НАУКОВІ ГОРИЗОНТИ

Засновник, редакція, видавець:
Поліський національний університет

Рік заснування: 1998

Рекомендовано до друку та поширення через мережу Інтернет Вченою радою Поліського національного університету (протокол № 2 від 27 вересня 2023 року)

Свідоцтво про державну реєстрацію друкованого засобу масової інформації
серії КВ № 24997-14937 ПР

Науковий журнал включено до категорії А Переліку наукових фахових видань України, у яких можуть публікуватися результати дисертаційних робіт на здобуття наукових ступенів доктора та кандидата ветеринарних, економічних, сільськогосподарських і технічних наук зі спеціальностей: 051 – Економіка; 071 – Облік і оподаткування; 072 – Фінанси, банківська справа та страхування; 073 – Менеджмент; 075 – Маркетинг; 076 – Підприємництво, торгівля та біржова діяльність; 101 – Екологія; 133 – Галузеве машинобудування; 201 – Агрономія; 202 – Захист і карантин рослин; 203 – Садівництво та виноградарство; 204 – Технологія виробництва і переробки продукції тваринництва; 205 – Лісове господарство; 206 – Садово-паркове господарство; 208 – Агроінженерія; 211 – Ветеринарна медицина (наказ МОН України № 1166 від 23 грудня 2022 р.)

Журнал представлено у міжнародних наукометричних базах даних, репозитаріях та пошукових системах: Інституційний репозитарій Поліського національного університету, AGRICOLA, CAB Abstracts and Global Health (CABI), Open Academic Journals Index (OAJI), Scopus, Index Copernicus, Реєстр наукових видань України, Національна бібліотека України імені В. І. Вернадського, Crossref, Directory of Open Access Journals (DOAJ), ERIH PLUS, MIAR, International System for Agricultural Science and Technology (AGRIS)

Адреса редакції:
Поліський національний університет
10008, б-р Старий, 7, м. Житомир, Україна
E-mail: info@sciencehorizon.com.ua
https://sciencehorizon.com.ua/uk

SCIENTIFIC HORIZONS

Founder, Editorial and Publisher:
Polissia National University

Year of foundation: 1998

Recommended for printing and distribution
via the Internet by the Academic Council
of Polissia National University
(Minutes No. 2 of September 27, 2023)

Certificate of state registration
print mass media
Series KV No. 24997-14937 PR

The scientific journal is included in category A of the List of scientific professional publications of Ukraine, in which the results of dissertations for the degree of doctor and candidate of veterinary, economic, agricultural, and technical sciences can be published in the following specialties: 051 – Economy; 071 – Accounting and Taxation; 072 – Finance, Banking and Insurance; 073 – Management; 075 – Marketing; 076 – Entrepreneurship, Trade and Exchange Activities; 101 – Ecology; 133 – Sectoral Engineering; 201 – Agronomy; 202 – Plant Protection and Quarantine; 203 – Horticulture and Viticulture; 204 – Technology of Production and Processing of Livestock Products; 205 – Forestry; 206 – Park and Gardening Management; 208 – Agricultural Engineering; 211 – Veterinary Medicine (Order of the Ministry of Education and Science of Ukraine No. 1166 of December 23, 2022)

The journal is presented international scientometric databases, repositories and scientific systems: Institutional Repository of Polissia National University, AGRICOLA, CAB Abstracts and Global Health (CABI), Open Academic Journals Index (OAJI), Scopus, Index Copernicus, Register of Scientific Publications of Ukraine, Vernadsky National Library of Ukraine, Crossref, Directory of Open Access Journals (DOAJ), ERIH PLUS, MIAR, International System for Agricultural Science and Technology (AGRIS)

Editors office address:
Polissia National University
10008, 7 Staryi Bvld., Zhytomyr, Ukraine
E-mail: info@sciencehorizon.com.ua
https://sciencehorizon.com.ua/en

НАУКОВІ ГОРИЗОНТИ

Том 26, № 9 2023

НАУКОВИЙ ЖУРНАЛ
Засновано 12 березня 1998 р.
Періодичність випуску: дванадцять разів на рік

SCIENTIFIC HORIZONS

Vol. 26, No. 9 2023

SCIENTIFIC JOURNAL
Year of establishment: Since March 1998.
Publication frequency: Twelve times a year

ЗМІСТ

ОГЛЯДОВА СТАТТЯ

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SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 9-20

UDC: 619:636.2:618.3: 591.111
DOI: 10.48077/scihor9.2023.09

Cow haemostasis and resistance of calves under hypoxia conditions

Mariia Kambur*
Doctor of Veterinary Sciences, Professor
Sumy National Agrarian University
40000, 160 Herasyma Kondratieva Str., Sumy, Ukraine
https://orcid.org/0000-0002-4864-5292
Andrii Zamazii
Doctor of Veterinary Sciences, Professor
Poltava National Agrarian University
36003, 1/3 Skovorody Str, Poltava, Ukraine
https://orcid.org/0000-0003-3138-0424
Viktor Kolenchenko
Postgraduate Student
Sumy National Agrarian University
40000, 160 Herasyma Kondratieva Str., Sumy, Ukraine
https://orcid.org/0009-00085-3472-9259
Oleksandr Demydko
Postgraduate Student
Sumy National Agrarian University
40000, 160 Herasyma Kondratieva Str., Sumy
https://orcid.org/:0000-0002-6433-315X
Yevheniia Livoshchenko
PhD in Veterinary Science, Associate Professor
Sumy National Agrarian University
40000, 160 Herasyma Kondratieva Str., Sumy, Ukraine
https://orcid.org/0000-0001-5826-4824

Article's History:
Received: 10.05.2023
Revised: 20.08.2023
Accepted: 27.09.2023

Abstract. Intensification of the livestock industry is impossible without effective animal reproduction. The solution to this problem is possible only if a viable offspring is obtained, and their body resistance and safety are increased, which determines the research relevance. In this regard, determining the influence of the haemostasis system and blood properties of cows on the growth and development of the foetus, and subsequently on the resistance of newborn calves, depending on the condition at birth, determined the research aim. The conditions of foetal growth and development

Suggested Citation:
Kambur, M., Zamazii, A., Kolenchenko, V., Demydko, O., & Livoshchenko, Ye. (2023). Cow haemostasis and resistance of calves under hypoxia conditions. *Scientific Horizons*, 26(9), 9-20. doi: 10.48077/scihor9.2023.09.

*Corresponding author

were found to be related to the activity of haemostatic factors and blood properties of cows. Hemocoagulation factors were active in animals that gave birth to calves in a state of hypoxia. The prothrombin time was 1.63, 1.40, 1.23 and 1.40 times lower, and the prothrombin haemostasis index was 1.53, 1.52, 1.35 and 1.46 times lower, respectively than in cows that gave birth to functionally active calves ($p<0.01$). The thrombin time of haemostasis, partially activated thrombin time and fibrinogen content in cows of the experimental groups were higher than in animals of the control group. The blood viscosity of cows in the control group was lower ($p<0.01$), and the blood coagulation of cows in the experimental groups was faster ($p<0.05$). The increase in coagulation properties of the blood of cows that gave birth to calves in a state of hypoxia occurred against the background of a decrease in the activity of the fibrinolytic system ($p<0.05$) and retraction of the blood clot. All this reduced the growth and development of the embryo and foetus. The placental ligamentous connection of foetuses born with signs of hypoxia was significantly greater than that of functionally active calves. The intensity and size of embryo growth were higher in the group of functionally active calves ($p<0.05$). The activity of leukocyte defence factors, and, accordingly, the resistance of the organism of functionally active calves at birth was higher. The percentage of activated leukocytes in the blood of calves was practically the same, and the microbial count was higher in calves of the control group ($p<0.05$). The results obtained in the course of the research can be implemented in the process of obtaining and rearing calves, and offered to all livestock farms in Ukraine

Keywords: clotting; blood; leukocytes; indices; fibrin

INTRODUCTION

Carrying a foetus into the female body is accompanied by the formation of a complex biological system. It is believed that the maintenance of its gravity is ensured by the mother's body, the foetus, and the placenta. A disturbance in only one of these systems triggers compensatory reactions in the other to maintain the conditions for foetal development. In solving this problem, the haemostasis system is of great importance. The stability of haemodynamic processes in the mother-placenta-foetus system is a condition that ensures the normal course of pregnancy. Circulatory disorders in this system lead to the development of foetal hypoxia. Intrauterine hypoxia is manifested by fetoplacental insufficiency. It leads to impaired blood circulation in the uteroplacental basin and characterises the flow of blood to the foetus.

O.S. Yaremchuk and R.L. Varpikhovskyi (2021) believe that obtaining viable offspring and preserving the number of animals for reproduction is an urgent problem of livestock production. According to O.W. Stasyshyn (2020), the solution to the problem is possible only if a viable offspring with a high level of resistance is obtained. According to L.G. Tunikovska (2020), the environment continuously affects the mother-placenta-foetus system by various factors, causing a change in the relationship between the female and foetus. S. Kohli *et al.* (2021) point out that the mother's body creates conditions for the foetus. They depend on her ability to provide the foetus with components that affect the course and outcome of pregnancy. The development of the foetus in the mother's body contributes to increased blood clotting in the vessels and activation of haemostatic factors.

S. Vlasenko *et al.* (2021) established that during foetal gestation, the aggregation properties of red blood cells and platelets change in the female body, and the effectiveness of blood clot resorption decreases. The effect of hypoxia on the haemostatic system, foetal oxygen supply and the condition of newborn animals remains to be determined. The formation of the provisional organs in the body of females associated with foetal gestation is accompanied by physiological and morphological changes in all body systems. Studies have not determined the condition of newborn animals and the intensity of embryo growth. L.V. Koreyba (2019) proved that the intake of nutrients and oxygen to the foetus is an additional burden on the female body. This is evidenced by the haematological parameters of highly productive cows in the dynamics of the dry period. Insufficient supply of Oxygen to the foetus is manifested by hypoxia. It affects the activity of haemostasis factors and blood rheology. I.A. Zhabchenko *et al.* (2019), H. Hamada and S.G. Matthews (2019) considered the perinatal aspects of pregnancy maintenance in the context of chronic stress and proved that polycythaemia, increased platelet, and erythrocyte aggregation occur in the case of oxygen supply disorders. They also point out that a violation of oxygen homeostasis is accompanied by activation of the platelet link of the blood coagulation process, and the number of blood plates in the blood increases due to young platelet forms.

The haemostatic system responds to hypoxia by increasing the activity of coagulation processes and decreasing fibrinolytic activity. U. Ezechukwu *et al.* (2014) established a relationship between blood viscosity and platelet aggregation in the blood of pregnant women. Deviations from the physiological level of activity of haemostasis processes and indicators of blood reactivity in the female body during foetal gestation affect haemodynamic and haemostasiological processes. They disrupt processes adaptively related to the functions of the fetoplacental complex. The effect of hypoxia on the

utilisation of oxygen, the activity of coagulation and anti-coagulation factors, and blood properties have not been studied. According to G. Gonzales *et al.* (2017), an increase in blood viscosity and a decrease in the speed of its movement through blood vessels is manifested by stagnation in pregnant women. Tissues are saturated with fluid, and inflammatory processes occur. These processes are especially threatening for cows with multiple placentas. M.D. Kambur *et al.* (2018) believe that postpartum complications are accompanied by intense blood clot formation in the placental vessels, which is detected by analysing its appearance.

The impact of negative factors during critical periods of pre-and postnatal growth and development of the organism manifests itself at different times, especially during periods of dominant growth of organs of different systems (Livoshchenko, 2016). The mother's and foetus' organism forms an "adaptive response" to the action of negative factors, which is accompanied by a change in the functions of regulatory systems. The consequence of the "adaptive response" of the foetus may be a decrease in the reproductive capacity of its offspring, a violation of the metabolism of biologically active substances under the influence of androgens, which are found in significant quantities in the mother's body during prenatal stress It causes suppression of the body's immune response to an immunodeficiency state, shortens the gestation period, reduces foetal weight, and causes the development of pathological processes that complicate pregnancy and the birth of a foetus with a low level of body resistance (Matviichuk, 2022).

Such issues as the effect of hypoxia on the haemostasis of pregnant cows, foetal growth and development, and the resistance of newborn animals remained unaddressed by researchers, which in combination determines the subsequent focus of adequate measures to preserve newborn animals and increase their viability, which was the research aim.

MATERIALS AND METHODS

The experiments were carried out during 2021-2022 at the farm "Vitalia", Chernecha Sloboda village, Buryn district, Sumy region, private joint-stock company "RADHOSP "Shevchenkivskyi", Shevchenko village, Konotop district, Sumy region, and "Chernihiv Main Enterprise for Livestock Breeding" (Chernihiv, Chernihiv region). Blood samples were tested in the clinical diagnostic laboratory "Sekhmet", Sumy. The devices of the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv, were used for the study. Indicators of oxygen homeostasis were determined in the clinical diagnostic laboratory of the Sumy Regional Children's Clinical Hospital (Sumy) and the laboratory of veterinary medicine (Lebedyn, Sumy region). Blood samples were prepared for the study at the Department of Anatomy, Normal and Pathological Physiology of Animals of Sumy National Agrarian University.

Cow parturition was observed during the study. According to the characteristics of the first breath, and its stability, the resulting calves and mother cows were divided into groups (n=5). Calves with an adequate respiratory process after birth were classified as functionally active animals. Cows – mothers of functionally active calves were assigned to the control group of animals (control). Calves born with meconium in the amniotic fluid, or a state of asphyxia and maternal cows were assigned to the first group (I). The second group (II) included mothers and their offspring who had inadequate, spontaneous respiratory movements after birth. The third group (III) included cows and calves born to them with spontaneous, adequate respiratory movements.

Blood samples were taken from the umbilical cord vessels in calves at birth and the jugular vein in cows. Asepsis and antisepsis rules were followed during the study. Blood samples were collected in vacuum containers with special tubes with heparin. Blood samples were urgently delivered on ice for testing in the laboratory. The diameter of the umbilical cord of newborn calves was measured, and body weight was determined by weighing. Growth, development of the embryo, foetus and resistance of the calves' organism were determined using the following formulas: placental connection was defined as the ratio of the umbilical cord cross-sectional surface to the body weight of the offspring; intensity of embryonic growth - by the value of the specific ratio of the body weight of newborns to the body weight of the parental pair; the value of embryonic growth, g, was defined as the ratio of the body weight of the offspring (kg) to the duration of gestation (days).

The oxygen balance of the animal organism was determined using a blood gas analyser Easy Blood JAS, Medica, (USA), platelet haemostasis – using a Coagulometer K 3002 OPTIC. Blood smears were prepared to determine the resistance of the calves' organism. A drop of blood was applied to the edge of a dry, degreased slide. The ground edge of the cover glass was brought to the drop at an angle of 45°. The angle formed by the glass was evenly filled with blood. By moving the right hand away from the body, the drop of blood was spread in a thin layer over the surface of the slide. The smear was air-dried and fixed. It was placed in a bath and methyl alcohol was applied to it with a pipette for 3-5 minutes. The smear was removed from the bath and dried. It was stained according to the Romanovski-Gimza method. The finished stain was pre-diluted with distilled water. For each ml of water, 2-3 drops of paint were added and poured onto the smear, which was kept in a humid chamber for 30-40 minutes. The stain was washed off with distilled water. The blood smear was air-dried. The leukocyte formula was counted in the prepared blood smears.

Indicators of leukocyte formula were used to determine the activity of leukocytes and indices of resistance of the calves' organism:

- Phagocytic activity – % of leukocytes involved in phagocytosis;
- The phagocytic count is the ratio of the number of bacteria absorbed to the number of cells involved in phagocytosis;
- Phagocytic index – % of phagocytes that absorbed bacteria;
- The phagocytosis completeness index was calculated by dividing the killed bacteria in the phagocytes by the total number of absorbed bacteria and multiplying by 100;
- Nuclear index was defined as the ratio of the percentage of monocytes and stick-nucleated neutrophils to segmented neutrophils;
- Resistance index = % lymphocytes: % segmented neutrophils;
- WBC, % – determined by dividing the number of active phagocytes by the number of leukocytes and multiplying by 100;
- CAF, 10^9/L – the number of active phagocytes as the product of the number of leukocytes divided by the number of lymphocytes and the phagocytic index divided by 100;
- Microbial count, 10^9/l, was defined as the product of CAF and phagocytic count;
- Leukocyte intoxication index (LII)=(2xP+C)/((Mo+L) xE+1);
- Leukocyte shift index, % (LSI)=(M+MM+PN):CH;
- Leukocyte index=(4xmol+3xun+2xPN)+1)+CHx (0+1);
- Neutrophil-lymphocyte ratio (NLC)=neutrophils: lymphocytes;
- Neutrophil shift index=M+U+PN:CH.

All experimental studies were carried out by modern methodological approaches and in compliance with relevant requirements and standards, in particular, they meet the requirements of DSTU ISO/IEC 17025:2005 (2006). The animals were kept, and all manipulations were performed following 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). The data were analysed using Microsoft Excel software. The reliability of the results was determined by the Student's criterion. Quantitative values were expressed as the arithmetic mean and its deviation (M±m).

RESULTS AND DISCUSSION

Various environmental factors affect foetal growth conditions. They disrupt the formation of the body's defence mechanisms, which makes it necessary to determine the resistance of newborn animals and carry out adequate correction. The process of embryogenesis is negatively affected by the disturbance of oxygen homeostasis in the body. Under the influence of hypoxia, during the germinal, embryonic and foetal periods, the body's supply of oxygen decreases and manifests itself in calves after birth (Table 1). The partial pressure of oxygen in the blood of newborn animals decreased depending on the severity of hypoxia by 1.45, 1.34, ($p<0.01$), 1.08 times and an average of 1.19 times ($p<0.05$) compared to functionally active calves. In cows of the first and second groups, the PO_2 value was significantly lower than in control animals ($p<0.05$).

PCO_2 in the blood of calves in hypoxia (groups I-III) was 1.73, 1.62, and 1.44 times higher than that of control calves ($p<0.01$), and 1.17 times higher than that of cows on average ($p<0.05$). The acidotic shift in blood pH of calves and cows of the experimental groups was not significant. Blood saturation of calves in the control group was 11.52±0.82 ml/dl. The oxygen saturation of calves' blood in the state of hypoxia was 1.52, 1.23, 1.13 times and on average 1.27 times less than O_2ct in functionally active calves, and A-aDO_2 on average 1.23 times ($p<0.05$).

Table 1. *Oxygen homeostasis in the blood of cows and newborn calves (M±m, n=5)*

Indicators	Measurement unit	Animal group				Average, per calve/cows I-III groups
		Control	I	II	III	
pH	log (C)	7.386±0.006	7.108±0.004	7.261±0.006	7.258±0.0015	7.209±0.006
		7.374±0.006	7.119±0.007	7.203±0.009	7.268±0.012	7.217±0.009
H+ ion content	mEq/l	52.01±2.031	63.02±3.011	54.03±2.01	49.01±2.05	55.33±3.01
		50.02±2.012	54.00±3.012	52.04±1.86	50.05±1.37	52.07±1.33
PO_2	mmHg	28.63 ± 0.97	19.84 ± 0.42**	21.31±1.23**	26.50±1.62	22.55±1.31*
		29.06±1.02	24.92±1.36*	26.02±1.36*	27.081±1.23	26.007±1.19*
PCO_2	mmHg	45.60±1.36	78.82±2.14**	73.85±3.78**	65.44±3.26*	72.70±3.20**
		46.40±0.74	58.12±2.46*	53.34±2.02	51.62±1.06	54.36±1.74*
TCO_2, general	mmol/l	28.81±0.79	32.42±1.07	31.14±0.78	30.86±0.96	31.47±1.86
		28.67±0.53	29.26±1.58	28.69±1.13	27.61±1.21	28.52±1.03

Table 1, Continued

Indicators	Measurement unit	Animal group				Average, per calve/cows I-III groups
		Control	I	II	III	
$\%SO_2$	%	80.66±4.02	66.82±2.24	74.86±2.74	82.08±3.31	74.35±2.55
		84.42±3.14	79.32±2.48	79.68±3.14	83.12±3.65	80.43±3.71
O_2 ct	ml/dl	11.25±0.97	7.85±0.62**	9.63±0.94*	10.42±0.68*	9.31±0.53*
		12.24±0.86	8.84±0.62	10.12±1.08	11.63±1.17	10.19±0.87
$A–aDO_2$	mmHg	58.18±2.04	43.89±1.43	45.30±1.71	52.60±2.30	47.26±1.71*
		60.02±1.68	55.49±1.63	56.58±1.46	58.70±2.52	56.92±2.14

Note: **p<0.05; **p 0.01; ***p<0.001 compared to the control group. Numerator – performance of calves. Denominator – indicators of cows*
Source: *compiled by the authors*

Under the influence of hypoxia, embryo growth is inhibited (Table 2). The placental ligament in calves exposed to hypoxia in the prenatal period was 1.91, 1.59 and 1.41 times higher (p<0.01) than in functionally active calves. The intensity of embryonic growth reached 0.035±0.001 g in calves of the control group. It was 1.45, 1.35, 1.30 and on average 1.35 times higher than in calves in the hypoxia condition. The embryonic growth rate was 0.120±0.002 g in the control group. In the embryos of the first – third groups, this indicator was 1.21, 1.13, 1.10 and on average 1.14 times less (p<0.05).

