the shadow economy and taxation of hidden incomes in the agricultural sector of the Republic of Azerbaijan. The methodology of the study was based on the analysis of statistical

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data, in particular, reports of the International Monetary Fund, the World Bank and the State Statistical Committee of Azerbaijan, as well as on a comparative analysis with the data of neighbouring countries: Georgia, Armenia, Turkey, Uzbekistan. Key findings indicated that around 40% of agricultural workers are employed in the informal sector, resulting in tax revenue losses of over 1 billion USD per year, which significantly undermines the financial stability of the state. The study also determined that the current tax policy does not encourage the formalisation of employment, and the lack of access to finance limits the opportunities for legal business development. The findings suggested the need for comprehensive reforms in the tax system, including simplification of taxation procedures, improved tax enforcement, and creation of incentives for shadow workers to move to the legal sector. The implementation of these measures can significantly improve the economic situation in agriculture, ensure fair conditions for all market participants and guarantee the social rights of workers, which will contribute to the stability and development of the agricultural sector

Keywords: agriculture; agribusiness; finance; corruption; anti-corruption mechanisms

INTRODUCTION

The shadow economy, or informal sector, encompasses all economic activities that are not officially registered and are not subject to government control or taxation. It includes a wide range of activities, from small home-based businesses to illegal trade, and often occurs due to high bureaucracy, corruption or lack of economic transparency. Overall, the shadow economy can significantly impact national economic development. It distorts the competitive environment, as legal businesses that comply with the law and pay taxes are forced to compete with illegal actors who can offer their goods or services at lower prices by avoiding tax liabilities. This creates an uneven playing field for businesses and undermines economic stability.

The shadow economy is a significant problem for many countries, including Azerbaijan, where it continues to grow, creating serious issues for the state budget and social development. Experts estimate that the share of the informal economy in the total economy can reach 40%, which leads to significant losses in tax revenues and disrupts fair competition between legal and illegal businesses (Mehdiyev, 2024). The issue of the shadow economy and tax evasion in agriculture was addressed by many scholars. For instance, the relationship between economic complexity, shadow economy and income inequality were studied by M. Pham *et al.* (2024). The authors determined that the growth of the shadow economy can contribute to income inequality, emphasising the need for proper regulation to improve social justice. A. Asllani and F. Schneider (2024) analysed the main reasons for the development of the informal economy in six EU countries and assessed policy measures that could help reduce its scale. The authors emphasised the importance of integrating the informal economy into the formal sector through tax reform and the fight against corruption.

The impact of the shadow economy and tax evasion on economic growth in Ghana was studied by P. Wiawe *et al.* (2024). The researchers analysed how illegal economic activity and tax abuse affected the national gross domestic product (GDP) using a quantitative approach.

The authors determined that the underground economy significantly reduced the national tax base, limiting public investment in economic development. The researchers suggested strengthening tax administration and transparency policies as a means of minimising losses to the economy. B. Bhuana and S. Wijaya (2024) addressed the role of the fight against corruption in mitigating the negative impact of the shadow economy and per capita income on tax revenues. The authors analysed the interaction between these factors based on data from countries with different levels of corruption. The study demonstrated that corruption control significantly improved the efficiency of tax systems, even in countries with high shadow economies. The authors emphasised that an effective anti-corruption policy increased public trust in the state, stimulating tax payments. Z. Manzoor and H. Ahsan (2024) conducted a systematic review of definitions and models for assessing the shadow economy. The authors analysed the existing approaches to measuring the shadow economy and pointed out the need to develop new models that would more accurately reflect its scale and impact.

The relationship between the shadow economy and financial sustainability in Palestine was studied by M. Abuamsha and L. Hattab (2024). The researchers determined that the growth of the shadow economy negatively affected the long-term financial sustainability of the country by reducing tax revenues. The authors recommended strengthening control over informal sectors and introducing effective financial mechanisms to combat these problems. The theoretical basis for reducing the share of the informal economy through financial instruments was considered by L. Rakhmanov (2024). The study substantiated the role of tax incentives, financial transparency and modernisation of the financial infrastructure in reducing the shadow sector. The author emphasised the need for government initiatives to encourage the formalisation of economic activity. N. Sreenu (2024) studied the uneven impact of exchange rate volatility on the shadow economy in the BRICS countries (Brazil, Russia, India, China, South

Africa). The study determined that currency volatility significantly increased the share of the shadow economy in the context of weak regulation. The authors proposed stabilising the foreign exchange market as a key tool to minimise this impact. A. Hashimova (2023) highlighted the main problems and their solutions for agriculture in Azerbaijan. The author emphasised that the shadow economy is a substantial issue for the development of the agricultural sector, hindering its modernisation and financing.

The economic aspects of agricultural contracting, including the impact of the shadow economy on the agricultural sector, were analysed by H. Bachev (2024). The study demonstrated that contractual relations contribute to a more rational allocation of resources and reduce risks for farmers but require an improved regulatory environment. K. Ben Kelmanson *et al.* (2019) explained the causes and extent of the shadow economy in Europe, proposed policy options to reduce it, and highlighted the importance of developing an effective tax system to reduce the informal economy.