Table 2. *Indicators of embryonic growth (M±m, n=5)*

Indicators	Calve group				
	Control	I	II	III	On average per 1-3rd calve groups
Placental connection, mm^2/kg	0.341±0.002	0.653±0.003**	0.542±0.008**	0.483±0.006**	0.559±0.004
Intensity of embryonic growth, g	0.035±0.001	0.024±0.002*	0.026±0.002*	0.027±0.003*	0.026±0.002*
Embryonic growth rate, g	0.120±0.002	0.099±0.003*	0.106±0.002*	0.109±0.003*	0.105±0.001*

Note: **p < 0.05; **p<0.01; ***p<0.001 compared to the control group*
Source: *compiled by the authors*

The appearance of the placenta indicates a violation of the conditions of foetal development under the influence of hypoxia.

In functionally active calves, the placenta is shiny. The vessels are clear, and filled, and capillaries are defined (Fig. 1).

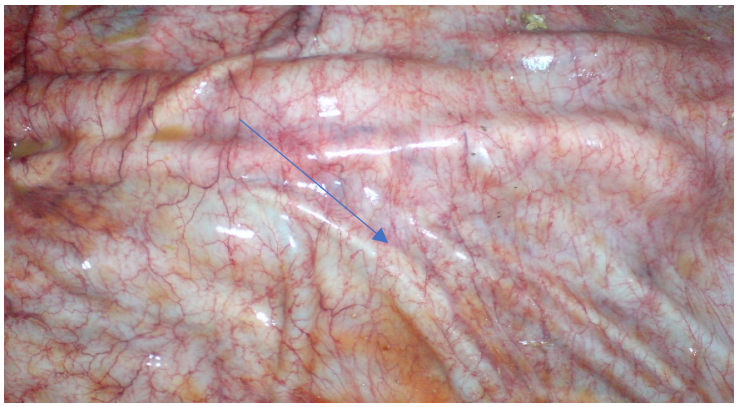

Figure 1. *Placenta of a functionally active calf*
Note: *placental vessels are clear, and capillaries are defined*
Source*: photographed by A.A. Zamaziy*

However, in calves born in a state of hypoxia, the placenta is not coloured, there are foci of haemorrhage and inflammation. The vessels are not clear, and the capillary system is not defined (Fig. 2).

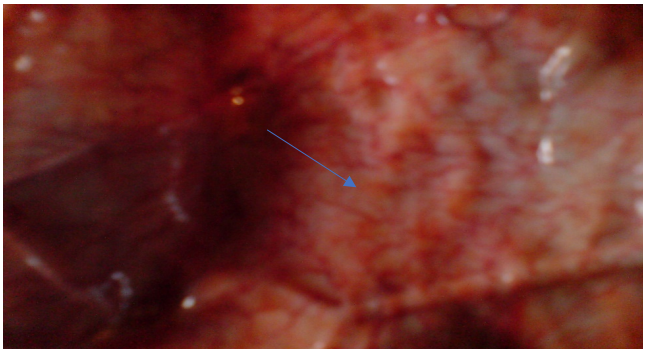

Figure 2. *Calf placenta in a state of hypoxia*
Note: *placental vessels are not clear, and the capillary system is vague*
Source*: photographed by A.A. Zamaziy*

The haemostasis of cows whose offspring were born with signs of impaired O_2 homeostasis was characterised by an increase in the activity of coagulation processes (Table 3). The prothrombin time was 48.20±1.42 s in cows of the control group. It was 1.63, 1.40, 1.23 and on average 1.40 times longer than in cows of the experimental groups (p<0.01). The prothrombin index of cows of groups I-III was 1.53, 1.52, 1.35 and on average 1.46 times lower than that of control cows (p<0.01). The thrombin time of haemostasis in control cows lasted longer, by 1.29, 1.23, 1.17 and 1.23 times on average (p<0.05). In cows of the control group, the partially activated thrombin time was 55.80±2.40 s. It was 1.34, 1.27, 1.40 and on average 1.28 times higher than the APTT of haemostasis in cows of the experimental groups.

Table 3. *Indicators of cow haemostasis (M±m, n=5)*

Indicators	Groups				
	Control	I	II	III	On average for animals of groups I-III
Prothrombin time, (TI), s	48.20±1.42	29.59±1.23**	34.24±1.81**	39.12±1.96*	34.32±1.21**
Prothrombin index, (PI), %	49.72±1.64	32.38±1.22**	32.89±1.53**	36.71±0.93**	33.99±0.83**
Internationally Normalised Ratio (INR), %	2.56±0.22	2.11±0.23	2.43±0.17	2.56±0.32	2.37±0.29
Thrombin time, s	36.84 ± 2.26	28.50±1.35*	30.09±2.17*	31.53±2.52*	30.04±1.94*
Activated partial thrombin time (APTT), s	55.80±2.40	41.60±1.90*	43.90±2.05*	45.10±1.82*	43.53±1.77*
Fibrinogen, g/l	2.46±0.18	3.93±0.29**	3.45±0.21*	3.14±0.28*	3.51±0.42*

Note: **p<0.05; **p<0.01; ***p<0.001 compared to the control group*
Source: *compiled by the authors*

Fibrinogen was determined in the blood of cows of groups I-III – 3.93±0.29 g/l, 3.45±0.21 g/l and 3.14±0.28 g/l. Its content was at the level of 2.46±0.18 g/l in cows of the control group. It was 1.61 (p<0.01), 1.41 and 1.28 times (p<0.05) less than in animals of the experimental groups. A probable increase in the content of fibrinogen in the blood, and activation of the coagulation process, affected its properties (Table 4).

Table 4. *Properties of cow blood (M±m, n=5)*

Indicators	Animal group				On average per 1-3rd calve groups
	Control	I	II	III	
Blood viscosity, Pa*s	5.68±0.92	7.19±0.83*	6.79±0.37*	6.38±0.82	6.79±0.63*
Blood coagulation rate, s	402±8.00	361±6.00*	374±9.00*	386±5.00*	373.67±6.03*

Table 4, Continued

Indicators	Animal group				On average per 1-3rd calve groups
	Control	I	II	III	
Fibrinolysis, min	5.35±0.25	6.38±0.32*	6.21±0.43*	5.68±0.36	6.09±0.43*
Thrombotest, art	4.22±0.34	5.81±0.641*	5.29±0.51*	5.17±0.32*	5.42±0.48*
Retraction of a blood clot, %	64.18±2.24	53.84±3.2*	57.63±2.15	59.41±1.81	56.96±1.79*
Platelet adhesion, %	37.88±1.24	48.28±2.04*	46.65±2.38*	42.19±1.95*	45.71±2.03*

Note: **p<0.05; **p<0.01; ***p<0.001 compared to the control group*
Source: *compiled by the authors*

In cows of the control group, blood viscosity was 5.68±0.92 Pa*s. In animals of the first group, it was 1.26 times higher, and in cows of the second and third groups, it was 1.19-1.12 times higher than this indicator of the blood of functionally active calves (p<0.05). Blood clotting in cows of the experimental groups was 1.20, 1.16 and 1.12 times faster (p<0.05) compared to the control. The breakdown of a blood clot took 1.21, 1.18 and 1.08 times longer in cows of the first to third groups. The blood thrombotest of cows of groups I-III was 1.36, 1.26, 1.20 and on average 1.28 times higher than in animals of the control group (4.22±0.34 st). Blood clot retraction was 64.18±2.24% in cows of functionally active calves, which was 10.40%, 6.60%, 4.80% and on average 7.27% higher than in animals of the experimental groups. The ability of blood platelets in the control group to adhere was 10.52%, 8.76%, 4.40% and 7.89% less than in cows with hypoxia. The phagocytic activity of neutrophils in the blood of functionally active calves reached 78.00±3.05 % (Table 5).

Table 5. *Blood leukocyte activity of newborn calves (M±m, n=5)*

Indicators	Animal group				On average for calves of groups I-III
	Control	I	II	III	
Phagocytic activity of neutrophils, %	78.02±3.05	68.04±3.52	72.02±2.68	74.00±3.22	71.35±2.19
Phagocyte count	7.12±0.48	5.22±0.64**	6.16±0.58*	6.58±0.56	5.99±0.83*
Phagocyte index	72.14±2.62	62.25±1.39	70.28±3.46	72.42±4.02	68.327 ± 3.46*
Phagocytosis completion index, %	73.91±4.03	56.10±2.70*	62.22±3.60*	68.50±3.30	62.27±3.13
Nuclear index	0.458±0.08	0.819±0.14**	0.739±0.22**	0.619±0.16*	0.725±0.19**
Resistance index	1.18±0.26	0.64±0.07**	0.81±0.21**	0.94±0.08*	0.80±0.079**
Percentage of active leukocytes (WBC), %	29.23±1.97	20.49±1.25**	22.43±1.37**	24.87±1.25*	22.58±1.14*
The number of active phagocytes (CAF), 10^9/l	2.38±0.42	2.51±0.37	2.76±0.34	2.88±0.32	2.72±0.31
Microbial count, 10^9/l	19.33±1.54	12.69±0.96**	14.19±0.82**	16.28±0.94*	14.39±0.99**

Note: **p<0.05; **p<0.01; ***p<0.001 compared to the control group*
Source: *compiled by the authors*

In animals in groups I-III, FAN was 10%, 6.0%, 4.0% and on average 6.67% less. The ability of phagocytes to neutralise foreign proteins, i.e., FF, was 7.12±0.84 in calves of the control group. This indicator of leukocyte activity in the blood of calves in the state of hypoxia was 1.37, 1.17, 1.08 and on average 1.19 times (p<0.05) less. The completeness of phagocytosis in the blood phagocytes of calves of groups I-III was 17.81%, 11.71%, 5.41% and on average 11.64% less than in calves of the control group (73.91±4.03%). The nuclear index of blood cells of control calves reached 0.46±0.08. It was 1.78, 1.61, 1.35 and on average 1.57 times (p<0.01) less than in calves in the state of hypoxia. The index of resistance in calves in the state of hypoxia was 1.80, 1.43, 1.24 and on average 1.46 times less (p<0.01) than in calves of the control group. The percentage of activated leukocytes, i.e., the number of leukocytes that participate in phagocytosis in the blood of calves of the control group was 1.43, 1.30 (p<0.01), 1.18 and on average 1.29 times higher than in animals of the experimental groups (p<0.05).

The absolute number of leukocytes involved in phagocytosis (CAF) was not significantly higher in

calves of the first – third groups, which is associated with a higher level of leukocytes in the blood of these calves. However, the microbial count in calves of the control group was significantly higher than in animals of the first to third groups (1.52, 1.36 ($p<0.01$), 1.15 times ($p<0.05$) and an average of 1.32 times ($p<0.01$)). The leukocyte index of intoxication (Table 6) of newborn calves of the control group was 2.66±0.84, which was 4.43, 3.69, 2.46 and on average 3.33 times higher than that of calves of the experimental groups ($p<0.001$). The leukocyte shift index was 0.74±0.078, 0.85±0.057, 0.94±0.171 and 0.84±0.068 on average, which was 1.55, 1.35, 1.23 and 1.36 times less than in functionally active calves, respectively. The leukocyte index, on average, in calves in a state of hypoxia (83.15±3.26) was not significantly higher than in calves of the control group – 82.55±3.17.

Table 6. *Resistance indices of newborn calves (M±m, n=5)*

Indicators	Animal group				On average for calves of groups I-III
	Control	I	II	III	
Leukocyte intoxication index (LII)	2.66±0.84	0.61±0.05***	0.73±0.059***	1.07±0.083***	0.80±0.052***
Leukocyte shift index (LSI), %	1.16±0.32	0.74±0.078	0.85±0.057	0.94±0.171	0.84±0.068
Leukocyte index	82.55±3.17	83.48±4.61	83.019±2.23	82.94±2.83	83.15±3.26
Neutrophil-lymphocyte ratio (NLC)	1.241±0.22	0.768±0.14**	0.96±0.22*	1.06±0.16*	0.93±0.13*
Leukocyte shift index	0.53±0.11	1.03±0.019**	0.98±0.062**	0.779±0.126**	0.93±0.171**

Note: **p<0.05; **p<0.01; ***p<0.001 compared to the control group*
Source: *compiled by the authors*

The ratio of neutrophils to lymphocytes (NLC) was 1.61, 1.32, 1.22 and on average 1.36 times higher in calves of the control group, and the neutrophil shift index was 1.94, 1.85, 1.47 ($p<0.01$) and on average 1.75 times higher in calves in the state of hypoxia. Effective management of this industry is possible only if viable offspring are obtained and their safety is improved. M. Sharan and S. Shalovylo (2018) point out that identifying the causes and mechanisms of prenatal losses, as well as their correction, is an urgent task for production. According to M.M. Zhelavskyi *et al.* (2021), a long period of foetal development in cows is accompanied by the negative impact of various stimuli that disrupt the relationship in the mother-embryo, mother-embryo, and mother-foetus system. The obtained results of the research allow us to assert that a violation of the foetal oxygen supply negatively affects the body's homeostasis and is manifested by a violation of the respiratory process in newborn calves.

The effect of hypoxia on the foetus in the prenatal period is manifested by impaired embryonic growth. Under the influence of hypoxia, the placental ligament in calves increased and was larger ($p<0.01$) than in functionally active calves. The intensity of embryonic growth was higher in functionally active calves. It was, on average, 1.35 times higher than in calves born in a state of hypoxia. The amount of embryonic growth in the embryos of the first to third groups was on average 1.14 times less ($p<0.05$). It is believed that only an external examination of the umbilical cord at birth allows us to assume that the conditions for foetal growth and development are impaired and should be used in production. K.G. Pringle *et al.* (2018) believe that the indicators of embryonic growth and development indicate the unevenness of this process, which is caused by a decrease in the supply of oxygen. L. Tremetsberger and C. Winckler (2015) point out that the inequality of embryonic development is manifested by the peculiarities of the morphological and functional connection between the foetus and the mother's body. S.T. Cohen *et al.* (2020) proved that embryogenesis in the cow foetus is accompanied by changes in the biochemical structure of the cell protoplasm and the intensity of all life processes. At each subsequent stage (embryonic → pre-foetal → foetal) of embryonic development, the organism does not remain morphologically, biochemically, and physiologically the same as it was in the previous period.

Researchers determine the percentage of losses in the process of foetal growth and development without determining the causes and their manifestation in newborn animals. According to P.T. Ruane *et al.* (2017), during the embryonic period, losses are up to 45%, and in the foetal period, this figure reaches 7-9%. At the stage of morula formation, embryonic mortality is 2-3%. In cows, the most critical period is 7-18 days of pregnancy, they say. The proportion of postplacental embryonic death is 5-8%. R.C. West *et al.* (2019) found that losses in the embryonic period are based on disorders of trophoblast differentiation. The developmental delay and death of blastocysts occurs on day 15-18 of gestation. Tissues of dead blastocysts remain in the uterus for up to 35-45 days. Such animals do not show signs of sexual desire

and do not inseminate for the next three months. The length of the service period increases, the number of calves produced per year decreases, and production indicators are negatively affected.

Y.Y. Shin *et al.* (2018) found that an increase in the placental mother-foetus connection was observed in the setting of foetal growth retardation. According to J. Liao *et al.* (2019), it is accompanied by compensatory processes, such as increased placental blood flow, vasodilation, and umbilical cord hypertrophy. It is believed that the thickening of the umbilical cord to the body weight of newborn animals indirectly characterises the level of metabolism and, accordingly, the body's resistance. The intensity of embryonic growth and its size were significantly lower in animals that developed under the influence of hypoxia. The course of cows' pregnancy, and, accordingly, the foetus, is influenced by feeding conditions and technological stresses. Up to 22% of newborns die and up to 40% of abortions occur when pregnant cows are not fed properly.

Despite the presence of numerous nutritional factors that negatively affect the bodies of cows and foetuses, L.H. Duntas (2020) points out that only vitamin and selenium deficiencies in the diet can cause embryonic death. J. Moake & M. Baylor (2021) believe that hypoxia is the most common factor affecting the growth and development of the embryo, foetus, and foetus. Calf hypoxia is accompanied by a significant decrease in partial pressure of oxygen in the blood, its saturation, on average, by 1.27 times ($p<0.01$). We believe that these results alone indicate a variety of measures aimed at restoring the viability of calves after birth, depending on the state of hypoxia. The partial pressure of CO_2 and the content of hydrogen ions in the blood significantly increased. Indicators of oxygen homeostasis in the body of animals allow us to make assumptions about the conditions of foetal development. These data are in line with the results of a study by J.J. Saugy *et al.* (2016). They indicate that hypoxia negatively affects homeostasis in humans. However, the authors do not determine the impact of hypoxia on the body depending on the severity of the process. We believe that such an approach subsequently reduces the effectiveness of measures aimed at preserving newborns and is not adequate for the state of the organism.

The biological system that ensures the prevention of blood loss and the preservation of the physiological norm of circulating blood parameters is defined as the haemostasis system. In an uncomplicated pregnancy, the female body undergoes several adaptive reactions aimed at ensuring an adequate course of the gestational period of foetal development. Changes in the haemostasis system can cause complications for both the female and the foetus. A significant restructuring of the vital activity of pregnant females is associated with changes in the blood system and haemostasis. According to C. Whyte *et al.* (2017), activated haemostatic factors have the properties of inflammatory mediators. Compensatory changes in the body of pregnant women bring haemostasis into a state of unstable tense equilibrium. F. Agoreyo *et al.* (2016) found that an increase in blood viscosity in pregnant females, in combination with haemo concentration and increased fibrinogen content, is manifested by microcirculatory disorders. Perhaps this leads to a decrease in uteroplacental blood flow and creates conditions for foetal hypotrophy, as evidenced by the appearance of the placenta of calves born in a state of hypoxia. The smaller the diameter of the vessels, the higher the viscosity of the blood flowing in them. Optimisation of the blood flow rate in the interventricular space is possible by reducing the number of platelet cells, especially red blood cells and platelets.

The process of physiological haemodilution is aimed at increasing the volume of circulating blood plasma. The process is most clearly manifested in the second trimester of pregnancy. During this period, the second wave of trophoblast invasion ends, and the further course of pregnancy is programmed Factors that negatively affect embryogenesis reduce the level of anabolic processes and suppress blood supply to the foetus. They lead to placental dysfunction (placental insufficiency), which is characterised by functional and morphological changes in the placenta. Such changes in the placental complex cause various pathologies in the foetus. Delayed intrauterine development and foetal hypoxia are detected. The rheological properties of the blood of cows that have given birth to calves in a state of hypoxia differ significantly from those of cows with functionally active calves. They are characterised by an increase in blood viscosity, coagulation, fibrinolysis, platelet aggregation properties and reduced blood clot retraction. The appearance of the placenta indicates a violation of O_2 homeostasis in the foetus. In calves born with signs of hypoxia, the placental capillaries are blurred and illegible. There are foci of inflammation and haemorrhage.

According to V.O. Melnyk and O.O. Kravchenko (2018), the most common complication is foetal retardation, which is detected in 40% of cases Increased blood viscosity in blood vessels, impaired microcirculatory blood flow is the basis for impaired oxygen homeostasis in the foetus. In such animals, hypoxia is manifested by impaired external respiration. A prerequisite for adequate external respiratory function is timely pneumatisation of the lungs and even distribution of inhaled air. Decreased pneumatisation of the lungs occurs against the background of inhibition of alveolar fluid resorption and intensity of surfactant synthesis. V.O. Velichko (2022), M.M. Zhelavskyi *et al.* (2022) indicate that impaired oxygen homeostasis, foetal growth retardation, determined by low birth weight, reduces the body's resistance. The processes of haematopoiesis and blood profile formation are disrupted, and the body's

resistance decreases, which necessitates determining the state of the newborn's body and carrying out appropriate correction.

CONCLUSIONS

Depending on the severity of the process, newborn hypoxia is accompanied by a decrease in partial pressure of oxygen, blood saturation and an increase in PCO_2 ($p<0.01$). The placental connection was significantly increased under the influence of hypoxia ($p<0.01$), and the intensity and size of embryonic growth were higher in functionally active newborn animals ($p<0.05$). Blood clotting and blood clot breakdown in cows with functionally active calves was significantly longer and viscosity was lower than in cows that gave birth to calves in a state of hypoxia ($p<0.05$). The phagocytic activity of neutrophils significantly affected the phagocytic number, phagocytic index, and completeness of phagocytosis ($p<0.05$), indicating a high level of resistance of functionally active calves.

The leukocyte index of intoxication and the leukocyte shift index of functionally active calves were significantly higher ($p<0.001$) than in animals in a state of hypoxia. The percentage of activated leukocytes, i.e., the number of leukocytes that participated in phagocytosis in calves of the control group was 1.43, 1.30 ($p<0.01$), 1.18 and on average 1.29 times higher than in animals of the experimental groups ($p<0.05$). The microbial count was 1.52, 1.36 ($p<0.01$), 1.15 ($p<0.01$) and on average 1.32 times higher in functionally active calves ($p<0.01$). In the future, research should identify adequate schemes and develop devices to correct the state of the body of newborn calves, their impact on the body's resistance, and increase the viability and safety of calves.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Гемостаз корів та резистентність організму телят за умов гіпоксії

Марія Дмитрівна Камбур
Доктор ветеринарних наук, професор
Сумський національний аграрний університет
40000, вул. Герасима Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0000-0002-4864-5292

Андрій Анатолійович Замазій
Доктор ветеринарних наук, професор
Полтавський державний аграрний університет
36003, вул. Сковороди, 1/3, м. Полтава, Україна
https://orcid.org/0000-0003-3138-0424

Віктор Анатолійович Коленченко
Аспірант
Сумський національний аграрний університет
40000, вул. Герасима Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0009-00085-3472-9259

Олександр Сергійович Демидко
Аспірант
Сумський національний аграрний університет
40000, вул. Герасима Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0000-0002-6433-315X

Євгенія Михайлівна Лівощенко
Кандидат ветеринарних наук, доцент
Сумський національний аграрний університет
40000, вул. Герасима Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0000-0001-5826-4824

Анотація. Інтенсифікація галузі тваринництва неможлива без ефективного відтворення тварин. Вирішення цієї проблеми можливе лише за умови отримання життєздатного приплоду, підвищення резистентності та збереженості його організму, що визначає актуальність досліджень. У зв'язку з цим визначення впливу системи гемостазу та властивостей крові корів на ріст і розвиток плода, а в подальшому на резистентність новонароджених телят залежно від стану при народженні визначило мету досліджень. Встановлено, що умови росту і розвитку плода пов'язані з активністю факторів системи гемостазу та властивостями крові корів. Фактори гемокоагуляції були активні у тварин, які народжували телят у стані гіпоксії. Протромбіновий час був у 1,63, 1,40, 1,23 і 1,40 раза нижчим, а протромбіновий індекс гемостазу – у 1,53, 1,52, 1,35 і 1,46 раза відповідно, ніж у корів, які народили функціонально активних телят ($p<0,01$). Тромбіновий час гемостазу, частково активований тромбіновий час та вміст фібриногену у корів дослідних груп були вищими, ніж у тварин контрольної групи. В›язкість крові корів контрольної групи була нижчою ($p<0,01$), а згортання крові корів дослідних груп було швидшим ($p<0,05$). Підвищення коагуляційних властивостей крові корів, які народили телят у стані гіпоксії, відбувалося на тлі зниження активності фібринолітичної системи ($p<0,05$) і ретракції кров'яного згустку. Все це знижувало ріст і розвиток ембріона та плода. Плацентарно-зв'язковий зв'язок плодів, народжених з ознаками гіпоксії, був достовірно більшим, ніж у функціонально активних телят. Інтенсивність та розміри росту ембріонів були вищими у групі функціонально активних телят ($p<0,05$). Активність лейкоцитарних факторів захисту і, відповідно, резистентність організму функціонально активних телят при народженні була вищою. Відсоток активованих лейкоцитів у крові телят був практично однаковим, а мікробне число було вищим у телят контрольної групи ($p<0,05$). Результати, отримані в ході досліджень, можуть бути впроваджені в процес отримання та вирощування телят, а також запропоновані всім тваринницьким господарствам України

Ключові слова: зсідання; кров; лейкоцити; показники; фібрин

SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 21-34

UDC: 636.592:661.158]366.5
DOI: 10.48077/scihor9.2023.21

Triazoline compounds influence on the meat productivity of turkey carcasses

Ruslan Dubin*
PhD in Veterinary Sciences, Associate Professor
Odesa State Agrarian University
65012, 13 Panteleimonivska Str., Odesa, Ukraine
https://orcid.org/0000-0003-3540-0816
Anatolii Palii
Doctor of Veterinary Sciences, Professor
National Scientific Centre "Institute of Experimental and Clinical Veterinary Medicine"
61023, 83 Pushkinska Str., Kharkiv, Ukraine
https://orcid.org/0000-0002-9193-3548
Pavlo Sharandak
Doctor of Veterinary Sciences, Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0002-5434-666X
Oksana Ivleva
PhD in Veterinary Sciences, Associate Professor
Volodymyr Dahl East Ukrainian National University
01042, 17 John Paul II Str., Kyiv, Ukraine
https://orcid.org/0000-0001-8090-4373
Mykola Todorov
PhD in Veterinary Sciences, Associate Professor
Odesa State Agrarian University
65012, 13 Panteleimonivska Str., Odesa, Ukraine
https://orcid.org/0000-0001-9260-567X

Article's History:
Received: 29.04.2023
Revised: 26.08.2023
Accepted: 27.09.2023

Abstract. The need to find effective means to improve the quality and production of meat products from poultry farming determines the relevance of the study. The introduction of drugs into the diet can increase the safety of livestock and improve the quality characteristics of poultry meat. The research aims to evaluate the impact of new 1,2,4-triazole derivatives on some blood parameters, as well as meat and slaughter qualities of poultry. The study was conducted between 2022 and 2023 at Odesa State Agrarian University. The trial was conducted to study the effect of new triazoline derivatives GKPF-109 at a dose of

Suggested Citation:
Dubin, R., Palii, A., Sharandak, P., Ivleva, O., & Todorov, M. (2023). Triazoline compounds influence on the meat productivity of turkey carcasses. *Scientific Horizons*, 26(9), 21-34. doi: 10.48077/scihor9.2023.21.

*Corresponding author

0.5 ml/bird per day on turkey performance and meat quality. The experiment was conducted on Big-6 turkeys aged 1-105 days. The following research methods were used: morphological and biochemical, organoleptic, physicochemical, and statistical. The effects of triazoline derivatives GKPF-109 on organoleptic (appearance, smell, consistency, fat condition, broth quality during meat cooking) and physicochemical studies of turkey meat for microbiological parameters were analysed and investigated following DSTU 3143:2013. The results showed that there was an increase in average daily weight by 13.1%, slaughter yield of gutted carcasses by 12.4%, semi-gutted carcasses by 12.3% and livestock preservation by 4%. An increase in the nutritional value of meat was noted due to an increase in muscle tissue protein by 7.5%, fat by 8.3%, and energy value by 4.8%. The organoleptic, bacteriological, and physicochemical parameters of turkey meat when using triazoline derivatives GKPF-109 in the diet met the requirements of DSTU 3143:2013. It was concluded that the addition of triazoline GKPF-109 derivatives to drinking water enhances haemopoiesis and has anti-inflammatory and hepatoprotective effects. The assessment of the quality of broiler turkey meat and broth at the end of the experiment does not allow to assert a decrease in their aroma and taste, which indicates the absence of a negative effect of triazoline derivatives GKPF-109 and methods of their application on the organoleptic characteristics of meat, which should be considered when keeping turkeys

Keywords: 1,2,4-triazole derivatives; turkeys; weight gain; red blood cells; haemoglobin; leukocytes

INTRODUCTION

A special place in biological science is given to the natural resistance of the organism, which is a component of immunology. A decrease in immunity and adaptation mechanisms in young animals and poultry may lead to an increase in mortality at an early age with a subsequent decrease in productive qualities in the future. Based on the above, the immunobiological state of poultry at an early age is of great interest at present. The study of changes in the body's immune response will allow for the effective use of new drugs to reduce the impact of stress in poultry. The problem of immunoprotection of disturbed homeostasis is to find and create effective immunoprotective agents and develop effective methods of immunodiagnostics. The relevance of pharmacocorrection is due to a significant increase in immunodeficiency states in animals, which cause many diseases, and the success of treatment depends on the choice of immune correction agents and methods.

The results of the research (Mesquita *et al.*, 2021) and production trials allow us to recommend immunomodulators for implementation in industrial poultry farming practice, which is economically profitable and contributes to a significant increase in poultry products. Sch. Bernd *et al.* (2020) believe that the pharmaceutical industry currently offers many immunomodulatory drugs for industrial poultry production, and their safety, and impact on poultry performance and poultry products have been studied. A. Disetlhe *et al.* (2019) point out that immunostimulants are used in poultry farming to normalise the immunodeficiency state. They can activate metabolism, increasing natural resistance, poultry growth energy, and feed conversion to ensure poultry safety, which has a positive impact on production profitability.

M. Alrawashdeh (2018) studied the development of immunostimulant drugs and their effect on the poultry body. Among such modern drugs are 1,2,4-triazole derivatives, which are used in industry, agriculture, veterinary medicine, and humane medicine. In veterinary practice, 1,2,4-triazole derivatives are used to provide immunomodulatory, cardio- and hepatoprotective, anti-inflammatory, antifungal and antiviral effects.

M. Korish & Y. Attia (2019) believe that the most important mechanism of pharmacodynamics of one of the 1.2,4-triazole derivatives, thiotriazoline, is its multifaceted effect on energy metabolism. Healthy and nutritious nutrition is the most important factor in public health. Ukraine offers a wide range of poultry meat. Poultry meat differs from cattle meat in terms of its high content of complete proteins (poultry, 19.5%; beef, 13.0%), and poultry fat has a lower melting point (36.5°C), which makes it easier for humans to digest. In addition, turkey meat is a low-allergenic product, which makes it ideal for baby food. Therefore, turkey meat is an excellent source of animal protein. The chemical composition and amino acid profile of turkey meat have been studied in several studies (Li *et al.*, 2017; Boz *et al.*, 2019; Gumulka & Poltowicz, 2020).

According to researchers (Hascik *et al.*, 2019; Cech *et al.*, 2021), compared to other poultry, turkeys have the highest carcass weight (>70%), consisting of >60% muscle tissue and <28% breast muscle. It contains a high amount of protein (28% vs. 14-18% in other poultry meat), moderate amounts of fat (2-5%), is rich in B vitamins and has lower cholesterol levels than other poultry meat.

The synthetic derivatives of 1,2,4-triazole include new compounds GKPF-109, the effect of which on the poultry organism is poorly understood, given the novelty of the triazole class as a medicinal and prophylactic agent. The research aims to improve the technological methods of increasing the productivity of broiler turkeys with derivatives of triazole compounds.

MATERIALS AND METHODS

The study was conducted in the period from 2022 to 2023 at Odesa State Agrarian University. The scientific

and economic experiment was conducted to study the effect of new triazoline derivatives GKPF-109 at a dose of 0.5 ml/bird per day on turkey productivity and meat quality. Derivatives of 1,2,4-triazole synthesised at Zaporizhzhia State Medical University were used for the study: GKPF-109 – Morpholin-4-ium-2-((4-amino-5-(3-metyl-pyrasol-5-il)-1,2,4-triasol-3-il)tio) acetate. The experiment was conducted on Big-6 turkeys aged 1-105 days. The technological parameters of turkey rearing were in line with the recommendations for working with this cross. Changes in the dynamics of the live weight of turkeys were measured weekly by weighing them. Live weight is an indicator of poultry development, summarising the impact of feeding and housing conditions.

The birds were housed, fed, and cared for and all manipulations were carried out following the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1986) and the General Ethical Principles for Experiments on Animals, adopted by the First National Congress on Bioethics (Kyiv, 2001). The experiments were conducted in compliance with the principles of humanity set out in the European Community Directive (Directive 2010/63/EU). The clinical and physiological condition of the turkeys was determined by daily inspection of the livestock, paying attention to behaviour, appetite and health status. The physiological state of turkeys was checked at the beginning and end of the experiment by morphological and biochemical parameters of turkey blood. On the 28th day and 105th day, 20 blood samples were taken from each group from the subcostal vein.

To determine the health status of turkeys, morphological blood analysis was performed according to the following indicators: blood content of red blood cells, leukocytes, haemoglobin, erythrocyte sedimentation rate (ESR), blood content of rods, segmented lymphocytes, eosinophils, monocytes (Sadovnikov *et al.,* 2009; Kanda *et al.,* 2020). In addition, the biochemical parameters of turkey blood (total protein, calcium, phosphorus, and reserve alkalinity (Sadovnikov *et al.,* 2009) were determined and studied.

For a more complete and objective assessment of the meat qualities of turkeys, anatomical cutting, and complete deboning of 10 female carcasses in each group was carried out. To assess the meat productivity, turkeys were slaughtered and anatomically processed. When slaughtering turkeys in a private yard, the bird is taken by the head and the throat is cut 1.5-3 cm below the earlobe. The duration of exsanguination is 1.53 minutes. The quality of the carcass depends on the thoroughness of feather removal. If there are skin tears and scratches on the carcass, the grade of the carcass is reduced, regardless of its fatness.

Sampling. Three meat samples weighing at least 200 g each were taken from each carcass. Processing and deboning of gutted turkey carcasses were carried out following DSTU 3143-95 Poultry meat (carcasses of chickens, ducks, geese, turkeys, guinea fowls). To do this, the wings were divided at the shoulder joint, the thighs at the hip joint, and the breast part at the junction of the thoracic and vertebral ribs (coracoid line). If necessary, the wing was divided into three parts, and the limb into two parts by joints separated by lines perpendicular to the axis of the respective bones.

The effect of new triazoline derivatives GKPF-109 on the zootechnical indicators of turkey development was studied; an anatomical examination of the internal organs of the bird was carried out. As a result of anatomical processing, the average carcass weight, slaughter yield, and yield of edible and inedible parts were determined. Parts of the carcass obtained during processing were deboned. The weight of meat and bone weight were considered during deboning, and the weight of edible parts of the carcass was determined by weighing, expressing them as a percentage of carcass weight.

Slaughter yield is the ratio of slaughter weight to live weight, expressed as a percentage. It is determined by which body parts are included in the slaughter weight. In poultry, the slaughter yield depends on the specifics of post-slaughter processing of the carcass: In ungutted poultry, it is the highest, as it includes the weight of the bloodless and plucked carcass with fat, head, limbs and internal organs; in semi-gutted poultry, the carcass weight includes fat but no intestines; in full gutting, both blood, feathers, down and intestines are removed, as well as all internal organs, and even the head to the second neck vertebra, limbs to the tarsus and wings to the elbow joint were removed following DSTU 3136:2017 Poultry for slaughter. Technical conditions.

Organoleptic (appearance, smell, consistency, fat condition, quality of broth during cooking) and physicochemical studies of turkey meat were carried out following DSTU 3143-95 (1995). The organoleptic characteristics of turkey meat and broth: appearance, aroma, colour, texture, juiciness, and taste were assessed on a 9-point scale by a 7-person panel. The meat was tested for microbiological parameters following DSTU 3143:2013 (2013). Statistical processing of the results was performed using the Statistica 6.0 software package. The significance of the difference in means was assessed by Student's t-test.

RESULTS AND DISCUSSION

Morphological and biochemical parameters of turkey blood. To evaluate the effect of new triazoline derivatives GKPF-109 on the body of turkeys, it is necessary to monitor the morphological and biochemical composition of the blood. This is because many factors can affect blood composition. Poultry blood tests are a valuable indicator that can be used to detect diseases of various etiologies at early stages of diagnosis (Table 1, Fig. 1).

Table 1. *Morphological parameters of turkey blood (n=10)*

Indicators	Age of the bird, day	Control	Studied
Haemoglobin, mmol/l	28	129±2.00	136±5.36**
	105	134±2.18	141±4.55***
Red blood cells, 10^{12}/l	28	1.94±0.02	2.26±0.08*
	105	1.98±0.03	2.33±0.1**
White blood cells, thousand/mm^3	28	20.16±0.3	19.84±0.2
	105	22.48±0.5	22.78±0.4*
ESR, mm/h	28	2.26±0.2	2.19±0.1
	105	2.40±0.3*	2.13±0.1
Rod-core, %	28	1±0.01	3±0.01
	105	5±0.01	1±0.01
Segmental, %	28	17±2	28±3
	105	21±3	26±3
Monocytes, %	28	0	0
	105	0	0
Lymphocytes, %	28	70±5	58±4
	105	76±7	72±6
Eosinophils, %	28	1±0.01	2±0.01
	105	3±0.01	1±0.01
Basophils, %	28	0	0
	105	0	0

Note: ** – P<0.05; ** – P<0.01; *** – P<0.001*
Source*: compiled by the author*

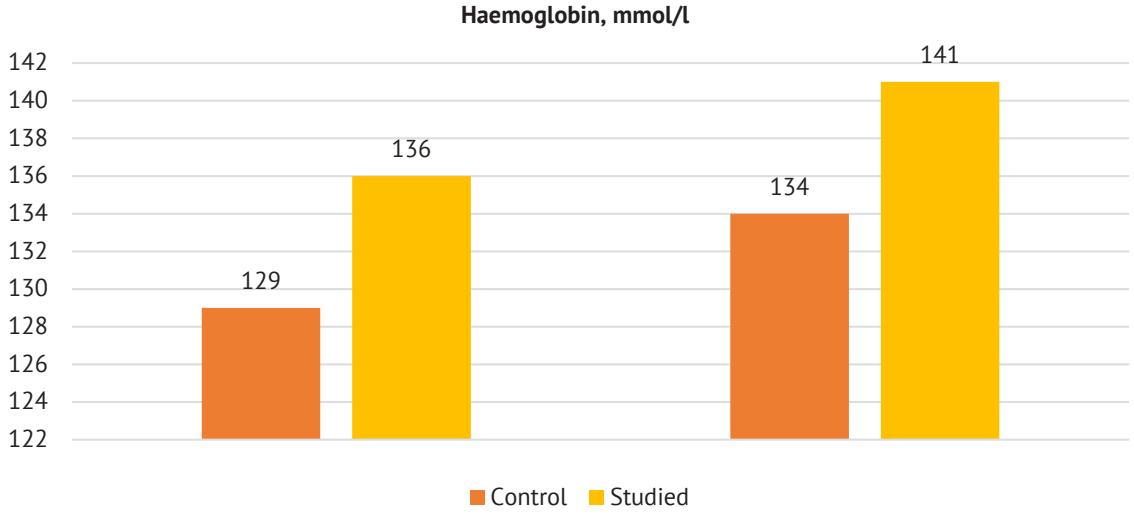

Figure 1. *Dynamics of haemoglobin concentration in the turkey blood*

The physiological state of the experimental turkeys treated with the triazoline compound GKPF-109 was assessed by their general appearance, behaviour, frequency of feeding and watering, as well as by the results of biochemical blood tests. During the study, the turkeys in the experimental group were observed to be clinically healthy, with good appetite, good response to external stimuli, and no signs of disease. The litters of the control and experimental groups were formed, without visible signs of digestive disorders, indicating physiological norms of the gastrointestinal tract.

The experiments (Table 1, Fig. 1) show that the haemoglobin content in the experimental group on day 28 (136 mmol/l) is 5.4% higher than in the control group (129 mmol/l). In addition, at 105 days, the haemoglobin content in the blood of the experimental group reached 141 mmol/l, which is 5.2% higher than in the control group. The analysis of Figure 2 shows that the red blood cell content in the experimental group on day 28 (2.26 10^{12}/l) and day 105 (2.33 10^{12}/l) increased by 16.5% and 17.7%, respectively, compared to the control group on day 28 (1.94 10^{12}/l) and on day 105 (1.98 10^{12}/l) (Fig. 2).

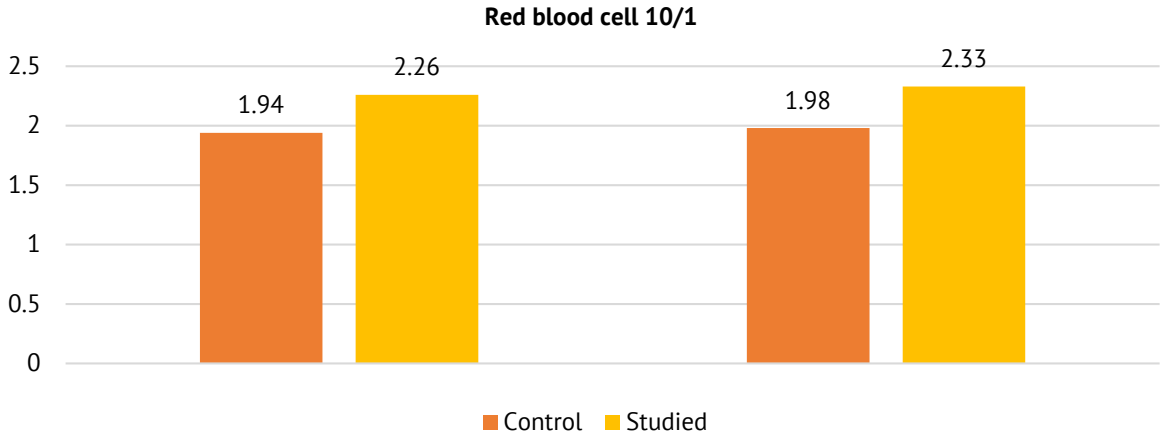

Figure 2. *Dynamics of red blood cells in the blood of turkeys, 10^{12}/l*

In addition, the results show that the leukocyte content of both the experimental and control groups is almost the same without any significant changes during the turkey-rearing period. However, no significant difference was observed between the two groups in terms of leukocyte monitoring (neutrophils, eosinophils, basophils, lymphocytes, and monocytes) ESR, mm/h (Fig. 3).