The study primarily aimed to analyse the causes and consequences of the shadow economy in Azerbaijani agriculture and to develop recommendations for overcoming obstacles to business formalisation in the agricultural sector. Thus, the study addressed the issue of assessing the real state of the shadow economy in agriculture in the Republic of Azerbaijan, as well as the effectiveness of measures taken to overcome it.

MATERIALS AND METHODS

This study analyses the measures aimed at combating the shadow economy and taxation of hidden incomes in the agricultural sector. To achieve the objectives, a combination of qualitative and quantitative research methods was used, which ensured a comprehensive approach to the study of this problem. At the initial stage, a systematic review of the scientific literature related to the research topic was conducted. The focus was on the papers dealing with the shadow economy, tax policy and the specifics of the agricultural sector. In the course of the analysis, various sources were studied, including articles, reports of international organisations, monographs and dissertations covering the experience of

different countries in combating the shadow economy (Medina & Schneider, 2017; World Bank, 2023a; International Monetary Fund, 2024).

For the quantitative analysis, statistical data (2021–2023) from official sources such as the International Monetary Fund (2024) and tax authorities and others were used (State Statistical Committee..., 2023; World Bank, 2023b). The data collected included indicators of economic activity, tax revenues, and information on informal employment in agriculture. For the analysis, descriptive statistics were used, which was used to identify significant links between the level of the shadow economy and tax revenues. To assess the effectiveness of measures to counteract the shadow economy, the methods of comparative analysis and SWOT analysis were applied. This was used to identify the strengths and weaknesses of the existing tax system, as well as the opportunities and threats associated with reforms in the agricultural sector. The comparative analysis was used to evaluate the experience of other countries, namely Georgia, Armenia, Turkey, and Uzbekistan, and adapt it to the specifics of the region under study. The collected data was processed using statistical software, including SPSS and Excel. This ensured that the necessary analyses could be carried out, the results visualised, and the information presented clearly.

RESULTS

Analysis of the state of the shadow economy in agriculture in Azerbaijan. The shadow economy in Azerbaijan's agriculture remains a serious problem that significantly affects the stability of the national economy and tax revenues. According to the International Monetary Fund (2024), the total shadow economy in Azerbaijan in 2023 was estimated at 30% of the country's GDP, with the agricultural sector accounting for about 25% (World Bank, 2023b). This indicates a significant size of the informal economy in agriculture, which can lead to loss of tax revenues and social instability. Table 1 shows the dynamics of changes in the shadow economy in agriculture in Azerbaijan in recent years. Significant fluctuations in the share of the shadow economy can be attributed to various factors, including changes in legislation, economic crises and external economic conditions.

Table 1. Dynamics of changes in indicators of the shadow economy in agriculture in Azerbaijan in 2020–2023

Year	GDP (billion USD)	Share of shadow economy (%)	Share of the agricultural sector in the shadow economy (%)
2020	49.5	32	27
2021	45.9	31	25
2022	46.6	30	26
2023	48.2	30	25

Source: compiled by the authors based on International Monetary Fund (2024) and World Bank (2023b)

The dynamics of the shadow economy in the agricultural sector have undergone certain changes in recent years, which can be attributed to both econom-

ic and political factors. The impact of the COVID-19 pandemic in 2020–2021 led to a decrease in farmers' incomes and an increase in informal employment due

to a decrease in demand for agricultural products and logistical difficulties. In 2020, certain tax incentives were introduced for the agricultural sector, which reduced the share of the shadow economy, but imperfect mechanisms for monitoring their implementation allowed certain entities to avoid official registration. Fluctuations in oil prices and other export commodities affected the national financial stability, which in turn affected investment in the agricultural sector. The simplification of registration procedures in 2022 contributed to some reduction in the shadow economy, but low awareness among farmers hampered this process. The decline in the shadow economy to 31% in 2021 can be attributed to a partial recovery in economic activity after the pandemic, as well as to the introduction of government incentives aimed at supporting small farmers. However, this positive trend could not be sustained in the long term. In 2023, the share of the shadow economy again reached 30%, which may be due to increased external economic pressure, fluctuations in export prices, and the lack of effectiveness of the implemented reforms. Fluctuations in oil prices and other export commodities affected the country's financial stability, which in turn affected the volume of investments in the agricultural sector. The simplification of registration procedures in 2022 contributed to some reduction in the shadow economy, but low awareness among farmers hampered this process.

The high level of informal employment in the agricultural sector is attributable to the following key factors. Many farmers do not have sufficient resources to provide formal employment for workers due to low commodity prices and high costs of production. In particular, the cost of registration, licensing and tax accounting can be a significant financial and time constraint for small farmers. Complicated administrative procedures and lack of transparency contribute to the fact that many farmers avoid formal registration. Many small farmers do not have access to credit or other financial instruments, driving farmers to operate outside the formal economy (Lanchenko & Ivchenko, 2024).