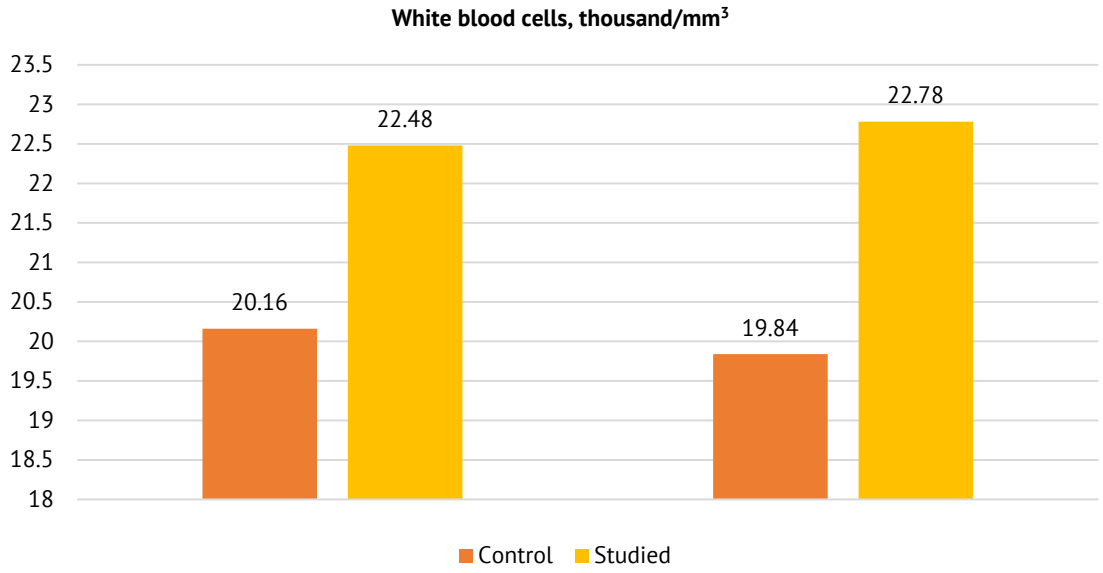

Figure 3. *Dynamics of leukocytes in the blood of turkeys, thousand/mm^3*

The results of the erythrocyte sedimentation rate (Fig. 4) show that on day 28, the experimental group (2.19 mm/h) was 3.1% lower than the control group (2.26 mm/h). At the same time, at 105 days, an analysis of the erythrocyte sedimentation rate shows that it is 11.25% lower in the experimental group (2.13 mm/h) than in the control group (2.40 mm/h). To maximise the production potential of poultry, body functions must be in perfect balance, known as homeostasis. In this context, knowledge of the levels of certain blood parameters provides important information to determine the state of balance of the body, which reflects the requirements for metabolic processes (Fig. 4).

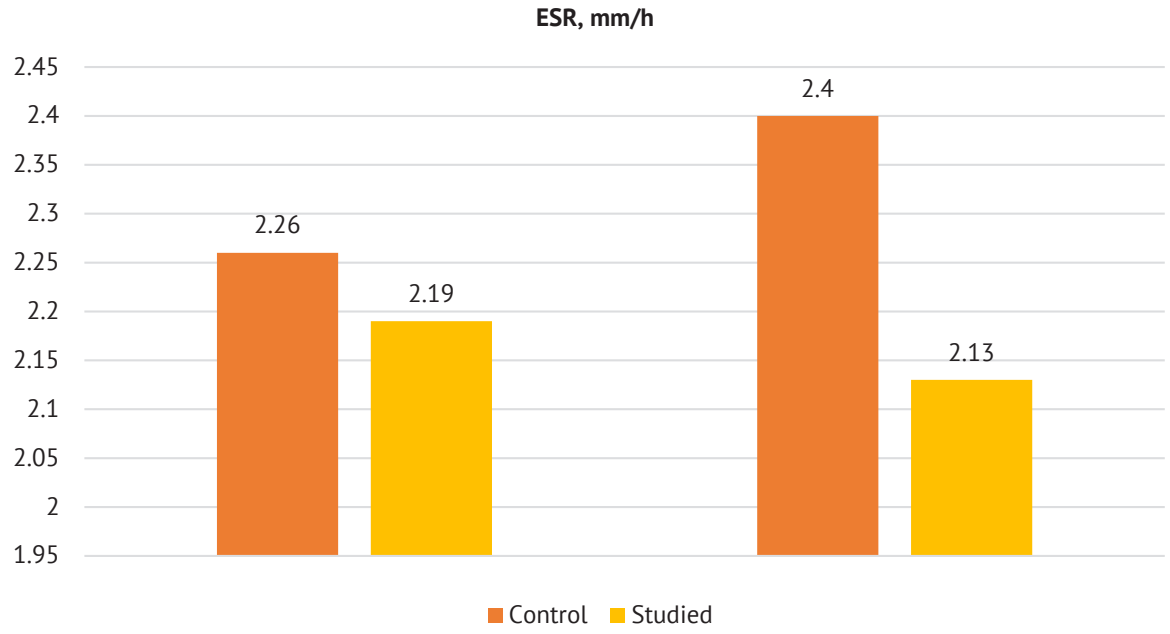

Figure 4. *Dynamics of ESR in turkey blood, mm/h*

The next stage of the study was the biochemical parameters of blood serum. Thus, age and related production processes in the rearing of poultry of meat type are important factors that can affect the intensity of metabolism and induce changes in the structure of serum proteins (Table 2).

Table 2. *Biochemical parameters of blood serum*

Indicator	Age, days	Control	Studied
Females (n=10)			
Total protein, g/l	28	49.37±0.15	50.13±0.19*
	105	49.74±0.26	51.2±0.3**
Calcium, mmol/l	28	4.15±0.01	4.21±0.02**
	105	3.99±0.06	4.20±0.01**
Phosphates, mmol/l	28	2.16±0.03	2.25±0.02*
	105	2.06±0.02	2.20±0.01
Ca:P ratio	28	1.92±0.02	1.87±0.02
	105	1.93±0.02	1.90±0.01
Reserve alkalinity, mmol/l	28	49.9±0.23	50.5±0.34
	105	50.2±0.13	50.41±0.21

Note: ** – P<0.05; ** – P<0.01; *** – P<0.001*
Source*: compiled by the author*

Poultry blood proteins are an important indicator in assessing health status and are the basis of general biochemistry, which allows identifying metabolic changes. The results in Table 2 and Figure 5 show that the total protein content on day 28 in the blood of turkeys of the experimental group (50.13 g/l) is 1.5% higher than in the control group (49.37 g/l), and on day 105 in the experimental group (51.2 g/l) is 2.9% higher than in the control group (49.74 g/l). Considering that blood proteins play an important physiological role in the body and in maintaining homeostasis, determining their concentration in sick poultry is of paramount importance for assessing the health status of the organism (Fig. 5).

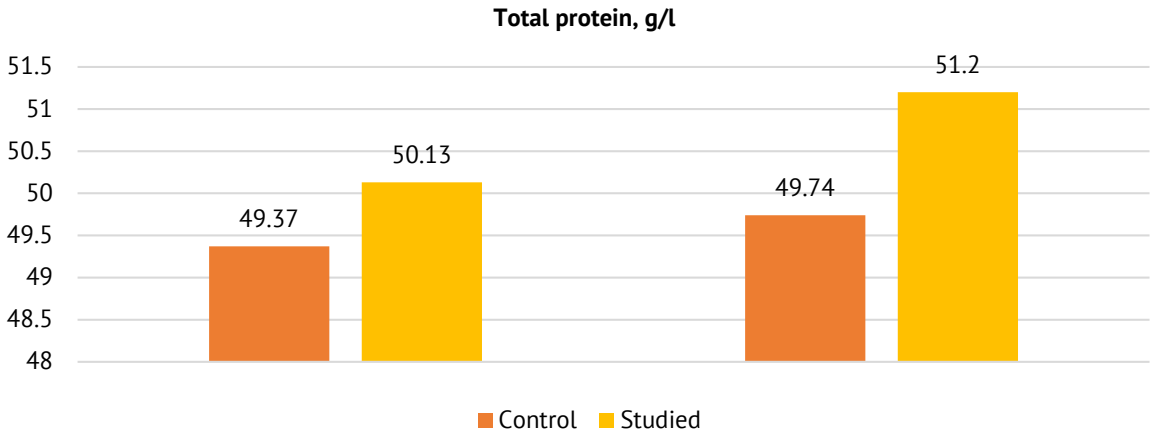

Figure 5. *Total protein content in turkey blood, g/l*

The data in Figure 6 show that the level of calcium and phosphorus in the blood of turkeys of the control group on day 28 (4.15 mmol/l and 2.16 mmol/l) is 1.5% and 4.2% lower, respectively, than in the experimental group (4.12 mmol/l and 2.25 mmol/l), At the same time, on the 105th day, the level of calcium and phosphorus in the blood of turkeys of the experimental group (4.20 mmol/l and 2.20 mmol/l) exceeded the levels in the control group (3.99 mmol/l and 2.06 mmol/l) by 5.3% and 6.8%, respectively.

In addition, Table 2 shows no significant changes between the levels of reserve alkalinity, mmol/l, in both the experimental and control groups at different age periods (Fig. 6).

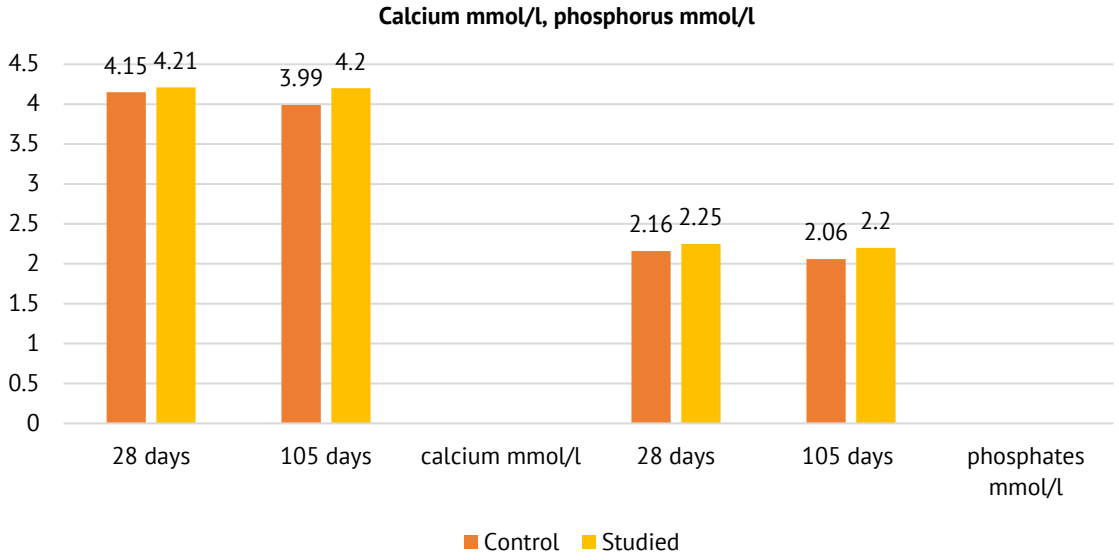

Figure 6. *Calcium and phosphorus levels in turkey blood, mmol/l*

At the end of the experiment, to test the efficiency of digestion and metabolism in turkeys, the general morphological composition of the blood was analysed, and the content of total blood protein showed a significant increase in the experimental group by 2.9% compared to the control group. This may indicate that GKPF-109 can increase metabolic efficiency in turkeys. In the biochemical analysis, a significant difference was observed in the increase in haemoglobin and red blood cells in the experimental group of turkeys by 5.2% and 17.7%, respectively.

Thus, the increase in the number of red blood cells, haemoglobin, and total protein in the blood of turkeys of the experimental groups suggests that the drug can stimulate the processes of erythropoiesis and protein synthesis without affecting the stability of haemopoiesis and the stability of the overall blood composition.

Turkey meat productivity and quality. Quantitative and qualitative characteristics of turkey meat are evaluated after slaughter. The addition of the triazoline compound GKPF-109 to the turkey diet leads to an increase in pre-slaughter weight, semi-gutted carcass weight and gutted carcass weight of the experimental group (9787 g, 8359 g and 7480 g) by 7.6%, 7.1% and 7.6%, respectively, compared to the control group (9100 g, 7806 g and 6950 g) (Table 3).

Table 3. *Turkey meat qualities*

Indicators	Control	Studied
Number of females	10	10
Pre-slaughter weight, g	9100±99.30	9787±95.61***
Semi-gutted carcass weight, g	7806±74.22	8357±76.49***
Of the pre-slaughter weight, %	85.78%	85.39%
Weight of gutted carcass, g	6950±62.73	7480±76.03***
Slaughter yield, %	76.37%	76.43%

Note: ** – P<0.05; ** – P<0.01; *** – P<0.001*
Source*: compiled by the author*

According to the results presented in Table 4, there is a large difference between the weight of different parts of turkey carcasses of the experimental and control groups: the weight of the breast in the control group (2589 g) is 7.9% lower than in the experimental group (2788 g), and the weight of the back of the experimental group (1446 g) is 7.1% higher than in the control group (1548 g). In addition, the hip weight was 7.8% higher in the experimental group (1240 g) than in the control group (1150 g), 143 g) by 8% and 6.3%, respectively (Table 4).

Morphological examination of the internal organs of turkeys (heart, spleen, glandular part of the stomach, muscular part of the stomach, kidneys, air sacs) revealed that in all groups they were in a state without any pathological changes (Table 5).

Table 4. *Meat quality and conformity of different parts of turkey carcasses*

Indicators	Control	Studied
Number of females	10	10
Pre-slaughter weight, g	9100±99.30	9787±95.61***
Weight of semi-gutted carcass, g	7806±74.22	8357±76.49***
Of the pre-slaughter weight, %	85.78%	85.39%
Weight of the alarmed turkey carcass, g	6950±62.73	7480±76.03***
Including breast	2589±40.4	2788±45.6**
%	37.25%	37.27%
Backbone	1446±36.8	1548±35.9**
%	20.80%	20.7%
Thighs	1150±24.7	1240±25.5**
%	16.55%	16.58%
Shin	805±14.1	807±14.3*
%	11.59%	11.63%
Wing	817±12.9	882±13.6**
%	11.76%	11.79%
Neck skin	143±7.7	152±8.3*
%	2.05%	2.03%

Note: ** – P<0.05; ** – P<0.01; *** – P<0.001*
Source*: compiled by the author*

Table 5. *Internal organ mass*

Indicators	Control	Studied
Pre-slaughter weight, g	9100±99.29	9787±95.60***
% of pre-slaughter weight	100%	100%

Table 5, Continued

Indicators	Control	Studied
Liver, g	83.72±1.11	92.11±1.26*
% of pre-slaughter weight	0.92%	0.94%
Spleen, g	4.55±0.2	5.12±0.2
% of pre-slaughter weight	0.05%	0.05%
Heart, g	25.48±0.75	29.50±0.81*
% of pre-slaughter weight	0.28%	0.3%

Note: ** – P<0.05; ** – P<0.01; *** – P<0.001*
Source*: compiled by the author*

The study results of internal organs showed that the absolute weight of the internal organs of the poults of the experimental group was significantly higher than that of the control group, which was noticeably manifested in the weight of the liver and heart, which increased to 92.11 g (10.02%) and 29.50 g (15.78%) compared to the control (P<0.05).

Organoleptic evaluation of turkey meat. When analysing the organoleptic characteristics of meat and the quality of broth when cooking turkey meat, it was found that the organoleptic characteristics of turkey meat were better in the experimental group than in the control group, when conducting a scoring assessment of appearance, aroma, colour, taste, a significant increase in the indicators of the experimental group (8.1, 8.3, 8.2 and 8.1) was found, which is more than in the control group (7.8, 8, 7.9 and 7.8) by 3.9%, 3.8%, 3.8% and 3.9%, respectively (Table 6).

Table 6. *Organoleptic characteristics of turkey meat and broth quality*

Indicators	Group	
	Control	Studied
	Meat	
Appearance	7.8±0.40	8.1±0.50*
Aroma	8.0±0.24	8.3±0.57*
Colour	7.9±0.25	8.2±0.54
Texture	7.8±0.54	7.8±0.83
Succulence	8.0±0.00	8.1±0.44
Taste	7.8±0.65	8.1±0.71*
Overall score	47.3±7.41	48.6±6.92
	Stock	
Appearance	7.7±0.70	8.0±0.63
Aroma	7.9±0.62	8.4±0.50**
Colour	8.1±0.25	8.3±0.44*
Taste	8±0.52	8.1±0.77
Richness	7.3±0.70	7.5±1.02
Overall score	39±5.09	40.3±6.46

Note: ** – P<0.05; ** – P<0.01; *** – P<0.001*
Source*: compiled by the author*

The results of the study (Table 6) show that the addition of GKPF-109 to the turkey diet leads to a significant increase in the results of the broth evaluation of turkey meat scores of the experimental group in terms of appearance, smell, colour, and taste (8, 8.4, 8.3 and 8.1) by 3.9%, 6.3%, 2.5% and 1.3% more than in the control group (7.7, 7.9, 8.1 and 8.1). The next stage of the study was the physical and chemical evaluation of turkey meat. The results presented in Table 7 show that the pH and ammonia-ammonia nitrogen content in white and red turkey meat does not differ significantly between the control and experimental groups, the peroxidase reaction is positive, and the content of ammonia and ammonium salts is negative. However, microscopy of smear prints from the surface layers of red and white turkey meat shows a significant decrease in the number of microorganisms in the experimental group compared to the control group by 36.4% and 42.1%, respectively.

Table 7. *Physical and chemical properties of turkey meat*

Indicators	Control group		Studied group	
	Red meat	White meat	Red meat	White meat
pH	6.01±0.16	5.97±0.10	5.91±0.09	5.85±0.09
Ammonia nitrogen (mg KaON)	1.12±0.07	1.08±0.09	0.98±0.08	0.88±0.06
Reaction to peroxidase	Positive	Positive	Positive	Positive
Ammonia and ammonium salts content	Negative	Negative	Negative	Negative
The number of microorganisms in one field of view of the smear prints				
External layer	3.30±0.90	3.80±0.98	2.10±1.04*	2.20±0.98*
Deep layer	3.1±1.14	2.90±1.04	2.10±1.54	2.10±0.94

Note: ** – P<0.05; ** – P<0.01; *** – P<0.001*
Source*: compiled by the author*

Poultry meat, in particular turkey meat, has a different degree of colour compared to meat from other farm animals, with muscle tissue ranging from light pink (white meat) to dark red (red meat) depending on the myoglobin content; red limb meat contains less protein, more fat and cholesterol, and white meat (breast muscles) contains more carnosine, glycogen, and adenosine triphosphate.

During the chemical analysis of turkey meat, it was observed that the myoglobin content in white muscles is 0.05 – 0.08%, and in red muscles – several times higher (Table 8).

Table 8. *Chemical analysis of turkey meat*

Indicators	Control		Studied	
	Red meat	White meat	Red meat	White meat
Moisture, %	75.12±1.96	70.75±2.59	74.50±1.66	70.12±1.90
Dry matter, %	24.88±1.96	29.25±2.59	25.50±1.66	29.88±1.90
Protein, %	23.81±1.56	31.21±1.79	25.28±1.73**	32.04±1.59*
Fat, %	4.4 ±0.69	3.85±0.58	3.84±0.71	3.34±0.63
Mineral substances, %	0.95±0.11	0.98±0.14	1.06±0.19	1.21±0.23
Calorie content, kcal/100 g	121.43	135.95	120.14	134.87

Note: ** – P<0.05; ** – P<0.01; *** – P<0.001*
Source*: compiled by the author*

Turkey meat is distinguished from other animal meat by its high protein content. There is a significant variation in the flavour and nutritional properties of meat in different turkey breeds. Given the importance of providing the population with high-quality meat raw materials, comprehensive research is needed to determine the quality and commercial and technological characteristics of meat. The results show that the red meat of turkeys of the first experimental group is higher than the red meat of turkeys of the first control group in terms of dry matter, protein and mineral content by 2.5%, 6.14% and 11.97%, respectively.

The results presented in Table 8 show that the highest water content was in the red meat of the control group turkeys – 70.75%, and the lowest – in the white meat of the experimental group turkeys – 70.12%. The results of the protein content analysis show that the protein content in the white meat of the turkeys of the experimental group is 32.04%, which is 2.1% higher than in the white meat of the control group (31.21%) (Table 8). The protein content in the red meat of the turkeys of the experimental group was also 10.2% higher than in the control group.

The fat content in the red and white meat of turkeys of the experimental group (3.84% and 3.34%) is lower than in the control group (4.49% and 3.85%) by 14.5 and 13.3%, respectively (Table 8). The analysis of the research results (Table 8) shows that the highest amount of minerals is contained in the white meat of turkeys of the experimental group – 1.21%, which is 23.5% higher than in the control group. Table 8 shows that the calorific value of white and red meat of turkeys in the experimental group (120.14 kcal/100 g and 134.87 kcal/100 g) is 1.1% and 0.8% lower than that of turkeys in the control group (121.43 kcal/100 g and 135.95 kcal/100 g).