The loss of tax revenues due to the shadow economy has serious consequences for the state budget. The lack of tax revenues limits the ability to finance programmes to support rural areas, such as infrastructure construction or the development of educational initiatives for farmers (Guliyeva & Azizova, 2022). Low levels of funding for roads, irrigation systems and market infrastructure impede the productivity and efficiency of the agricultural sector. Lack of funds limits the ability to implement sustainable land use practices, leading to land degradation. The shadow economy also significantly affects the competitiveness of agricultural enterprises. Informal enterprises often do not comply with international standards, which limits their access to foreign markets. Shadow enterprises cannot benefit from government subsidies or loans

for modernisation, which reduces their productivity and product quality. Due to the lack of transparency, such enterprises rarely secure profitable contracts or receive foreign investment.

The significant size of the shadow economy in Azerbaijan's agriculture negatively affects several aspects of economic development. The lack of control over informal income leads to significant losses in tax revenues. According to estimates, annual budget losses due to informal employment in agriculture can reach billions of dollars. The existence of shadow businesses that do not comply with tax obligations and standards creates an unequal playing field for legal businesses. This can reduce investment in the agricultural sector and worsen its competitiveness in the international market. The high level of the shadow economy can also cause social tension, as workers do not have access to social guarantees, health insurance and pensions, which negatively affects the quality of life of the rural population and the overall social atmosphere.

Informal employment in agriculture is one of the main factors contributing to the development of the shadow economy. According to studies, up to 40% of agricultural workers are employed in the informal sector, which makes monitoring and taxation difficult. Workers employed in the shadow economy often have less knowledge and skills, which leads to lower productivity (Chornyi & Chorna, 2017). The lack of training and investment in labour force development affects the quality of agricultural products. Shadow businesses usually do not have access to credit and other financial instruments, which limits their ability to invest and grow. Informal sector workers have no legal protection, causing a threat of exploitation and social exclusion. An analysis of the level of shadow economy in Azerbaijan's agriculture points to the need for comparison with other countries in the region. Azerbaijan has a higher level of shadow economy in the agricultural sector than most of its neighbouring countries. These data indicate that the shadow economy in the agricultural sector of Azerbaijan is a serious obstacle to agricultural development compared to neighbouring countries. The need for comprehensive reforms in the tax system and formalisation of economic activity is urgent.

Thus, the shadow economy in agriculture in Azerbaijan is a complex problem that requires a systematic approach to address. The main measures that can be taken to reduce the shadow economy include formalising employment, improving tax policy and stimulating investment. Developing programmes that facilitate the transfer of informal workers to the legal sector, simplifying taxation procedures for small and medium-sized agricultural enterprises, and introducing tax incentives for enterprises willing to invest in formalising operations can help reduce the shadow economy in the agricultural sector, increase tax revenues and improve the socio-economic situation in the country (Table 2).

Table 2. Comparison of the level of shadow economy in agriculture in Azerbaijan with other countries in the region (2023)

Country	Share of shadow economy (%)
Azerbaijan	25
Georgia	20
Armenia	22
Turkey	23
Uzbekistan	30

Source: compiled by the authors based on State Statistical Committee of the Republic of Azerbaijan (2023)

The share of the shadow economy in the neighbouring countries also shows differences that depend on several specific factors. For instance, the share of the shadow economy in Armenia is 22%, which is lower than in Azerbaijan (25%). This can be attributed to the better level of government control over tax compliance in Armenia, as well as targeted support programmes for farmers that encourage business formalisation. In Turkey, where the share is 23%, technological solutions for monitoring agricultural activities are being more actively implemented, which also contributes to reducing the shadow economy. At the same time, Uzbekistan has a higher rate (30%), which can be explained by complex administrative procedures, low access to finance for farmers, and a high tax burden. Thus, the difference in the level of shadow economy between the neighbouring countries reflects both differences in government policies and the specific economic conditions of each country. These data indicate that the shadow economy in Azerbaijan's agricultural sector is a significant challenge to agricultural development compared to its neighbours. The need for comprehensive reforms in the tax system and formalisation of economic activity is urgent.

Thus, the shadow economy in Azerbaijan's agriculture is a complex problem that requires a systematic approach to address. The main measures that can be implemented to reduce the shadow economy include: the formalisation of employment and – the development of programmes to facilitate the transfer of informal workers to the legal sector. Improving tax policy – simplifying taxation procedures for small and medium-sized agricultural enterprises. Stimulation of investment – introducing tax incentives for enterprises willing to invest in formalising their operations. These steps can help reduce the shadow economy in the agricultural sector, increase tax revenues and improve the socio-economic situation in the country.

Assessment of measures to counteract the shadow economy. Combating the shadow economy in agriculture in Azerbaijan is an important component of the state policy aimed at stabilising the economy and ensuring transparency in the agricultural sector. To this end, the Azerbaijani government is implementing

numerous policies and programmes to reduce the shadow economy and encourage the formalisation of agricultural enterprises. One of the main strategies is to improve tax administration. As part of this initiative, the government is introducing new technologies to automate tax collection, which allows for more effective control of budget revenues and reduces tax fraud. For instance, since 2020, electronic platforms for registering farms have been introduced, which simplifies the tax payment process and reduces the administrative burden (International Monetary Fund, 2024).

Social programmes are also substantial in countering the shadow economy. They are designed to improve the living standards of the rural population and provide access to financial resources. In 2021, the government launched a programme to support small farmers, which includes subsidies for the purchase of machinery and seeds, helping to legalise their activities and reduce their dependence on shadow practices. It also includes unemployment programmes that provide financial support to those who have lost their jobs in the agricultural sector (World Bank, 2023b). Regulatory reforms are also an important aspect of the fight against the shadow economy. The Azerbaijani government contribute to the simplification of legislation to make it more understandable and accessible to farmers. This includes reducing the number of documents required to do business and shortening the time it takes to process them. For example, in 2020, the number of licences required to conduct agricultural activities was reduced, which helped to reduce bureaucratic obstacles.

Educational programmes are also important to raise awareness among farmers about the importance of legal employment and taxation. Training seminars and workshops for farmers help them better understand the benefits of operating legally, including access to credit, social guarantees and markets. In 2021, such programmes reached more than 10,000 farmers, indicating a growing awareness of the importance of legalising their activities (State Statistical Committee..., 2023). The effectiveness of these measures can be assessed through a SWOT analysis, which allows identifying strengths, weaknesses, opportunities and threats (Table 3).

Table 3. SWOT analysis of measures to counteract the shadow economy in agriculture in Azerbaijan

Category	Elements
Advantages	State support in the form of subsidies and training programmes for farmers. Growing awareness of the benefits of legalisation among farmers. Introduction of new technologies to improve tax administration. Openness to international investment.
Disadvantages	High level of corruption in the tax authorities, which hinders transparency. Insufficient training of personnel to administer the new systems. Limited access to information on tax legislation for small farmers.
Possibilities	Attraction to international investment in agriculture. Partnership with NGOs to raise awareness. Expansion of support programmes for small farmers, including access to credit and technology.
Threats	Economic crises that may reduce funding for government programmes. Competition from the informal sector. Changes in legislation that may complicate business operations.

Source: compiled by the authors

This SWOT analysis reflects a comprehensive approach to assessing measures to counteract the shadow economy in agriculture in Azerbaijan. Strengths point to positive trends that may contribute to the legalisation of activities. However, problems related to corruption and lack of training need to be prioritised. Attracting international investment and cooperation with civil society organisations could be important drivers of development, although threats such as economic crises could significantly affect programme implementation.

For a more detailed assessment of the effectiveness of measures to combat the shadow economy in

agriculture, it is necessary to monitor and analyse indicators such as the dynamics of tax revenues, the level of formalisation of employment, and changes in the business environment. For instance, data analysis shows that tax revenues from agricultural enterprises increased in 2023 compared to the previous year, which indicates the effectiveness of the reforms implemented. Table 4 shows the dynamics of tax revenues from agricultural enterprises in 2021-2023. The growth of tax revenues after the introduction of electronic platforms and other reforms is an important indicator of the effectiveness of these measures.

Table 4. Tax revenues from agricultural enterprises for 2021-2023

Year	Tax revenues (AZN million)	Growth (%)
2021	200	-
2022	230	15
2023	265	15.2

Source: compiled by the authors based on State Statistical Committee of the Republic of Azerbaijan (2023)

The introduction of electronic platforms in 2022 was an important step in the fight against the shadow economy. Compared to 2021, tax revenues in 2022 increased by 15%. This growth can be attributed to increased transparency in accounting for farmers' income and reduced opportunities for tax evasion. In 2023, the positive dynamics continued with a further 15.2% increase in revenues, which demonstrates the sustainability of the reforms implemented. Electronic platforms simplified access to government services, such as farm registration and reporting, reducing administrative burdens for farmers. Furthermore, subsidies provided to small and medium-sized farms have contributed to their economic activity, which has also contributed to the growth of tax revenues. For

instance, farmers who legalised their activities gained access to financial support, which expanded production and, consequently, increased their tax liabilities. The author's assessment shows that measures aimed at digitalisation and financial support have demonstrated a positive impact on tax revenues. However, to maintain this momentum, it is necessary to continue to improve the electronic administration system and increase the amount of subsidies to encourage business formalisation.

Table 5 shows how state subsidies are distributed among farmers in different categories. The role of subsidies in supporting business formalisation is important, as they encourage farmers to legalise their activities to receive financial assistance.