The demand for immunostimulants has been growing rapidly in recent years, driven by the widespread

prevalence of immunodeficiencies in poultry production. These immunostimulants are most commonly used in poultry farming to improve the effectiveness of poultry vaccination and poultry productivity. During these studies, the authors noted that the results obtained by scientists after the use of immunostimulants in poultry rearing in all cases were positive, namely the impact on reducing the time of fattening, and the safety of poultry during fattening. In our research, we used haemolytic, organoleptic methods and anatomical examination of poultry to develop rational regimens for the use of immunostimulants – derivatives of the triazoline series. When choosing immunostimulants, preference was given to the triazoline derivatives GKPF-109, synthesised in recent years in Ukraine. Fedotov *et al.* (2023) point out that heterocyclic 1,2,4-triazole compounds are structural fragments of many synthetic drugs that exhibit antifungal (fluconazole, itraconazole), antidepressant (trazodone alprazolam), hepatoprotective, wound healing and antiviral (thiotriazoline) effects, which contain a 3-pyridine fragment as a substituent and lead to the appearance of substances with high pharmacological activity.

R. Dubin *et al.* (2022) and A. Paliy *et al.* (2018) found that after the introduction of new triazoline compounds, the general condition of broiler chickens was positively affected compared to the control group. The experimental chickens had an improved appetite, and the compounds had a positive effect on the gastrointestinal tract during the transition from one feed to another. An important aspect of the use of triazoline compounds in our studies was that the emphasis was placed on studying the properties and developing schemes for the use of this compound, which allowed us to develop more reasonable general schemes for the use of the compound to stimulate haematological parameters and organolipid properties of poultry carcasses.

Similar results were obtained for the feeding of triazoline compounds to broiler chickens in the experiments of O. Orobchenko *et al.* (2022), where a positive effect on reducing the time of poultry fattening was found, which is important for industrial meat poultry production. The developmental dynamics of broiler turkeys, which were used in the experiment to study the effect of triazoline compounds on meat productivity, showed that the introduction of GKPF-109 caused a large difference between the weight of different parts of the carcasses. The use of the triazoline compound GKPF-109 did not affect the morphological parameters of blood – haemoglobin, red blood cells, leukocytes, and proteins were within the physiological norm. In the experimental group, there was an increase of 8.14% in the number of red blood cells and 5.2% in the total protein content compared to the control group.

When studying the effect of the triazoline compound GKPF-109 on the meat quality of turkey poults, a positive effect was found. In the experimental group, the increase in muscle tissue increased protein by 7.5%, fat by 8.3%, and energy value by 4.8%. The results of our studies are consistent with the data of C. Hautefeuille *et al.* (2020), and M. Arif *et al.* (2018), when Carcesel was used in experiments in the diet of broiler chickens, it increased the pre-slaughter live weight and carcass weight of broiler chickens compared to the control, which was 2.76-5.02% and 2.93-5.89% higher, muscle tissue weight – by 3.32-6.75%, pectoral muscle weight – by 3.53-7.83%, and the weight of edible parts of the carcass – by 3.33-6.47%. According to T. Hofmann *et al.* (2021), the higher productivity of broilers in the experimental groups was due to the protein quality index (PQI) in broiler chickens of the experimental groups, which was 3.92-7.84% higher than in the control, and the culinary and technological index of meat was 1.81-3.61% higher. During the experiment, a positive effect on the nutritional value of meat was observed, which increased due to an increase in muscle tissue protein – by 7.5%, fat – by 8.3%, and energy value – by 4.8%.

The results of our organoleptic study are consistent with the studies of Sch. Bernd *et al.* (2020), A. Fatenok-Tkachuk *et al.* (2017) and S. Kabene & S. Baadel (2019). Thus, according to the authors, feeding broiler chickens with various immunostimulant preparations significantly affects the digestibility of organic matter by 3.92%, protein by 7.80%, fat by 5.35%, fibre by 6.7%, and BEV by 1.7%. They are characterised by a high average daily weight gain of 3.11-10.79% and live weight at the time of slaughter by 3.7-10.29% better feed conversion than control broiler chickens, which allows for fuller use of the biological resources of their meat productivity: to increase not only the pre-slaughter weight and carcass weight, but also the slaughter yield of gutted carcasses by 2.52-2.68%, and to increase the yield of edible parts from the carcass to 85.0-86.1%. Thus, the results obtained by the authors indicate a positive effect of the triazoline compound GKPF-109 in the diet of turkeys during all periods of development due to a higher content of red blood cells, leukocytes, haemoglobin, total protein in the blood, which accelerates the main biochemical processes in their body.

CONCLUSIONS

The addition of a triazoline compound GKPF-109 to the diet of turkeys at a dose of 0.5 ml/bird per day increases the slaughter yield of gutted carcasses by 12.4%, and semi-gutted carcasses by 12.3%. In the study of morphological and biochemical parameters of turkey blood, an increase in haemoglobin in the blood of turkeys of the experimental group was observed by 8.14%, the number of red blood cells in the blood was higher by 8.29%, the content of total protein – by 5.2%. When examining the internal organs, it was observed that the absolute weight of the internal organs of the experimental group was significantly higher than that of the control group, which was noticeably manifested

in the weight of the liver and heart, which increased to 92.11 g (by 10.02%) and 29.50 g (by 15.78%) compared to the control (P<0.05). Organoleptic, bacteriological, and physicochemical parameters of turkey meat showed an increase in the nutritional value of meat due to an increase in muscle tissue protein by 7.5%, fat by 8.3%, and energy value by 4.8%. During the evaluation of the broth, the scores of turkey meat from the experimental group in terms of appearance, smell, colour, and taste (8, 8.4, 8.3 and 8.1) were 3.9%, 6.3%, 2.5% and 1.3% higher than in the control group (7.7, 7.9, 8.1 and 8.1). The results show that the red meat of turkeys of the first experimental group is higher than the red meat of turkeys of the first control group in terms of dry matter, protein, and mineral content by 2.5%, 6.14% and 11.97%, respectively. Prospects for further research are to determine the effect of the drugs on poultry infection with diseases of bacterial aetiology.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Вплив триазолінових сполук на м'ясну продуктивність тушок індиків

Руслан Анатолійович Дубін
Кандидат ветеринарних наук, доцент
Одеський державний аграрний університет
65012, вул. Пантелеймонівська, 13, м. Одеса, Україна
https://orcid.org/0000-0003-3540-0816

Анатолій Павлович Палій
Доктор ветеринарних наук, професор
Національний науковий центр «Інститут експериментальної і клінічної ветеринарної медицини»
61023, вул. Пушкінська, 83, м. Харків, Україна
https://orcid.org/0000-0002-9193-3548

Павло Василійович Шарандак
Доктор ветеринарних наук, професор
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0002-5434-666X

Оксана Вікторівна Івлева
Кандидат ветеринарних наук, доцент
Східноукраїнський національний університет імені Володимира Даля
01042, вул. Іоанна Павла II, 17, м. Київ, Україна
https://orcid.org/0000-0001-8090-4373

Микола Іванович Тодоров
Кандидат ветеринарних наук, доцент
Одеський державний аграрний університет
65012, вул. Пантелеймонівська, 13, м. Одеса, Україна
https://orcid.org/0000-0001-9260-567X

Анотація. Необхідність пошуку ефективних засобів підвищення якості та збільшення виробництва м'ясної продукції птахівництва зумовлює актуальність дослідження. Введення препаратів до раціону дозволяє підвищити збереженість поголів'я та покращити якісні характеристики м'яса птиці. Мета дослідження – оцінити вплив нових похідних 1,2,4-тріазолу на деякі показники крові, а також м'ясні та забійні якості птиці. Дослідження проводилося у 2022-2023 роках в Одеському державному аграрному університеті. Дослід проводився з метою вивчення впливу нових похідних триазолу GKPF-109 у дозі 0,5 мл/птицю на добу на продуктивність індиків та якість м'яса. Дослід проводили на індичатах кросу Біг-6 віком 1-105 днів. Використовували такі методи дослідження: морфологічні та біохімічні, органолептичні, фізико-хімічні та статистичні. Вплив похідних триазолу GKPF-109 на органолептичні (зовнішній вигляд, запах, консистенція, стан жиру, якість бульйону під час варіння м'яса) та фізико-хімічні дослідження м'яса індиків за мікробіологічними показниками було проаналізовано та досліджено згідно з ДСТУ 3143:2013. Результати показали, що відбулося збільшення середньодобових приростів на 13,1 %, забійного виходу потрошених тушок на 12,4 %, напівпотрошених тушок на 12,3 % та збереженості поголів'я на 4 %. Відмічено підвищення харчової цінності м'яса за рахунок збільшення вмісту білка в м'язовій тканині на 7,5 %, жиру на 8,3 % та енергетичної цінності на 4,8 %. Органолептичні, бактеріологічні та фізико-хімічні показники м'яса індиків за використання в раціоні похідного триазолу GKPF-109 відповідали вимогам ДСТУ 3143:2013. Зроблено висновок, що додавання похідних триазолу GKPF-109 до питної води посилює гемопоез, має протизапальну та гепатопротекторну дію. Оцінка якості м'яса та бульйону індичат-бройлерів наприкінці досліду не дозволяє стверджувати про зниження їх аромату та смаку, що свідчить про відсутність негативного впливу похідних триазолу GKPF-109 та способів їх застосування на органолептичні показники м'яса, що слід враховувати при утриманні індиків

Ключові слова: похідні 1,2,4-тріазолу; індики; приріст маси; еритроцити; гемоглобін; лейкоцити

SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 35-43

UDC 614.9.616.6-002.618.636.09
DOI: 10.48077/scihor9.2023.35

Inflammatory and anti-inflammatory cytokines in the endometrium of cows during anaphrodisia and estrous cycle

Iryna Bondarenko*
PhD in Veterinary Science, Associate Professor
Sumy National Agrarian University
40021, 160 H. Kondratiev Str., Sumy, Ukraine
https://orcid.org/0000-0002-1019-3446
Andriy Lazorenko
PhD in Veterinary Science, Associate Professor
Sumy National Agrarian University
40021, 160 H. Kondratiev Str., Sumy, Ukraine
https://orcid.org/0000-0002-0916-3901
Yurii Musiienko
PhD in Veterinary Science, Associate Professor
Sumy National Agrarian University
40021, 160 H. Kondratiev Str., Sumy, Ukraine
https://orcid.org/0000-0002-9735-4758
Oleksandr Panasenko
PhD in Veterinary Science, Associate Professor
Sumy National Agrarian University
40021, 160 H. Kondratiev Str., Sumy, Ukraine
https://orcid.org/0000-0003-1257-6341

Article's History:
Received: 9.05.2023
Revised: 24.08.2023
Accepted: 27.09.2023

Abstract. Infertility and sexual cycle disorders in cows are an urgent and widespread problem in cattle breeding, causing significant losses to farms. At the same time, a detailed understanding of the cellular and molecular mechanisms in the bovine uterus is crucial to explain and avoid infertility in dairy cows. The research aims to determine the concentration of tumour necrosis factor (TNFα), interleukin-1 (IL-1) and interlekin-4 (IL-4) in endometrial homogenates during oestrus, proestrus, corpus luteum and anaphrodisia caused by endometritis, as well as lactation. The research material was homogenates of cow endometrium taken from the horns of the uterus in its upper third. The methods used to determine the content of IL-1, IL-4 and TNFα in endometrial samples were enzyme-linked immunosorbent assay with a ratio (tissue homogenate-phosphate-salt buffer). The results of the study revealed changes in the content of IL-4, IL-1 and TNF-α in the functional layer of the endometrium during anaphrodisia and certain stages of the oestrous cycle. A significant increase in the content of TNF-α

Suggested Citation:
Bondarenko, I., Lazorenko, A., Musiienko, Yu., & Panasenko, O. (2023). Inflammatory and anti-inflammatory cytokines in the endometrium of cows during anaphrodisia and estrous cycle. *Scientific Horizons*, 26(9), 35-43. doi: 10.48077/scihor9.2023.35.

*Corresponding author

and a decrease in the levels of IL-1 and IL-4 in the endometrium compared to the same indicator during oestrus were found. No significant difference in the level of IL-1 during proestrus compared to the corpus luteum was found, as a result of the extinction of the transient phlogogenic reaction inherent in the oestrous endometrium at this time, due to the cytokine balance, which prepares the best conditions for zygote implantation. It was found that the level of IL-1 during oestrus significantly increases relative to the corpus luteum and proestrus stages. It was determined that the concentration of IL-4 in the functional layer of the endometrium of cows suffering from afterbirth retention and endometritis is lower than the same indicator during oestrus, which confirms the inhibition of cell proliferation and differentiation. The data obtained can be used to optimise the correction of sexual cycling in cows with anaphrodisia

Keywords: cows; anaphrodisia; sexual cycle; endometrium; TNF-α; IL-1; IL-4

INTRODUCTION

Maintaining and improving the reproductive performance of cows and heifers is one of the key parameters for the economic success of dairy farming. According to M. Bekara & N. Bareille (2019), infertility is a significant problem for dairy farming, as the associated extended calving intervals, reduced milk yields and increased culling rates contribute to huge economic losses. According to P. Lonergan *et al.* (2019), the causes of infertility are diverse and complex and, in addition to controlling the sexual cycle, efficiency of oestrus detection and proper breeding management, monitoring uterine health is a key factor in the proper reproductive performance of a dairy herd.

In the postpartum period in dairy cows, a high risk of clinical or subclinical diseases of the reproductive tract is present, which, following O. Pascottini & S. LeBlanc (2020), is accompanied by glucose intake, hypocalcaemia, lipid mobilisation from body fat, ketosis and the expression of proinflammatory cytokines that affect the immune response and change rapidly but differently in individual cows. At the same time, according to H. Pothmann *et al.* (2021), inflammatory processes and mRNA expression of cytokines at the end of the postpartum period can be considered as multifocal or widespread throughout the endometrium, regardless of the health of the uterus.

I. Sheldon *et al.* (2019) analysed infectious factors and endometrial inflammation in the postpartum period in cows with clinical and subclinical endometritis to determine the main causes of deterioration of the structural and functional state of the uterine mucosa and showed that inflammatory processes that continue in the postpartum period can impair fertility, lead to anaphrodisia, and interfere with embryo development. N. Naim *et al.* (2020) have shown that the reproductive function is realised through the activation of cambial tissues, which include the endometrium, and the physiological restructuring of the latter depends on coordinated intercellular interaction through the cytokine system.

F. Pinaffi (2018) found an important role of gonadal hormones and cytokines in preparing the endometrium for implantation and activating the transformation of epithelial cells and stromal fibroblasts into decidual cells. The cytokines IL1, IL6 and TNFα are considered (Drillich & Wagener, 2018) as mediators of nonspecific inflammatory processes, but their activity is also physiologically enhanced during the early postpartum period with a high degree of mRNA expression of cytokines and acute phase proteins during the third week after delivery, regardless of the health status of the animals.

The cytokine system is quite informative about the intercellular interaction of the endometrium, in connection with the prediction of zygote nidulation. According to P. Abeysinghe *et al.* (2023), binding of interleukin-1 to the receptors of maternal placental cells ensures zygote nidulation, proliferation of placental barrier cells, embryogenesis, and an increase in the percentage of zygotes that reach the blastocyst stage. N. Nijkang (2019) points out that the main function of interleukin-4 is to regulate stem cell proliferation by inducing mitogen-activated protein kinases, which transmit intracellular signals that cause cell differentiation, metabolic changes, apoptosis, and stimulate luteal cells to secrete progesterone. Q. Huang (2021) has demonstrated that zygote nidulation occurs when proinflammatory cytokines are expressed, but subsequently, pregnancy proceeds physiologically in the event of a shift in the cytokine balance towards immunosuppressive cytokines (interleukin-4, interleukin-10), as the latter inhibits cellular immunity responses, whereas a significant increase in interleukin-4 indicates a violation of peripheral tolerance and a shift in the local immune response towards Th-1, which causes infertility, and the ratio of interleukin-1 to interleukin-4 is a prognostic indicator in assessing the course of fertilisation and pregnancy.

According to M. Lappas (2017), cytokines affect the regulation of numerous types of intercellular interactions: granulosa cell proliferation, expression of gonadotropin receptors, and, conversely, cytokine imbalance leads to desynchronisation of cell differentiation, implantation disorders and infertility. N. Naim *et al.* (2020) investigated the cytokine-regulated endothelial-haemostatic interaction during maternal placental remodelling, implantation, and pregnancy. Q. Huang *et al.* (2021) note that during endometrial proliferation and differentiation, cytokines stimulate the functional activity of cells and humoral regulation of intercellular and intersystemic connections.

Thus, the analysis of the cytokine profile of tissue extracts of the uterine mucosa of cows at different stages of the sexual cycle will make it possible to determine the fullness of sexual cyclicity, the prerequisites for the development of infertility and will help to optimise prognostic tests for the effectiveness of sexual function correction. The research aims to investigate the content of certain pro- and anti-inflammatory cytokines in cow endometrial tissue extracts at different stages of the sexual cycle and in anaphrodisia following endometritis and after partum retention.

MATERIALS AND METHODS

The research was carried out within the framework of the research work "Study of cellular, biochemical and molecular genetic mechanisms of infectious diseases, metabolic disorders and immunocompensatory processes of counteracting biotic and abiotic factors in obstetric-gynecological, andrological and surgical pathology in animals" (state registration number 0116U005121).

The selection of experimental samples of the uterine mucosa of cows was carried out in farms with different housing technologies in the period from 2016 to 2022: by the tethered method (Shorthorn cow breed, OJSC breeding plant "Mikhailovka", Lebedynsky district, Sumy region with a productivity of <6000 kg and SFH "Vitalia", Burynsky district, Sumy region, Simmental breed of cows with productivity of >6000 kg), as well as the untethered method (LLC AF "Vladana", Ukrainian Black-and-White cows and LLC AF "Lan", Sumy district, Sumy region, Holstein breed, with a productivity of >6000 kg).

The material for the study was fragments of endometrial tissue (2-6 grams) taken from the upper third of the uterine horns of euthanised cows from experimental farms. Endometrial tissue samples were taken from euthanised animals without gynaecological pathology of the reproductive organs, aged 3 to 10 years: in heat (n=5), during the corpus luteum (n=5), proestrus (n=5) and from cows that did not come into heat for more than 60 days after endometritis (n=5) and after delayed afterbirth (n=5).

The selected endometrial samples were washed with 0.9% sodium chloride saline with a theoretical osmolarity of 308 mosmol/l, pH 5.0-7.0. Subsequently, the washed samples were weighed on an electronic analytical balance of the 1st accuracy class AS 82/220 R2, labelled, packed in polystyrene containers, and subjected to cryopreservation at – 20°C. To determine the concentration of cytokines IL-1, IL-4 and TNFα, endometrial samples were homogenised in the cold with the addition of 0.01 M phosphate-salt buffer (pH 7.4) containing 1% Triton X-100 solution in a ratio of 1:40, followed by placement in a +4°C refrigerator for 120 minutes (Lazorenko & Izdepsky, 2012).

The endometrial homogenates were then centrifuged at 3000 rpm for 15 min. In the supernatant, the level of cytokines IL-1, IL-4 and TNFα was determined by enzyme-linked immunosorbent assay using diagnostic test kits manufactured by Peninsula Laboratories Inc, (USA). The actual content of IL-1, IL-4 and TNFα in endometrial samples was determined by considering the ratio (tissue homogenate-phosphate-salt buffer).

Experimental studies were performed by modern methodological approaches, following the requirements of DSTU ISO/IEC 17025:2005 (2006). The animals were kept, and all manipulations were carried out following the Order of the Ministry of Health of Ukraine No. 416/20729 "On Approval of the Procedure for Conducting Animal Tests in Research Institutions" (Law of Ukraine No. 249, 2012) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986). The digital material obtained in the research was processed by variational statistical methods using statistical techniques (STATISTICA 10.0 for Windows) with the determination of the arithmetic mean (M), the statistical error of the arithmetic mean (m), and the probability of difference (p) between two variation series according to the Student's criterion with values of $p \leqslant 0.05$, $p \leqslant 0.01$, and $p \leqslant 0.001$.

RESULTS AND DISCUSSION

The complex pathogenetic aspects of the interaction of anti-inflammatory and pro-inflammatory cytokines during endometrial remodelling and post-morbid state in cows are not well understood. Therefore, the studies that detail the above processes are new and relevant. The data presented in Figure 1 indicate that during oestrus, the concentration of TNFα in the endometrium is the lowest, amounting to 5.03±2.16 pg/ml, while during proestrus and the corpus luteum, its level increases, compared to the oestrous phase, by almost 4.2-4.3 times (21.15±3.08 pg/ml and 21.63±4.69 pg/ml, $p<0.01$), respectively.