Table 5. Distribution of subsidies for farmers in 2023

Category of subsidies	Number of recipients	Amount of subsidies (million AZN)
Seed subsidies	5,000	20
Subsidies for equipment	2,500	15
Fertiliser subsidies	4,000	10
Other subsidies	3,000	5
Total	14,500	50

Source: compiled by the authors based on State Statistical Committee of the Republic of Azerbaijan (2023)

The analysis shows that subsidies for seeds and machinery had the greatest impact on farm formalisation. Recipients of these subsidies demonstrated higher levels of legalisation than those who did not receive support. For instance, among farmers who received subsidies for machinery, the level of business registration increased by 25%, expanding access to markets and credit. Seed subsidies also increased productivity, which had a positive impact on farmers' incomes and increased their interest in legalising their activities. In comparison, farmers who did not receive subsidies

showed significantly lower registration rates, remaining mostly in the informal sector. This suggests the importance of financial support as an incentive to formalise farms. The author's assessment indicates that expanding subsidy programmes and making them more accessible could be effective in combating the shadow economy in the agricultural sector. Table 6 on the results of the educational programmes shows a great interest in the training and a high level of satisfaction among participants. This underscores the importance of raising awareness of business legalisation in agriculture.

Table 6. Results of educational programmes in 2023

Application type	Number of participants	Satisfaction (%)
Seminars on tax awareness	8,000	85
Business planning training	4,000	90
Women support programs	2,000	88
Total	14,000	88

Source: compiled by the authors based on State Statistical Committee of the Republic of Azerbaijan (2023)

Participation of farmers in tax awareness workshops and business planning training can significantly influence the conduct of farmers, including the legalisation of employment and increased tax revenues. Workshops that raise awareness of tax obligations and the benefits of legalising activities can motivate farmers to register their businesses and employees. This is particularly important in rural areas where many farmers operate informally. In addition, participation in business planning training helps farmers to better organise their activities, which can also facilitate the registration of new farms and ensure more efficient resource management. Participants' satisfaction with the training plays an important role in further behavioural change. Farmers who have had a positive experience with the programmes are more inclined to make changes in their activities, such as farm registration and tax compliance. For instance, a high level of satisfaction with business planning training (90%) may correlate with a greater willingness of farmers to legalise their business and seek new development opportunities, such as attracting investment or expanding production. In 2023, new subsidy programmes for farmers were also introduced to improve productivity and encourage the legalisation of agricultural activities. For instance, 400

million manats were allocated to support small and medium-sized farms (World Bank, 2023b).

Combating the shadow economy in Azerbaijan's agriculture is an important step towards ensuring economic stability and transparency in the agricultural sector. Measures taken by the government, such as the introduction of electronic platforms to simplify farm registration, subsidies and educational programmes, have shown some positive results. There has been an increase in tax revenues and an increase in farmers' awareness of the benefits of legalising their activities. Overall, the assessment of the effectiveness of these measures demonstrates a gradual impact on the formalisation of economic activity. Over the past three years, tax revenues from the agricultural sector have increased, and the level of farm registration has also shown positive dynamics. This indicates that the initiatives taken are helping to reduce the size of the shadow economy. At the same time, significant levels of corruption and imperfections in some administration mechanisms remain significant challenges.

The importance of these measures is determined by their contribution to national economic stability. Reducing the shadow economy strengthens the financial stability of the state, creates conditions for fair

competition and provides social guarantees for agricultural workers. Transparency of economic activity also helps to attract international investment and increase public confidence in government programmes. However, to be more effective in combating the shadow economy, subsidy programmes, for small and medium-sized farms, should be expanded and access to finance should be simplified. Mechanisms for monitoring the activities of agricultural sector entities should be improved, with a focus on combating corruption. It would be advisable to intensify educational activities aimed at raising awareness among farmers about the benefits of formalising their activities and to simplify tax and administrative procedures to encourage business legalisation. Thus, the measures taken are important for stabilising Azerbaijan's economy and creating a transparent environment for the development of the agricultural sector. Further efforts should be made to improve existing programmes and develop new initiatives that will help formalise economic activity and increase the efficiency of public resources.

Recommendations for tax policies improvement.

The agricultural sector in Azerbaijan, similarly to many countries, faces challenges related to the shadow economy and hidden incomes. To overcome these challenges, it is necessary to implement a comprehensive approach that includes improving tax policy, increasing transparency and formalising farmers' incomes. One of the top priorities is to simplify farm registration procedures. Currently, the existing bureaucracy is often a barrier for new farmers wishing to legalise their activities. The government should reduce the complexity of registration processes, including simplifying the requirements for documents and shortening the timeframe for their review. This will allow more farmers to register their farms and thus reduce the shadow economy. To facilitate income formalisation, it is necessary to introduce support programmes for farmers willing to legalise. These programmes could include training courses to teach farmers how to keep books and prepare tax returns. Studies show that education is one of the key factors that influence farmers' willingness to legalise their income. Such programmes must be accessible and understandable, as not all farmers have the necessary level of knowledge in financial matters.