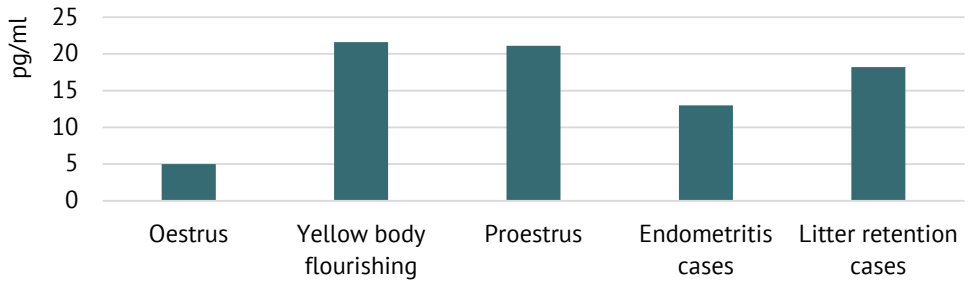

Figure 1. *TNF-α content in cow endometrial homogenates, pg/ml*

Source: *compiled by the authors*

The study of TNF-α content in endometrial homogenates of cows, during anaphrodisia and after endometritis and retained litter, indicates high expression, with the accumulation of this cytokine in the endometrium. In particular, the concentration of TNF-α in endometrial homogenates was significantly higher in patients with endometritis – 2.6 times to 13.07±1.98 pg/ml ($p<0.01$) and 3.6 times after afterbirth to 18.21±3.99 pg/ml ($p<0.01$), compared to the index during oestrus.

At the same time, the level of endometrial TNFα decreased by 1.7 and 1.2 times in cows with endometritis and after delayed afterbirth, respectively, compared to the same indicator in the corpus luteum, respectively. Concerning the proestrus, the content of TNFα in the endometrium decreased by 1.6 times in animals with endometritis and by 1.2 times after delayed birth, respectively. The data shown in Figure 2 demonstrate an increase in the level of IL-1 in cow endometrial homogenates during oestrus (8325.46±69.18 pg/ml), almost 2-fold, compared to the corpus luteum (4153.82±74.23 pg/ml) and proestrus (4092.57±476.05 pg/ml), $p<0.001$, respectively.

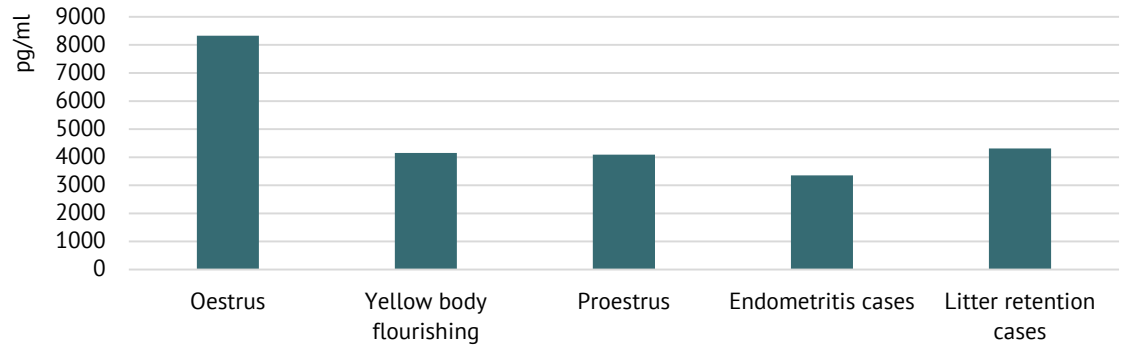

Figure 2. *IL-1 content in cow endometrial homogenates, pg/ml*
Source: *compiled by the authors*

The absence of a significant difference in the content of endometrial IL-1 in proestrus compared to corpus luteum is obviously due to the attenuation of the temporary inflammatory response inherent in the endometrium in oestrus at this period of the sexual cycle and a shift in the cytokine balance in favour of immunosuppressive cytokines, which creates optimal conditions for zygote nidulation. The concentration of IL-1 in the endometrium of anaphroditic cows suffering from endometritis and afterbirth retention was 2.5 times lower than that of cows in heat, 3351.71±1422.42 pg/ml and 3412.43±430.12 pg/ml, respectively, $p<0.01$, respectively, which is associated with the inhibition of the transformation of stromal fibroblasts and epithelial cells into decidual cells, and the impossibility of endometrial remodelling necessary for implantation. Thus, studies have shown that the key proinflammatory cytokine in the endometrium of cows during oestrus is IL-1, while from 7 to 18 days of the sexual cycle its concentration decreases significantly, almost reaching the level of animals that do not show sexual cyclicity, and TNFα takes over the role of the dominant proinflammatory cytokine, the expression of which increases significantly during this period. As can be seen from the data presented in Figure 3, the level of IL-4 in endometrial homogenates during oestrus reaches its maximum value – 407.07±45.78 pg/ml, while during the corpus luteum it begins to show a tendency to decrease by almost 1.4 times (295.31±42.16 pg/ml), and during proestrus it undergoes a significant decrease by 3.4 times (118.56±32.34 pg/ml), $p<0.001$, respectively. At the same time, a significant difference in the concentration of endometrial IL-4 was found between days 7-8 and 17-18 of the sexual cycle, amounting to 2.5 times ($p<0.01$), respectively.

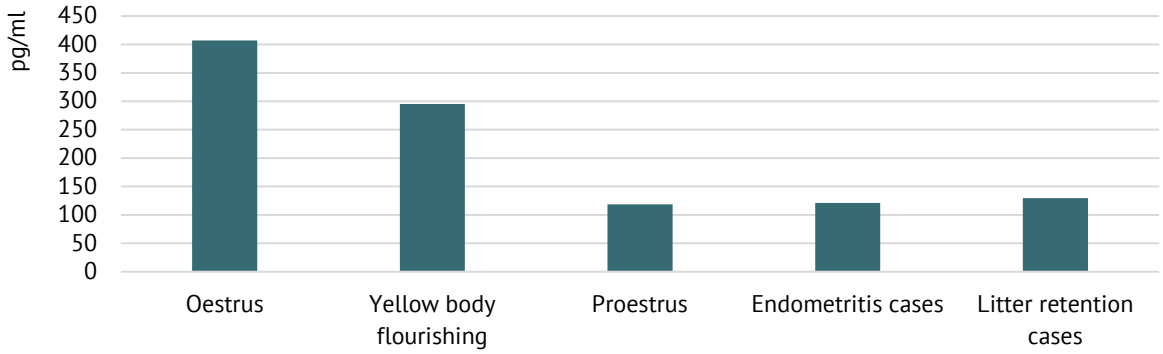

Figure 3. *IL-4 content in cow endometrial homogenates, pg/ml*
Source: *compiled by the authors*

The high concentration of this cytokine during oestrus is probably a consequence of the inhibitory effect of IL-4 on proinflammatory cytokines, the content of which at this time significantly increases, and in particular, the level of IL-1. The level of IL-4 in the endometrial homogenates of cows in the state of anaphrodisia and suffering from endometritis (121.03±52.36 pg/ml) and afterbirth retention (129.58±58.28 pg/ml) underwent a significant decrease compared to the indicator during the oestrous phase of the cycle by 3.4 and 3.2 times, $p<0.001$, respectively.

To assess the cytokine imbalance between proinflammatory IL-1 and anti-inflammatory IL-4, the ratio between IL-1 and IL-4 was calculated as an index reflecting the balance between both interleukins and the balance of cytokine expression. The ratio of IL-1 to IL-4 is an objective criterion for predicting the type of tissue regeneration, since in the event of a local imbalance of these cytokines, the regeneration mechanism undergoes a pathophysiological shift and causes tissue remodelling defects and infertility (Fig. 4)

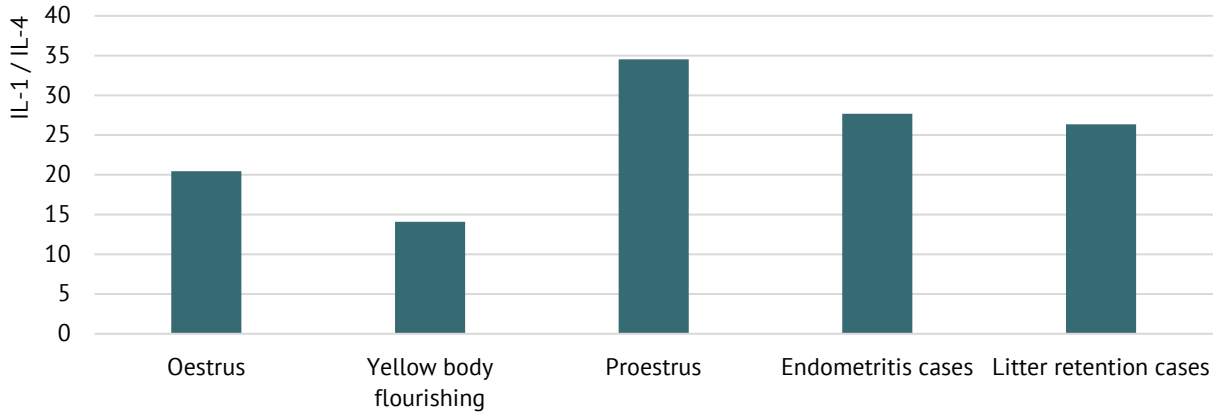

Figure 4. *IL-1 to IL-4 ratio index in cow endometrial homogenates*
Source: *compiled by the authors*

The highest index of the ratio between IL-1 and IL-4 was found to be during proestrus (34.53), while during oestrus it was 20.45, and during the corpus luteum, this ratio had a minimum value of 14.08. The obtained results demonstrate the role of IL-1 and IL-4 in the process of remodelling since changes in concentration confirm the activation of physiological endometrial remodelling during proestrus and inhibition of remodelling during corpus luteum. The index of the ratio between IL-1 and IL-4 in animals with endometritis and retained litter was 27.69 and 26.35, respectively, remaining significantly lower than the same index in the period of proestrus. The abovementioned changes indicate the inhibition of regenerative and restorative reactions due to the extinction of the metabolic and secretory activity of the postmorbid endometrium.

It is likely that in the present study, the increase in TNFα during active endometrial remodelling during days 7(8) to 17(18) of the sexual cycle is due to increased reproduction of endometrial components. In particular, studies (Abeysinghe *et al.,* 2023) have shown that TNFα initiates the production of other cytokines and prostaglandins, which causes stimulation, proliferation, and differentiation through the pleiotropic effect of different cell types.–Furthermore, according to N. Nijkang (2019), TNFα can induce apoptosis of immunocompetent cells, connective tissue cells, as well as circulating and exudative neutrophils through the activation of cellular caspases that destroy intracellular components. TNF-α, like IL-1 and IL-6, is a powerful stimulator of acute-phase protein synthesis in the event of tissue damage, so even a slight increase in TNF-α levels causes mobilisation of energy reserves and stimulates the body's defence responses.

Thus, the increase in TNFα content in the endometrium from day 7 to day 18 of the sexual cycle indicates the influence of this cytokine on the differentiation and active proliferation of cells of the functional layer of the uterine mucosa, and the activation of humoral regulation of intercellular connections and intersystemic cellular associations. According to M. Segura-Benítez *et al.* (2022), this finding details the peculiarities of intercellular matrix remodelling during the stages of the oestrous cycle and clarifies the optimisation of implantation conditions. There is no characteristic cell proliferation and differentiation in the postmorbid state, as evidenced by a decrease in TNFα. S. Warashina *et al.* (2022) note that this cytokine is a powerful stimulator of acute phase protein synthesis and, in the pathological process, even a slight increase in it causes mobilisation of energy reserves and stimulates the body's defence responses. At the same time, a significant increase in TNFα content in the case of reproductive dysfunction, according to H. Ragsdale *et al.* (2019), indicates its involvement in the regulation of reproductive processes, as high levels of TNFα activate the biosynthesis of oestrogens by cytotoxic and proliferative cell receptor types.

According to F. Almughlliq *et al.* (2019), this illustrates the disorganisation of the connective tissue matrix of the postmorbid endometrium, since anaphrodisia results in a significant decrease in the concentration of connective tissue markers – glycoproteins and glycosaminoglycans, which is a consequence of the decrease in secretory cells due to the induction of their apoptosis under conditions of TNF-α expression. It is known that IL-1 exerts its biological effect during intercellular contact through specific and non-specific receptors on the surface of target cells involved in the process of implantation and pregnancy. Several studies (Kuhla *et al.,* 2019; Koh *et al.,* 2020), that record an increase in IL-1 and TNFα levels during the corpus luteum, i.e., at the beginning of the inhibition stage, are contradictory to other studies (Abeysinghe *et al.,* 2023), who indicate a maximum increase in IL-1 in endometrial cells during the follicular phase compared to the luteal phase. These circumstances give rise to the need to detail these studies, as they do not reflect the overall concentration of cytokines in endometrial tissue.

In the present study, an increase in endometrial IL-1 was detected during hunting. This trend, according to researchers (Huang *et al.,* 2021), is likely due to the ability of this cytokine to stimulate the expression of release factor genes that enhance the production of gonadotropic hormones, followed by the induction of the synthesis of estrogens and other gonadal hormones, activation of the transformation of stromal fibroblasts and epithelial cells into decidual cells, which in general details the process of intercellular matrix remodelling and ensures nidulation.

Studies conducted by M. Lappas (2017), and H. Ragsdale *et al.* (2019) indicate that IL-1 stimulates the transformation of endometrial epithelial cells and stromal fibroblasts into decidual cells, the expression of releasing factor genes, and can affect homeostasis by increasing corticotropin-releasing factor, adrenocorticotropic hormone, and others. At the same time, N. Nijkang *et al.* (2019) point out that the release of hypothalamic monoamines and neuropeptides under the influence of endogenous IL-1 disrupts the synthesis of gonadotropic and sex hormones, thereby causing anaphrodisia.

Opposite to proinflammatory IL-1 and TNFα is IL-4, a pleiotropic cytokine produced by various immune cells during intercellular interaction and involved, on the one hand, in enhancing tissue alteration and destruction, and, on the other hand, in limiting the inflammation focus, increasing tissue barrier functions, activating regeneration, forming the body's defence response, and inhibiting proinflammatory cytokines. According to N. Nijkang *et al.* (2019) and N. Turner *et al.* (2022), it activates the attenuation of the immune response, and catalyses the endometrium's tolerance to the inflammatory process, thereby including the regeneration phase and restoration of the function of the maternal part of the placenta. The results of our research are consistent with the findings of other scientists.

In particular, as shown in studies (Taylor *et al.,* 2021), interleukin-4 is found in endometrial stromal phagocytes and actively regulates stem cell proliferation by inducing mitogen-activated protein kinases. The latter transmits an intracellular signal that causes cell differentiation, metabolic changes, and apoptosis. In addition, IL-4 stimulates luteal cells to increase progesterone. The findings of a significant decrease in IL-4 in endometrial tissue are consistent with the results of studies (Rasheed & Hamid, 2020) and may indicate a lack of cell proliferation and differentiation, and as a result, the inability of postmorbid endometrium to nidulation.

Individual studies (Sheldon *et al.,* 2020) have shown that IL-4 levels in physiological pregnancy decrease compared to pregnancies that are pathological and end in abortion. A significant increase in IL-4 levels indicates a violation of peripheral tolerance and a shift in the local immune response towards IL-1, which causes infertility. Assessment of serum cytokine levels (proinflammatory IL-1 and regulatory IL-4, which is an inducer of activation of the immune system cellular chain) reflects the dynamics of intercellular interaction and the nature of the pathological process (Salas-Huetos, *et al.,* 2020). According to J. Wisler *et al.* (2022), excessive levels of IL-1 destroy intercellular interactions, causing infertility, due to the excessive development of the endometrial inflammatory response during the stage of excitation. The results of studies obtained by M. Segura-Benítez *et al.* (2022) indicate that physiological zygote nidulation is possible under conditions of a balance between the immunomodulatory and immunosuppressive effects of the endometrium, in the implementation of which components of the cytokine system are directly involved.

M. Zargar *et al.* (2020) point to a specific balance between the anti-inflammatory components of the immune system and pro-inflammatory cytokines. It is this balance of inflammatory and anti-inflammatory cytokines that determines the course of inflammation, the development of possible complications, and organ and tissue dysfunctions. F. Almughlliq *et al.* (2019) proved that full endometrial regeneration is possible under conditions of a balanced ratio of IL-1, TNF-α and their antagonist IL-4, which play an important role in the formation of regenerative and repair reactions. In the case of their local imbalance, the regeneration mechanism acquires a pathophysiological shift and causes tissue remodelling defects. The index of the ratio of IL-1 to IL-4 in cows with endometritis and afterbirth retention was significantly lower compared to the index of proestrus.

The cytokine ratio of IL-1, TNF-α and IL-4 is an objective criterion for predicting the type of tissue regeneration. I. Sheldon *et al.* (2020), having studied the ratio of IL-1 and IL-4, proved that cytokine balance is a prognostic indicator in assessing the course of pregnancy. An

excessive increase in the concentration of IL-1 against a sharp decrease in IL-4 leads to abortion. Whereas, according to P. Abeysinghe *et al.* (2023), the correction of the above ratio towards a decrease in IL-1 concentration against the background of an increase in IL-4 ensures a physiological course of pregnancy. Thus, further research on a detailed understanding of the cellular and molecular mechanisms occurring in the bovine endometrium under physiological and pathological conditions is crucial to explain and avoid infertility in dairy cows.

CONCLUSIONS

During oestrus, the concentration of TNFα in the endometrium is as low as possible, amounting to 5.03±2.16 pg/ml, while during proestrus and corpus luteum, its level increases by almost 4.2-4.3 times (21.15±3.08 pg/ml and 21.63±4.69 pg/ml, $p<0.01$), respectively.

The concentration of TNF-α in endometrial homogenates of cows in the state of anaphrodisia is significantly higher in animals that have suffered from endometritis 2.6 times (13.07±1.98 pg/ml, $p<0.01$) and 3.6 times after afterbirth retention (18.21±3.99 pg/ml, $p<0.01$) compared to the indicator during oestrus. The level of IL-1 in cow endometrial homogenates increased during the oestrus period (8325.46±69.18 pg/ml), almost 2 times compared to the corpus luteum (4153.82±74.23 pg/ml) and proestrus (4092.57±476.05 pg/ml), $p<0.001$, respectively.

The concentration of IL-1 in the endometrium of animals in the state of anaphrodisia and suffering from endometritis and retention of afterbirth is 2.5 times lower than that of cows in heat, 2.5 times (3351.71±1422.42 pg/ml and 3412.43±430.12 pg/ml), $p<0.01$, respectively. The content of IL-4 in endometrial homogenates of cows during the heat reaches maximum values – 407.07±45.78 pg/ml, while during the oestrus it begins to show a tendency to decrease by almost 1.4 times (295.31±42.16 pg/ml), and during proestrus it undergoes a significant decrease by 3.4 times (118.56±32.34 pg/ml), $p<0.001$, respectively.