Addressing the experience of other countries, such as India and Brazil, it is possible to assess how such reforms have contributed to the fight against the shadow economy in the agricultural sector. India conducted substantial economic reforms in the 1990s, addressing the reduction of state control and developing market mechanisms in agriculture. Efforts were made to improve infrastructure and increase transparency in agricultural policy. This improved the efficiency of the tax system, by reducing barriers for small farmers. However, the reforms faced difficulties due to uneven access to resources and difficulties in monitoring in large

regions. At the same time, the shift in production patterns towards high-quality agricultural products has become an important development factor, partly leading to an increase in foreign trade and a reduction in informal economic activity in the agricultural sector (Sreenu, 2024). Brazil has also implemented reforms in the agricultural sector to improve transparency and reduce the impact of the shadow economy. Investments in infrastructure and electronic platforms for farmers have reduced opportunities for tax evasion. However, the reforms faced challenges due to large regional differences in access to new technologies and monitoring systems. These examples demonstrate that the effectiveness of reforms in the agricultural sector aimed at reducing the shadow economy often depends on a combination of infrastructure investment, access to modern technology, and proper monitoring.

The private sector can actively engage in educational programmes for farmers in accounting and tax returns through various mechanisms. One possible form of cooperation is partnerships with educational institutions and non-governmental organisations. Businesses can cooperate with universities and colleges to develop specialised courses that are targeted at farmers, accounting for their level of knowledge and needs. This approach will ensure access to quality financial education that meets the requirements of farmers. Another important area is the development of digital platforms and mobile applications. Private companies can create online courses, video tutorials, and interactive tools for farmers who want to learn the basics of accounting, tax returns, and financial planning. This will allow access to training regardless of location and time, which is especially important for farmers who often work in conditions of limited access to traditional educational resources.

The private sector can also participate in financing and sponsoring training programmes. Large agricultural enterprises or financial institutions could invest in seminars, workshops and courses for small farmers. This would not only increase financial literacy among farmers but also contribute to a more transparent and efficient agricultural economy. The private sector can also offer farmers consulting services in accounting and taxation. This can be either individual consultations or group training to help farmers better understand complex financial and tax procedures. This approach allows for practical advice and assistance from experts. Lastly, financial institutions can create specialised financial instruments for farmers to help them legalise their income and optimise their tax liabilities. Training loans or other financial products targeted at farmers would help support them in adapting to new financial realities and legal requirements. Thus, the involvement of the private sector in educational programmes for farmers can significantly improve their financial literacy and contribute to the legalisation of income and the development of the agricultural sector as a whole.

The introduction of electronic record-keeping systems is an important step in fighting the shadow economy and increasing transparency in the agricultural sector. Expansion of the functionality of existing farm registration platforms, including the introduction of automatic tax liability calculators and integration with accounting systems, will significantly reduce the possibility of errors in filling out tax returns. This will not only ease the tax payment process but also improve accessibility and convenience for farmers, especially those with limited accounting experience. The use of technology, such as blockchain, can further enhance this process by providing a high level of transparency and trust in agricultural financial transactions. Blockchain allows for the creation of an immutable and open record of all financial transactions, making it difficult to manipulate data or evade taxation (Vazov *et al.*, 2022). Each transaction processed through such a system is recorded in blocks that cannot be changed or deleted without the consensus of all participants. This significantly reduces the risk of corrupt practices, as all data is available for inspection by tax authorities and other regulatory authorities.

Automation of accounting and tax processes on such platforms reduces the administrative burden on farmers. By automatically calculating tax liabilities and generating the necessary reports, farmers can focus on running their businesses rather than on routine administrative tasks. This also reduces human error and misunderstandings with the tax authorities, which can prevent fines and penalties. As a result, the tax payment process becomes more efficient, which in turn increases tax revenues to the budget. Therefore, technologies such as blockchain, as well as automated systems for accounting and tax payments, can significantly increase transparency and trust in the agricultural sector. They reduce the administrative burden on farmers, reduce the likelihood of corruption schemes and increase the efficiency of tax collection, which is an important step in the fight against the shadow economy.

The creation of a reward system for farmers who comply with tax regulations can be a powerful incentive to legalise income and increase transparency in agriculture. The idea of introducing tax rebates for those who voluntarily declare their income has already shown positive results in some countries. Such a system encourages farmers to be more open, reduces costs, and at the same time helps to bring them into the formal economy. In addition, these measures can help create a more equitable environment for all market participants by encouraging fair competition. Involving the community in monitoring compliance with tax regulations is also an important step in ensuring transparency and increasing trust in the system (Shimchenko & Berezanska, 2023). Establishing community councils that monitor farmers' activities allows for effective monitoring and assessment of compliance with tax requirements. Importantly, these councils should cooperate with the

tax authorities to ensure a constant exchange of information and feedback. Such radical measures can create mechanisms for detecting and responding promptly to violations, which will help to avoid serious offences and abuses at a later stage.

Public councils are also central in ensuring transparency in the use of public funds. They can oversee how tax revenues are spent and check the effectiveness of government programmes and initiatives in agriculture. Moreover, creating channels for registering complaints from farmers will allow controlling bodies to respond to problems promptly and ensure faster resolution of conflict situations. This will not only help to promote compliance with tax regulations but also ensure constructive interaction between the state, business and the public. Deep involvement of the public and independent supervisory bodies in the process of monitoring tax regulations can significantly improve the transparency of the agricultural sector, reduce the level of abuse and corruption schemes, and contribute to more efficient use of resources collected through taxes.

Reforming the existing taxation mechanisms in the agricultural sector is another important step towards improving the situation. The tax system should be adapted to the specifics of agricultural production, in particular, consideration should be given to reducing tax rates for key agricultural products. The introduction of tax holidays for new farms could also be a good incentive for the development of the agricultural sector. These changes will help to increase farmers' incomes and increase the overall level of investment in agriculture. Changes in tax administration are also an integral part of the reform. Government agencies should introduce new technologies to improve tax administration processes. The introduction of electronic platforms for filing tax returns will make life easier for farmers and reduce corruption risks in the tax authorities. The up-to-date data and statistics provided by these systems will also help the state to plan its budget and manage resources more effectively.

Finally, ensuring transparency and accountability in the use of tax revenues is critical for the credibility of government institutions. The public should have access to information on how public funds, which come from taxes on the agricultural sector, are spent. Regular reports on the use of budgetary funds can increase trust in the system and motivate farmers to legalise their activities, as they will be confident that their contributions are going to the development of their industry. Thus, the comprehensive implementation of these recommendations will help reduce the shadow economy and increase the efficiency of taxation in the agricultural sector of Azerbaijan. A systematic approach involving the active participation of all stakeholders – government agencies, farmers and the community – will create a transparent and efficient tax system that meets the needs of the modern agricultural sector.

DISCUSSION

The results of the study indicate a significant level of informal employment in the agricultural sector of Azerbaijan, which accounts for approximately 40% of total employment in the sector. This indicator underlines that a significant part of economic activity remains outside the scope of official accounting and control, which has a negative impact on the state budget, social stability and competitiveness. At the same time, such phenomena are typical not only for Azerbaijan but also for many developing countries.

Comparison of the results with the study by Y. Wang *et al.* (2024) indicates that the shadow economy is often accompanied by environmental challenges in developing countries. The authors argue that poor governance and weak institutional frameworks worsen the impact of the informal sector on ecosystems. The results confirm this thesis, as informal employment in the agricultural sector of Azerbaijan limits the possibility of implementing environmentally sustainable practices. On the other hand, E. Atar and Ş. Kirboz (2024) demonstrated that structural reforms in local governance can reduce the size of the shadow economy, including the agricultural sector. This correlates with the findings of the study on the need to reform the tax system and implement measures to formalise employment.

It is also necessary to address the international experience of reforms in the field of local governance, such as in the study by M. Strokan (2024) on the transformation of post-Soviet states. V. Boshnakov and V. Goev (2024) analysed the impact of corruption and tax pressure on the shadow economy in Bulgaria. The authors concluded that high levels of corruption and tax abuse are the main factors contributing to the preservation of the informal sector. The findings also identify corruption as one of the key problems that hinders the effectiveness of the fight against the shadow economy in Azerbaijan. However, in contrast to the findings of A. Al Qudah (2024), emphasised the importance of educational campaigns to raise tax awareness in the context of Middle Eastern countries, this study found that even with such programmes, the lack of financial incentives for farmers to support their legalisation remains a key obstacle. D. Enste (2024) noted that even in industrialised countries the shadow economy can be significant due to tax pressure and complexity of legislation, which encourages entrepreneurs to evade taxes, which was also highlighted in this study. An important aspect is the relationship between tax reforms and government efficiency. According to S. Wijaya and I. Dewi (2024), governance effectiveness significantly moderates the impact of the shadow economy on tax revenues. This paper confirms this conclusion, as the introduction of electronic platforms for farmers' registration and tax administration in Azerbaijan has contributed to an increase in revenues.

The results also confirm the findings of S. Gnanon (2023), which states that the shadow economy weakens the effectiveness of tax reforms in developing countries, creating a vicious circle of low revenues and limited opportunities to finance public services. At the same time, A. Polese *et al.* (2023) draw attention to the specifics of post-Soviet countries, where cultural and historical features have a significant impact on the formation of the informal sector. This aspect also requires further research in the context of Azerbaijan. Given the impact of the shadow economy on competitiveness, as emphasised by A. Naghizadeh *et al.* (2023), future research should focus on a detailed analysis of financial incentives that could contribute to the formalisation of economic activity.

Several results of the study differ from the conclusions of T. Lichard *et al.* (2021) who emphasised the role of households as a source of data for estimating the shadow economy. In this study, the focus is on the formalisation of business units, while the household aspect was not considered in detail. The results of the study are also in line with the findings of D. Němec *et al.* (2021), who argued that corruption significantly worsens the state of the fight against the shadow economy. In the context of Azerbaijan, corruption in the tax authorities remains a key obstacle to the effective administration of reforms. It is also worth considering an interdisciplinary approach, integrating economic analysis methods with sociological research, as recommended by N. Canh and S. Thanh (2020). Comparison of the research results with international studies indicates the need to adapt the successful practices of other countries to the specifics of Azerbaijan. A systematic approach to reforming the tax system, including fighting corruption and simplifying registration procedures, can significantly reduce the size of the shadow economy and increase the competitiveness of the agricultural sector.