The concentration of IL-4 in endometrial homogenates of anaphroditic cows suffering from endometritis (121.03±52.36 pg/ml) and retention of afterbirth (129.58±58.28 pg/ml) undergoes a significant decrease compared to the indicator during the oestrous phase of the cycle by 3.4 and 3.2 times, $p<0.001$, respectively. The highest index of the ratio between IL-1 and IL-4 was found to be during proestrus – 34.53, while during oestrus it was 20.45, and during the corpus luteum, this ratio was 14.08, while in animals with endometritis and retention of the litter, it was 27.69 and 26.35, respectively, remaining significantly lower than the same index of proestrus. Thus, the prospect of further research is the need to use the data obtained to develop reasonable methods for correcting the sexual cyclicity of cows in the post morbid state.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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Уміст прозапальних та протизапальних цитокінів у ендометрії корів за анафродизії та естрального циклу

Ірина Вікторівна Бондаренко
Кандидат ветеринарних наук, доцент
Сумський національний аграрний університет
40021, вул. Г. Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0000-0002-1019-3446

Андрій Борисович Лазоренко
Кандидат ветеринарних наук, доцент
Сумський національний аграрний університет
40021, вул. Г. Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0000-0002-0916-3901

Юрій Володимирович Мусієнко
Кандидат ветеринарних наук, доцент
Сумський національний аграрний університет
40021, вул. Г. Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0000-0002-9735-4758

Олександр Сергійович Панасенко
Кандидат ветеринарних наук, доцент
Сумський національний аграрний університет
40021, вул. Г. Кондратьєва, 160, м. Суми, Україна
https://orcid.org/0000-0003-1257-6341

Анотація. Неплідність та порушення статевої циклічності в корів, є актуальною і поширеною проблемою в скотарстві, що завдає істотних збитків господарствам. Водночас, детальне розуміння клітинних і молекулярних механізмів у матці великої рогатої худоби має вирішальне значення для пояснення та уникнення неплідності у молочних корів. Метою досліджень було визначити концентрацію фактору некрозу пухлин (TNFα), интерлейкіну – 1 (IL-1) та интерлейкіну – 4 (IL-4) у ендометріальних гомогенатах за еструсу, проеструсу, розквіту жовтого тіла й анафродизії спричиненої прехворюванням на ендометрит, а також затримку посліду. Матеріалом для дослідження були гомогенати ендометрію корів, відібраного з рогів матки у верхній її третині. Використані методи визначення вмісту IL-1, IL-4 та TNFα у зразках ендометрію – імуноферментний твердофазний ELISA аналіз із урахуванням співвідношення (тканинний гомогенат-фосфатно-сольовий буфер). За результатами досліджень з'ясовано зміни умісту IL-4, IL-1 та TNF-α, у функціональному шарі ендометрія за анафродизії та окремих стадій естрального циклу. Встановлено істотне зростання вмісту TNF-α та зниження рівнів IL-1 і IL-4 у ендометрії відносно аналогічного показника в період еструсу. З'ясовано відсутність вірогідної різниці рівня IL-1 за проеструсу порівняно з розквітом жовтого тіла, як наслідок згасання в цей час, притаманної естральному ендометрію транзиторної флогогенної реакції, через цитокіновий баланс, що готує найкращі умови для імплантації зиготи. Встановлено, що рівень IL-1 за еструсу вірогідно збільшується, відносно стадії розквіту жовтого тіла та стадії проеструсу. Визначено, що концентрація IL-4 у функційному шарі ендометрія корів, які перехворіли на затримання посліду та ендометрит, є нижчою за аналогічний показник за еструсу, що підтверджує пригнічення проліферації й диференціації клітин. Отримані дані, можуть бути використані для оптимізації корекції статевої циклічності корів із анафродизією

Ключові слова: корови; анафродизія; статевий цикл; ендометрій; TNF-α; IL-1; IL-4

SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 44-51

UDC 638.124.428.144.54
DOI: 10.48077/scihor9.2023.44

Influence of the food protein on the development of hypopharyngeal glands, fat body, quality and lifespan of honeybees

Oleksandr Mishchenko
Head of the Laboratory
National Scientific Centre "Institute of beekeeping named after P.I. Prokopovich"
03143, 19 Akademika Zabolotnogo Str., Kyiv, Ukraine
https://orcid.org/0000-0001-9970-8540
Volodymyr Postoienko
Doctor of Agricultural Sciences, Professor
National Scientific Centre "Institute of beekeeping named after P.I. Prokopovich"
03143, 19 Akademika Zabolotnogo Str., Kyiv, Ukraine
https://orcid.org/0000-0002-6515-7004
Olesya Lytvynenko
PhD in Biological Sciences
National Scientific Centre "Institute of beekeeping named after P.I. Prokopovich"
03143, 19 Akademika Zabolotnogo Str., Kyiv, Ukraine
https://orcid.org/0000-0001-6643-2285
Alla Ivanyshyn*
PhD, Associate Professor
Lviv Polytechnic National University
79013, 12 Bandery Str., Lviv, Ukraine
https://orcid.org/0000-0002-3302-7889
Kristin Afara
National Scientific Centre "Institute of beekeeping named after P.I. Prokopovich"
03143, 19 Akademika Zabolotnogo Str., Kyiv, Ukraine
https://orcid.org/0000-0002-9180-2281

Article's History:
Received: 12.05.2023
Revised: 25.08.2023
Accepted: 27.09.2023

Abstract. Research on the anatomical and physiological characteristics of bees (the state of fat body, hypopharyngeal glands) in connection with changes in natural and climatic conditions (soil composition, prolonged droughts, prolonged rains, cold weather, environmental disasters) impoverishment of fodder base for bees due to the decrease of sown areas of honey crops leading to the use of bees' feeding, is relevant. The research aims to study the influence of food protein on the development of hypopharyngeal glands, fat body and life expectancy of honeybees. The zootechnical

Suggested Citation:
Mishchenko, O., Postoienko, V., Lytvynenko, O., Ivanyshyn, A., & Afara, K. (2023). Influence of the food protein on the development of hypopharyngeal glands, fat body, quality and lifespan of honeybees. *Scientific Horizons*, 26(9), 44-51. doi: 10.48077/scihor9.2023.44.

*Corresponding author

(the setting of the scientific research, the feeding of the bee families, the power of the bee families, productivity), ethological (orientation in the bee-entrance area), micrometrical (physiological and morphological characteristics of the parts of the bee's body), microscopic (analysis of hypopharyngeal glands and fat body of bees) and statistical (biometric data processing) methods were used in the study. The significant difference in their degree of development depending on the carbohydrate or protein feeding was identified. After feeding sugar syrup to bees, signs of protein malnutrition and the state of development of hypopharyngeal glands corresponding to the I-II degrees were noted. Alveoli of hypopharyngeal glands were reduced, and underdeveloped, with marked spaces, whereas after feeding protein food in the form of sugar syrup with bee pollen the bees had well-developed hypopharyngeal glands. The alveoli of the hypopharyngeal glands of the researched bees filled out, grew milky, with no space between them, and were able to produce larval food. Such a state of the glands' development corresponds to the IV degree of the development of hypopharyngeal glands. The research results indicate that the development of the fat body of bees happens at a young age when bees consume protein food the most. It has been proven that protein feeding of the bee colonies of experimental groups contributed to the better development of hypopharyngeal glands in both old and young bees, which resulted in better provision of food for larvae and more active development of the bee colonies. Protein feeding of bees in early spring under conditions of limited supply of protein food contributes to the production of larger larvae, which in turn contributes to the production of more complete bees

Keywords: Ukrainian steppe bee; protein feeding; bee larvae; the power of the bee colony; hypopharyngeal glands; alveoli of hypopharyngeal glands; fat body

INTRODUCTION

The creation of optimal conditions for the life of bee colonies requires various types of food for feeding. The search for the most effective food resources and their impact on bee productivity remains relevant. R. Brodschneider *et al.* (2019) and K. Khamid (2021) proved that increasing the number of bees in the colony in spring is of exceptional importance in preparing it for pollination of crops, using productive honey collections, as well as in autumn for increasing the number of young bees, which will have to overwinter and raise several generations of bees' next spring.

The life activity of the honeybee colony, particularly rearing of brood, successful overwintering, disease resistance, flight-collecting and pollinating activity depends to a large extent on the food stocks in the nest and the flow of the protein food – bee pollen. K. Ulutaş & A. Özkirim (2018), S. Ahmad *et al.* (2021), and M. Carroll *et al.* (2021) proved that for normal life activity, each bee colony needs a balanced diet, which should include a complex of proteins, fats, and carbohydrates, as well as mineral compounds and water. Honeybees use carbohydrates and lipids to replenish energy reserves and protein compounds – for growth and development; and minerals, vitamins and water are inseparable components of metabolic processes.

R. Pudasaini *et al.* (2020), M. Lee *et al.* (2019), and V. Corby-Harris *et al.* (2019) dealt with questions of dependence of the development and functional activity of hypopharyngeal glands on the age of bees and the availability of the protein food in the nest. There is a direct link between feeding, physiological state, and life expectancy of honeybees whereas the stocks of food are important (Lan *et al.,* 2021). The use of protein food has an impact on physiological development and contributes to the continuation of life of young bees. A statistically valid correlation between the life expectancy of bees and their physiological state, particularly close between the degree of development of the fat body and life expectancy, is present. M. Thakur & V. Nanda (2020), Li *et al.* (2019), and U. Toprak *et al.* (2020) noted that the presence of a sufficient amount of protein compounds increases the lifespan of worker bees, has a positive effect on the development of young bees and brood.

The study of lipid metabolism has received much attention for many years (Feregrino-perez *et al.,* 2018; Chen, 2018). The fat body develops quickly among the bees that take part in feeding the larvae, it is highly developed among larvae and also old queen bees. It is the most developed in the body of bee larva where its proportion corresponds up to 60% of the mass of the larvae. The research data (De Souza *et al.,* 2019; Almasri *et al.,* 2020; Frizzera *et al.,* 2020) indicates that although in the adult bee, the fat body occupies a relatively small part, this organ plays an important role in the physiological state of bees. The fat cells of summer and winter generations of bees are distinct in their structure. G. Galvani *et al.* (2019), M. Kunc *et al.* (2019), and A. Strachecka *et al.* (2021) have proven that in summer the fat cells form a thin and almost clear layer and among winter bees they create a thick yellow-white cover where cells, apart from fat droplets, also contain protein bodies. The main biochemical processes take place in the fat body as well as stockpiling of nitrogenous substances in the autumn period that define the life expectancy and safekeeping of bees during overwintering. The degree of development of the fat body is a reliable physiological indicator of the state of bees and their resistance. The metabolism in the body of bees is closely linked to the state of the fat body as one of the important vital organs of bees.

The studies on the impact of the protein feed on the development of bee colonies and their physiological state play an important part both in the conservation of honeybees and the increase in their productivity and are relevant for the beekeeping of Ukraine. The research aims to study the influence of the protein feed on the development of the hypopharyngeal glands, fat body and life expectancy of bees during the period of the low level of flow of bee pollen into the nest of the bee colony.

MATERIALS AND METHODS

This study is a part of the fundamental study and was conducted according to the plan of research work on the topic: "To study mechanisms and characteristics of the production of the bee wax and royal jelly by Ukrainian steppe breed and Carpathian breed of bees" (the number of the state registration 011U6001455) during 2016-2020.

The study was conducted on the experimental apiary of the National Scientific Centre "Institute of Beekeeping named after P.I. Prokopovych" on the standard bee colonies of the Ukrainian steppe breed (*Apis mellifera sossimai)*. The bee colonies met the standard of the Ukrainian steppe breed, as confirmed by the results of the exterior evaluation.

The entire bee colonies as well as separate individually marked bees of the Ukrainian steppe bees were the objects of research. The caring for the experimental bee colonies was equal and conducted using a generally accepted method (Brovarskyi *et al.,* 2017). The research was conducted under the provisions of the "Basic Ethical Principles for the Animal Experiments", adopted at the First National Bioethics Congress (Reznikov, 2003) and "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (1986).

The following groups of the bee colonies were formed: I group (control) – the bees of this group received feeding in the form of sugar syrup in the ratio 1:1 both in larval stage and after birth; II group – bees in larval stage received protein feeding (sugar syrup in the ratio 1:1 with addition of 15% bee pollen that was soaked in water for 6 hours), and after birth went on sugar syrup nutrition; III group – bees is larval stage received sugar syrup and after birth went on protein nutrition; IV group – bees received protein feeding both in larval stage and after birth.

Physiological characteristics were determined according to the degree of development of the fat body (on the Maurizio scale) and brood food (HP) glands (on the Hess scale) determined by 4 levels of their development. Fifty bees aged one day were placed in laboratory holding cages and were kept on the grid above the nest of the strong colony. Food and water were changed every two days. The holding cages were examined every day with an accounting of dead bees. To study the development of brood food (HP) glands, 10 bees from each holding cage were taken on the 10th, 20th, and 30th days. Hypopharyngeal glands were dissected and examined under a digital microscope and photographed afterwards. The studies were conducted with repeatability of two measurements. The mass of the newly born bees was determined on the torsion scale VT 500.

The amount of bee bread and sealed brood was determined with the help of a frame grid with the size of quadrants 5x5 cm. For the convenience of accounting for bee bread (given that it doesn't always occupy big solid areas) the frame grid was additionally divided into smaller quadrants 2.5x2.5 cm. Biometric data processing was conducted on a PC with the help of MS Excel software using built-in statistical functions.

RESULTS AND DISCUSSION

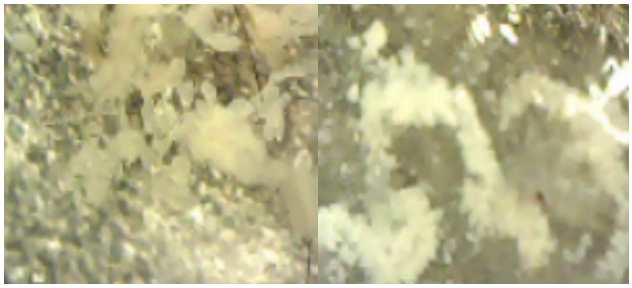
According to the results of the biometrical analysis of the level of development of hypopharyngeal glands of the bees from the research group colonies prevailed per degree of development of these glands over the bees of the control group on average by 0.86 points (P>0.01). Among young bees that were born in spring, the variance by the degree of development of hypopharyngeal glands was by 0.92 points more in the research group with probability P>0.01 (Table 1).

After examining the microscope of dissected hypopharyngeal glands of bees, authors identified the significant difference in the level of their development depending on feeding with carbohydrate or protein food (Fig. 1)

Table 1. *Physiological development of bees in research and control colonies*

Rates	Old bees		Young bees			
	The level of development of hypopharyngeal glands, points		The level of development of hypopharyngeal glands, points		Live body weight of bees, mg	
	Research	Control	Research	Control	Research	Control
n	100	100	100	100	80	80
Lim	2-5	1-4	2-4	1-4	78-116	70-100
X±m	3.4±0.10	2.6±0.15	3.1±0.14	2.1±0.13	93.2±1.12	90.8±1.10
Cv, %	21.4	39.95	26.88	36.35	9.55	9.42
P	≤0/01		≤0.01		≥0.05	

Source*: compiled by the authors*



Control group Studied group

Figure 1. *Hypopharyngeal glands of bees of control and research group*

The figure above shows that among the bees of a control group that received sugar syrup, there are signs of protein deficiency and the level of the development of hypopharyngeal glands meets the criteria of degree I-II. The Alveoli of hypopharyngeal glands are underdeveloped, with significant gaps and they remained clear throughout the whole research.

Quite a different situation was observed among bees of a research group that were fed with the protein mix in the form of sugar syrup with bee pollen both in the larval stage and after birth. They have sufficiently well-developed hypopharyngeal glands. The Alveoli of the hypopharyngeal glands of the researched bees became rounded and cloudy, no gaps were observed between the alveoli, and they had a milky white colour and were able to excrete brood food. Such a state of glands meets the criteria of IVth degree of development of hypopharyngeal glands of bees with sufficient stocks of protein feed in the nest. The fat body covers the internal organs and body walls of bees and contains fat, glycogen, and protein. In spring those substances flow into the brood food (HP) glands and are spent on producing the food for larvae. The data of research on the development of the fat body is provided in Table 2.

Table 2. *The dynamics of development of the fat body of bees*

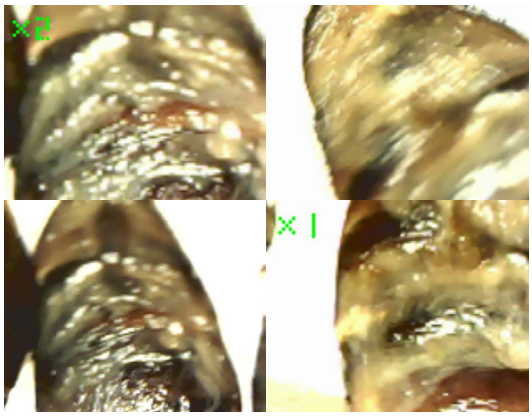
Bees' age, days	Research Group		Control group	Reliability of the differences, td
	M±m	% before the control	M±m	
1	1.2±0.03	86	1.5±0.04	4.0
3	1.7±0.05	85	2.1±0.05	4.1
6	2.1±0.05	78	2.5±0.05	7.5
12	2.1±0.06	95	2.2±0.02	1.0
18	2.0±0.05	94	2.1±0.05	1.4
24	2.1±0.06	100	2.1±0.82	0.0

Source*: compiled by the authors*

As seen from the data in the table, the highest rate of the development of the fat body among bees, 2.5 points, was observed at the age of 6 in the control group where the frames filled with protein food were not removed. At the same time, the degree of development of a fat body of the 6-day-old bees in the research group was 2.0 points. Statistical processing allowed to identify sufficiently stable accuracy of difference (td=7.5).

Among the bees older than 6 days the development of the fat body with the removal of the frames with protein food or without there was almost no difference. Such phenomena point to the fact that the development of this organ takes place at a young age when bees mostly consume protein food.

The dissected bee terga abdomen is presented in Figure 2.



Control group Studied group

Figure 2. *Fat body of bees of research and control group*

Source*: compiled by the authors*

Among the bees of the control group, the fat tissue has white rounded cells without visible inclusions, with visible chitin and meets the criteria of the I-st degree of development. Among the bees of the research group, the fat tissue is multi-layered, plicate, with yellow-filled inclusions and meets the criteria of III-IV degree of development. As a result of the study, the life expectancy of bees with protein feeding in the larval stage and after birth turned out to be significantly higher than among the bees of the control group that received the sugar syrup as extra-nutrition. The life expectancy of bees accounted for 47.09±1.72 days and 22.8±1.75 days in the research and control group respectively. The difference is statistically accurate (P<0.001), (Table 3).

Table 3. *The life expectancy of bees*

Groups	n	lim	M±m	Cv, %	P
I	100	1.0 – 33.0	22.80±1.75	44.1	-
II	100	1.0 – 41.0	27.67±1.61	37.2	0.001
III	100	1.0 – 70.0	37.00±2.65	38.4	0.001
IV	100	1.0 – 73.0	47.09±1.72	30.5	0.001

Source*: compiled by the authors*

The life expectancy of bees in the second group was significantly higher even though these bees received sugar syrup after birth but protein food in the larval stage. The bees of the third group had a relatively long lifespan – 37.00±2.65 days. As for the behaviour of bees, the bees from all groups behaved differently, the restless behaviour was observed among the bees with a short lifespan.

Life expectancy is a breed characteristic with seasonal variation and is directly proportional to the state of development of hypopharyngeal glands and fat body where the reserve nutrients are stored. Therefore, the study of the influence of alimentary factors on the life expectancy of bees is an important rate in their breeding. Studying the effect of feeding with pollen from 38 plant species on bees, M. Kunc *et al.* (2019) showed that pollen prolongs the life expectancy of bees by up to 100% compared to control, and improves some indicators of their physiological state. Ukrainian and foreign science and practice have a great deal of experience in the field of visual qualitative evaluation of the degree of development of the fat body of honeybees.

According to L. Bortoloţti *et al.* (2020), the fat body is an important metabolic organ and is decisive in the quality and activity of the course of physiological and biochemical processes in the body of honeybees. That is why the degree of fat body development is the main indicator of the biological state of the body of honeybees and affects their lifespan.

R. Balkanska *et al.* (2018) and B. Moumeh *et al.* (2020) proved that a sufficient amount of protein compounds increases the lifespan of worker bees, and has a positive effect on the development of young bees and brood. Scientific research dedicated to studies of the life of separate specimens and bee colonies as a whole demonstrates that during the overwintering with favourable conditions, the biological lifespan of bees is directly related to the presence of the reserve nutrients in their body. Meanwhile, what matters the most is the degree of development of the fat body that also determines the ability of bees to rear the brood intensely in early spring. That is why in spring they consume a large amount of protein food to provide the physiological development of the biological mode of the overwintering process and prepare the young spring generation to replace them.

F. Azzouz-Olden *et al.* (2018), I. Kumari & R. Kumar (2019) it has been proven that meeting the nutritional needs for protein compounds is critically important for the general condition of the bee colony, especially during the period of active development. The main area of accumulation of the reserve nutrients is the fat body of the bee. In its granules, the fatty-protein compounds appear where the reserve nutrients (proteins, fats, and carbohydrates) are stored and later used in the process of overwintering the bee colony. The fat body feeding only carbohydrate food remains relatively thin, with a limited amount of nutrients. With protein feeding it develops as multi-layered and contains large amounts of protein, fat, and glycogen. The fat body of bees serves as an area where extra amino acids are preserved which are accumulated in the active period and used under protein deficiency. In that regard, it can be concluded that the fat body is one of the most important elements in the process of metabolism and has an influence on the activity of all physiological processes. That is why the degree of the development of the fat body is one of the most important characteristics that defines the biological state of the body of a bee and its life expectancy. It is experimentally proven that the protein food in the form of a mixture of sugar syrup with bee pollen has a positive influence on the life expectancy of bees.

Thus, this research proved that protein feeding of bee colonies of the research groups contributed to the better development of hypopharyngeal glands both among old and young bees. This in turn contributed to the better release of brood food for larvae and more active development of the research group of colonies.

CONCLUSIONS

According to the conducted research, it was established that protein feeding of bees in early spring under the conditions of a limited supply of protein food contributes to the obtaining of larger larvae, which in turn contributes to the production of more complete bees.

During the study of the influence of protein food on the development of hypopharyngeal glands and fat body, when comparing the physiological state of bees, it was established that the hypopharyngeal glands and fat body of bees that consumed pure candy, that is, had only protein supplements, and where a deficiency of protein food was observed, had the lowest degree of development. In addition, it has been confirmed that the development of the fat body occurs at a young age when bees consume protein food.

The results of the study show that the average lifespan of bees with protein feeding in the larval stage and after birth was significantly longer. The average lifespan of bees with protein feeding in laboratory conditions was more than two times longer than the lifespan of bees with protein starvation. The rational feeding of bees is a decisive factor in their productivity. Meeting nutritional needs for proteins is an important condition for the general state of the bee colony, especially during the period of active development.

The studies of the influence of various factors including food, state of the colony, pesticides, and honeybee races that affect the physiology of bees are essential for the further investigation of the development and functionality of the hypopharyngeal glands and fat body of bees.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

None.