CONCLUSIONS

The study conducted a comprehensive analysis of measures to combat the shadow economy and taxation of hidden incomes in the agricultural sector of the Republic of Azerbaijan. The main objective of this study was to identify the factors that contribute to the existence of the shadow economy in the agricultural sector, as well as to assess the effectiveness of the measures already implemented to reduce its scale. The main findings indicate that about 40% of agricultural workers are employed in the informal sector, resulting in tax revenue losses of more than 1 billion USD per year. This figure undermines the financial stability of the state and creates an uneven playing field for businesses, where legal enterprises are forced to compete with informal ones.

The study revealed several qualitative indicators that confirm the existence of systemic problems in tax policy. A low level of awareness among entrepreneurs about the benefits of legal employment and the

existing tax system, as well as the lack of financial resources for small and medium-sized agricultural enterprises. As a result, most entrepreneurs believe that running a business in the shadows is less risky and more profitable. The analysis identified strengths, such as government support in the form of subsidies and training programmes, and the gradual introduction of digital technologies in tax administration. However, high levels of corruption, complexity of administrative procedures, lack of awareness among farmers of the benefits of formal economic activity, and limited access to financial resources remain significant challenges. This creates barriers to the legalisation of business activities in agriculture.

Recommended measures to overcome these challenges include simplification of farm registration procedures, which would reduce time and financial costs for small entrepreneurs. Expanding educational programmes would help to increase tax awareness and financial planning skills among farmers. Increasing government subsidies will help support entrepreneurs who are ready to legalise their activities by meeting their needs for machinery, seeds and fertilisers. The digitalisation of tax administration will minimise corruption risks and increase the transparency of economic processes. The development of tax incentives, such as tax holidays or discounts for small businesses, will be an important tool to attract farmers to the legal sector. An important aspect is to address international experience that demonstrates the effectiveness of combining

digitalisation, educational measures and financial incentives in the fight against the shadow economy. However, the implementation of the proposed measures requires overcoming key challenges, such as high levels of corruption and imperfect institutional systems.

Limitations of this study include the reliance on available statistics that may not fully reflect the true extent of the shadow economy and the lack of analysis of some regional and social factors. Further research should conduct an in-depth analysis of the impact of regional differences, environmental challenges, and socio-cultural aspects, as well as on the long-term effects of the proposed reforms. In the future, it is worth analysing opportunities to improve the results obtained. One area for future research could be to study international experience in combating the shadow economy, particularly in countries with similar economic conditions. It is also necessary to analyse the impact of the shadow economy on the socio-economic development of rural areas to develop a comprehensive policy that would not only reduce shadow activities but also improve the living conditions of the population. This will help to create a more sustainable agricultural sector capable of developing and adapting to current economic challenges.

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CONFLICT OF INTEREST

None.

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**Комплексний аналіз протидії тіньовій економіці
та оподаткування прихованих доходів
в аграрному секторі Азербайджанської Республіки**

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Анотація. Метою дослідження було виявлення основних причин тіньової економіки в аграрному секторі Азербайджану, зокрема факторів, що сприяють неформальній зайнятості, та оцінка ефективності заходів, спрямованих на її скорочення. У дослідженні проведено комплексний аналіз заходів протидії тіньовій економіці та оподаткування прихованих доходів в аграрному секторі Азербайджанської Республіки. Методологія дослідження ґрунтувалася на аналізі статистичних даних, зокрема, звітів Міжнародного валютного фонду, Світового банку та Державного статистичного комітету Азербайджану, а також на порівняльному аналізі з даними сусідніх країн: Грузії, Вірменії, Туреччини, Узбекистану. Основні результати дослідження свідчили, що близько 40 % працівників сільського господарства зайняті в неформальному секторі, що призводить до втрат податкових надходжень у розмірі понад 1 млрд. доларів США на рік, що суттєво підриває фінансову стабільність держави. Дослідження також визначило, що чинна податкова політика не стимулює формалізацію зайнятості, а відсутність доступу до фінансування обмежує можливості для розвитку легального бізнесу. Отримані результати свідчать про необхідність комплексного реформування податкової системи, включаючи спрощення процедур оподаткування, покращення податкового контролю та створення стимулів для переходу тіньових працівників до легального сектору. Реалізація цих заходів може суттєво покращити економічну ситуацію в сільському господарстві, забезпечити справедливі умови для всіх учасників ринку та гарантувати соціальні права працівників, що сприятиме стабільності та розвитку аграрного сектору

Ключові слова: сільське господарство; агробізнес; фінанси; корупція; антикорупційні механізми

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