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Вплив протеїну корму на розвиток підглоткових залоз, жирового тіла, якість та тривалість життя медоносних бджіл

Олександр Антонович Міщенко
Завідувач кафедри
ННЦ «Інститут бджільництва імені П.І. Прокоповича»
03143, вул. Заболотного, 19, м. Київ, Україна
https://orcid.org/0000-0001-9970-8540

Володимир Олексійович Постоєнко
Доктор сільськогосподарських наук, професор
ННЦ «Інститут бджільництва імені П.І. Прокоповича»
03143, вул. Заболотного, 19, м. Київ, Україна
https://orcid.org/0000-0002-6515-7004

Олеся Миколаївна Литвиненко
Кандидат біологічних наук
ННЦ «Інститут бджільництва імені П.І. Прокоповича»
03143, вул. Заболотного, 19, м. Київ, Україна
https://orcid.org/0000-0001-6643-2285

Алла Василівна Іванишин
Кандидат технічних наук, доцент
Національний університет «Львівська політехніка»
79013, вул. С. Бандери, 12, м. Львів, Україна
https://orcid.org/0000-0002-3302-7889

Крістін Дарвішівна Афара
ННЦ «Інститут бджільництва імені П.І. Прокоповича»
03143, вул. Заболотного, 19, м. Київ, Україна
https://orcid.org/0000-0002-9180-2281

Анотація. Дослідження анатомо-фізіологічних особливостей бджіл (стан жирового тіла, підглоткових залоз) у зв'язку зі змінами природно-кліматичних умов (склад ґрунту, тривалі посухи, затяжні дощі, похолодання, екологічні катаклізми), збідненням кормової бази бджіл через зменшення посівних площ медоносних культур, що призводить до використання бджолами підгодівлі, є актуальним. Метою дослідження є вивчення впливу кормового протеїну на розвиток підглоткових залоз, жирового тіла та тривалість життя медоносних бджіл. У роботі використовували зоотехнічні (постановка наукових досліджень, годівля бджолиних сімей, сила бджолиних сімей, продуктивність), етологічні (орієнтування в зоні бджолосімей), мікрометричні (фізіолого-морфологічна характеристика частин тіла бджоли), мікроскопічні (аналіз підглоткових залоз і жирового тіла бджіл) та статистичні (біометрична обробка даних) методи дослідження. Виявлено достовірну різницю в ступені їх розвитку залежно від вуглеводної чи білкової годівлі. Після згодовування бджолам цукрового сиропу відмічали ознаки білкової недостатності та стан розвитку гіпофарингеальних залоз, що відповідає I-II ступеню. Альвеоли підглоткових залоз були зменшені, недорозвинені, з помітними проміжками, тоді як після згодовування білкового корму у вигляді цукрового сиропу з бджолиним пилком бджоли мали добре розвинені підглоткові залози. Альвеоли підглоткових залоз досліджуваних бджіл наповнилися, стали молочними, без проміжків між ними, і були здатні виробляти личинковий корм. Такий стан розвитку залоз відповідає IV ступеню розвитку підглоткових залоз. Результати досліджень свідчать, що розвиток жирового тіла бджіл відбувається в молодому віці, коли бджоли найбільше споживають білкового корму. Доведено, що білкова підгодівля бджолосімей дослідних груп сприяла кращому розвитку підглоткових залоз як у старих, так і у молодих бджіл, що призвело до кращого забезпечення личинок кормом і більш активного розвитку бджолосімей. Білкова підгодівля бджіл ранньою весною в умовах обмеженої пропозиції білкового корму сприяє отриманню більших личинок, що, в свою чергу, сприяє отриманню більш повноцінних бджіл

Ключові слова: українська степова бджола; білкова підгодівля; личинки бджіл; сила бджолиної сім'ї; підглоткові залози; альвеоли підглоткових залоз; жирове тіло

SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 52-60

UDC 636.084/.087
DOI: 10.48077/scihor9.2023.52

High yielding cows metabolism peculiarities under climate change conditions with the use of feed additive with protein protected from rumen degradation

Igor Sediuk*
PhD in Agricultural Sciences, Head of Laboratory
Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine
61026, 1-A Tvarynnykiv Str., Kharkiv, Ukraine
https://orcid.org/0000-0003-1765-2868
Galyna Prusova
PhD in Agricultural Sciences, Senior Research Fellow
Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine
61026, 1-A Tvarynnykiv Str., Kharkiv, Ukraine
https://orcid.org/0000-0002-2604-5720
Anatoliy Tkachov
Junior Research Fellow
Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine
61026, 1-A Tvarynnykiv Str., Kharkiv, Ukraine
https://orcid.org/0000-0002-6325-4724
Victoria Petrash
PhD in Agricultural Sciences, Senior Research Fellow
Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine
61026, 1-A Tvarynnykiv Str., Kharkiv, Ukraine
https://orcid.org/0000-0001-9114-6117
Iryna Tkachova
Doctor of Agricultural Sciences, Chief Researcher
Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine
61026, 1-A Tvarynnykiv Str., Kharkiv, Ukraine
https://orcid.org/0000-0002-4235-7257

Article's History:
Received: 25.04.2023
Revised: 25.08.2023
Accepted: 27.09.2023

Abstract. The research relevance is determined by the problem of thermal load on the body of dairy cows by increasing the content of protein protected from breakdown in the rumen. The research aims to determine the metabolism and level of delivery of various forms of protein and energy to the body of highly productive cows in the diets of cows in the second half of lactation under conditions of climate change. Research methods include zootechnical (assessment of feed intake, rationing, selection of

Suggested Citation:
Sediuk, I., Prusova, G., Tkachov, A., Petrash, V., & Tkachova, I. (2023). High yielding cows metabolism peculiarities under climate change conditions with the use of feed additive with protein protected from rumen degradation. *Scientific Horizons*, 26(9), 52-60. doi: 10.48077/scihor9.2023.52.

*Corresponding author

groups, organisation of the experiment, determination of milk production), biochemical (determination of the content of basic nutrients in feed, milk quality indicators), statistical, and mathematical. The compensation of protein in the diet of high-yielding cows under climate change conditions was carried out using the protein feed additive TEP-mix with protein protected from rumen breakdown. It has been proven that its inclusion in the cow's diet can increase average daily milk production by 9.1% in the cold season (0-10°C) and by 28.0% in the summer (21-36.4°C). In addition, the fat and protein content in milk increased in cows receiving the high-protein feed additive TEP mix. It was found that the increase in protein nutrition of cows from 23.18% to 33.53% with the use of the feed additive TEP-mix with protein protected from scar breakdown ensures a reduction in the impact of climate change on the metabolism of dairy cows. Thus, it has been proved that the use of specific protein feed sources that regulate the level of non-cutting protein in the diet can be considered a factor in combating heat stress and increasing milk production of dairy cows throughout the physiological cycle of lactation, including in extreme conditions of high temperatures

Keywords: metabolism; dairy cows; heat stress; protected protein

INTRODUCTION

Studies of the physiology of cow nutrition show that animals exposed to a significant excess of thermal stress have a negative nitrogen balance mainly due to a decrease in feed intake, which makes this study relevant. O. Borshch (2021) noted that nitrogen deficiency can be compensated for by increasing the protein content in the dry matter of the diet. At the same time, such an increase is significantly limited by the inability of rumen microorganisms to quickly process soluble crude protein that has been supplied with dietary components to the cow's rumen into microbial protein, and the resulting excess nitrogen leads to its absorption into the bloodstream, which negatively affects metabolism, dry matter intake and milk production. This means that the solution to increasing the intake of protein in the body of a dairy cow lies not in the plane of physical quantity, but rather in the plane of dietary protein quality and, in particular, its rumen solubility. M. Gauly & S. Ammer (2020), A. Iqbal *et al.* (2021) and P. Skliarov *et al.* (2022) pointed out that most modern protein nutrition systems when determining the protein requirement of animals, do not proceed from the content of crude and digestible protein in the diet, but from the amount of protein that is broken down in the small intestine and is defined as the sum of the breakdown and non-breakdown protein fractions in the rumen. This approach to protein nutrition for high-yielding cows is based on providing the animal body with readily soluble nitrogen compounds in feed protein and non-protein nitrogen sources based on the proteolysis of microbial protein and feed protein.

J. Garner *et al.* (2022) noted that rumen degradable protein is a source of nitrogen for microorganisms that use it to synthesise their protein amino acids, which provide 50% to 90% of cows' amino acid requirements after absorption in the small intestine. With high milk production, the synthesis of milk protein from microbial amino acids accounts for only 40-50% of milk protein, the rest must be provided by non-hydrolysed rumen protein. J. Meneses *et al.* (2021) considered that this was largely impossible to achieve by feed selection. Therefore, to protect protein from rumen degradation, feeds, especially high-protein feeds, are treated with various physical and chemical methods.

O. Borshch (2021) points out that at high and even average levels of cow milk production, protein synthesis by rumen microorganisms does not meet the animals' need for amino acids, while a simple increase in the protein content of feed (not considering protein breakdown) causes to excessive breakdown in the rumen and ultimately to its irrational use, while a high proportion of dietary proteins that are not broken down in the rumen but digested in the intestine improved the productivity of dairy cows even in adverse climatic conditions.

I. Suprun & Y. Kurylenko (2022) determined that determining the amino acid requirements for high-yielding cows in both the productive and dry periods requires in-depth knowledge of the protein composition of feed and its role in rumen fermentation. Q. Shan *et al.* (2020) found that during lactation, the mammary glands require a significant number of amino acids, the metabolism of which is a rather complex process, amino acids can be converted into other amino acids or used for energy through oxidation, but most of the amino acids absorbed from the blood are used by the mammary glands for milk protein synthesis. Scientists (Van den Bossche *et al.,* 2023) found that methionine intake improves milk production and antioxidant capacity, reduces lymphocyte apoptosis, promotes *Bcl-2* gene expression in lymphocytes and suppresses the *Bax* gene. Moreover, methionine perfusion has been shown to increase milk protein synthesis.

It is practically impossible to regulate the supply of methionine and lysine from the soluble part of crude protein, as it is impossible to control the quantity and quality of rumen microorganisms to increase the synthesis of individual amino acids. That is why the issue of amino acid nutrition of dairy cows has recently been addressed by adding special feed additives to the diet, which use protected methionine. However, as noted above, an increase in the level of protein in the diet of cows should be accompanied by an increase in the

intake of not only methionine but also other essential amino acids, because the use of protected methionine alone is not sufficient to improve the quality of protein nutrition. Given the research relevance, the research aims to determine the peculiarities of metabolism and delivery of various forms of protein and energy to the body in the diets of highly productive cows in the second half of lactation under different climatic conditions.

MATERIALS AND METHODS

The possibility of reducing the negative impact of high temperature on the metabolism and productivity of animals with the help of the protein feed additive TEP-mix (Arnika Feed LLC, Ukraine) with a protein protected from rumen cleavage was investigated in the conditions of Pechenizke LLC, Chuhuiv district, Kharkiv region. The scientific and economic experiment was conducted during 2022 (January-September) on high-yielding cows of the Ukrainian Red and White dairy breed, considering productivity, with an average live weight of 550-600 kg in the second, third and fourth lactations. Control and experimental groups of 70 cows were formed. During the experiment (243 days), the control group was fed a basic diet with the addition of sunflower meal, and the experimental group - a basic diet with the addition of TEP-mix. The diets of both groups were balanced so that the concentration of the main nutrients was as similar as possible, with a difference of 1% to 3%. The difference was in the use of a traditional protein supplement in the control group – sunflower meal with a low degree of protein protection (15.2%) against rumen degradation, and in the experimental group – a high-energy protein supplement TEP mix with a high degree of protein protection (65.3%).

Before the experiment, feed samples were taken to determine their chemical composition and nutritional value in the laboratory for assessing the quality of feed and animal products of the Institute of Animal Husbandry of the National Academy of Agrarian Sciences of Ukraine. Considering the chemical composition and nutritional value of the feed, the main diet of the experimental animals of both groups was equally complete and balanced in terms of nutrition, the amount of organic and mineral substances and had no significant differences, except for the quality of protein nutrition, in particular, the level and ratio of protected protein, which was 10.35% higher than in the control group.

The diet of the control group of cows in the second half of lactation included 8.0 kg of corn silage, 12.0 kg of triticale + oats haylage, 1.0 kg of pea straw, 5 kg of beer pellet, and 7.6 kg of mixed fodder. This diet contained 17.66 kg of DM, 186.7 MJ of ME, and 2841 g of CP, including 2182 g of DM and 658 g of NDF, or 23.18% of the total crude protein. The diet of the experimental group of cows included 8.0 kg of corn silage, 12.0 kg of triticale + oats haylage, 1.0 kg of pea straw, 5 kg of beer pellets, 1.7 kg of TEP-mix, and 5.8 kg of mixed fodder (1.6 kg of sunflower meal was replaced by a high-energy protein supplement TEP-mix in the amount of 1.7 kg). The diet of the experimental group contained 17.50 kg of DM, 185.5 MJ of ME, 2836 g of CP, including 1885 g of DM and 951 g of NDF, or 33.53% of the total crude protein.

The daily feed intake of the experimental animals during the experimental feeding was almost complete – the residues were only 1 to 3% of the target. Housing conditions, feeding, and watering regimes, and microclimate parameters were the same between the groups of experimental animals. The cows were kept in a typical four-row barn in a tethered manner. Milking was carried out twice a day using a milk pipeline, and control milking was carried out using portable buckets and a milk meter. Feed distribution was carried out by mobile feeders, and manure removal was carried out by scraper conveyors. Watering was carried out by automatic drinkers. Cows were fed with complete feed mixtures twice a day. The research plan is shown in Table 1.

Table 1. *Research plan*

Research stage	Research period, days	Air temperature, °C	Group	Amount heads	Feeding conditions
I	83	Up to +10	Control	70	Basic ration (BR) + sunflower meal (NRP-23.18%)
			Studied	70	Basic ration (BR) + TEP-mix supplement (NRP-33.53%)
II	60	From +20 to +30	Control	70	OP + sunflower meal (NRP-23.18%)
			Studied	70	OR + TEP-mix additive (NRP-33.53%)
III	100	More than +30	Control	15	OP + sunflower meal (NRP-23.18%)
			Studied	15	OR + TEP-mix additive (NRP-33.53%)

Source: *compiled by the authors*

During the experiment, the following parameters were determined: actual chemical composition and nutritional value of feed; actual feed consumption – daily, by conducting control feeding during two adjacent

days, to determine the difference between the specified amount of feed and its residues in the context of each group; level of milk production of cows – monthly, by conducting control milking with subsequent selection of average milk samples to determine its quality. Milk was analysed for chemical composition, nutritional and energy value, and physical and technological properties using a Bentley-150 device, and the content of somatic cells in milk was determined using a Somacaunt 150 device using the flow cytometry method.

The economic efficiency of milk production with the use of various feed additives with protected protein in cattle diets was calculated by the difference between the cost of feed and sold products. The statistical processing of the research results was carried out using biometric methods to determine the level of probability of the difference (Baranovsky *et al.,* 2017).

All experimental studies were carried out by modern methodological approaches and in compliance with relevant requirements and standards, in particular, they meet the requirements of DSTU ISO/IEC 17025:2005 (2006). The animals were kept, and all manipulations were carried out by the Order of the Ministry of Health of Ukraine No. 416/20729 "On Approval of the Procedure for Conducting Animal Experiments in Research 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

It was found that an increase in the non-degradable protein fraction in cow diets by 10.35% in January-March at an average air temperature of +7°C increased the average milk yield of cows by 9.1% (Table 2). Thus, at the beginning of the experiment, cows in the control group had an average milk yield of 19.6 kg of milk (with an average fat content of 3.93% and protein content of 2.98%), and in the experimental group – 19.6 kg of milk (with an average fat content of 3.93% and protein content of 3.01%). This means that there was no difference between the average milk yield and fat in the experimental and control groups and minimal and unreliable difference in protein. During the 83 days of the first stage of the experiment, the daily productivity of cows in the control group increased by only 0.2 kg. In the experimental group, the daily productivity of cows increased by 1.80 kg (9.1%). At the same time, there was a tendency to increase both protein and fat in milk.

Table 2. *Dynamics of milk production of experimental cows at the first stage of the experiment*

Indicator	Actual air temperature 0°C – +10°C		
	control	studied	+/- relative to control
Cows in the group, heads	70	70	-
Research length, days	83	83	-
Average daily natural milk yield at the beginning of the period, kg	19.6±0.56	19.8±0.47	+0.2
Average daily natural milk yield at the end of the period, kg	19.8±0.51	21.6±0.51	+1.8
Average mass fraction of fat in milk at the beginning of the period, %	3.93±0.009	3.93±0.008	0
Average mass fraction of fat in milk at the end of the period, %	4.07±0.006	4.14±0.008	+0.07
Average mass fraction of protein in milk at the beginning of the period, %	2.98±0.005	3.01±0.007	+0.03
Average mass fraction of protein in milk at the end of the period, %	3.22±0.003	3.27±0.006	+0.05

Source: *compiled by the authors*

In the second stage of the study, from 26 March to 26 May (60 days), with a gradual increase in the average air temperature to +21°C, the effect of experimental feeding became even more significant. Feeding a diet with a 10.35% higher degree of protection against protein breakdown in the rumen was found to increase the milk yield of each cow in the experimental group by another 1.5 kg of milk, while in the control group, this figure decreased by 1.1 kg. On average, during the second stage of the study, the cows of the experimental group produced 4.4 kg more milk, or 23.5% more (Table 3).

Table 3. *Dynamics of milk production of experimental cows at the second stage of the experiment*

Indicators	Actual air temperature +11°C – +20°C		
	control	studied	+/- relative to control
Cows in the group, heads	70	70	-
Research length, days	60	60	-

Table 3, Continued

Indicators	Actual air temperature +11°C – +20°C		
	control	studied	+/- relative to control
Average daily natural milk yield at the beginning of the period, kg	19.8±0.51	21.6±0.51	+1.4
Average daily natural milk yield at the end of the period, kg	18.7±0.40	23.1±0.52	+4.4
Average mass fraction of fat in milk at the beginning of the period, %	4.07±0.006	4.14±0.008	+0.07
Average mass fraction of fat in milk at the end of the period, %	4.12±0.003	4.19±0.008	+0.07
Average mass fraction of protein in milk t the beginning of the period, %	3.22±0.003	3.27±0.006	+0.05
Average mass fraction of protein in milk at the end of the period, %	3.24±0.003	3.29±0.006	+0.05

Source: *compiled by the authors*

The productive effect of the experimental feeding was even more pronounced at the third stage of the research when the air temperature rose to +24.5°C – +36.4°C. Thus, within 60 days of the experimental stage, the average milk yield of each cow increased by 1.3 kg of natural milk, while in the control group, this figure decreased by 2.3 kg (Fig. 1). The amount of fat and protein in the milk of cows in the experimental group also increased by 0.14% and 0.06%, respectively, while in the control group, the fat content in milk increased by only 0.08% and the protein content decreased by 0.03% (Figs. 2-3). Thus, the productivity of cows in terms of basic fat content in the experimental group increased by 2.64 kg, and in the control group – decreased by 2.2 kg (Fig. 4).

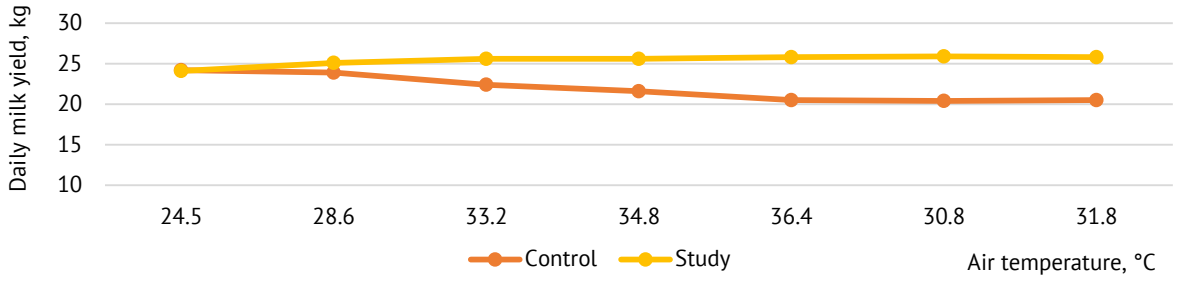

Figure 1. *Average daily milk yield of natural milk*

Source: *compiled by the author*

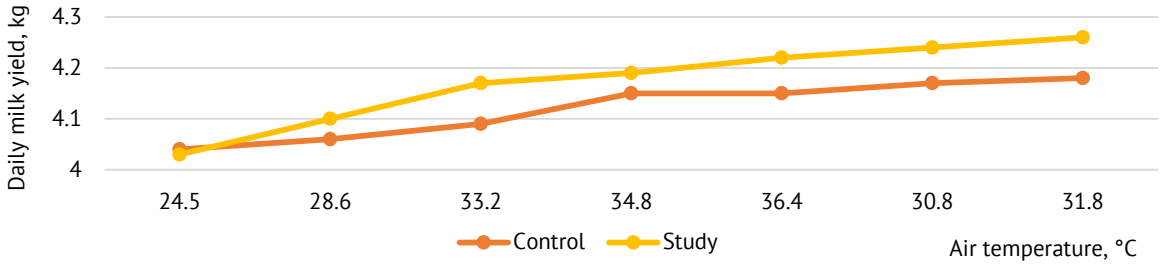

Figure 2. *Mass fraction of fat in milk, %*

Source: *compiled by the author*

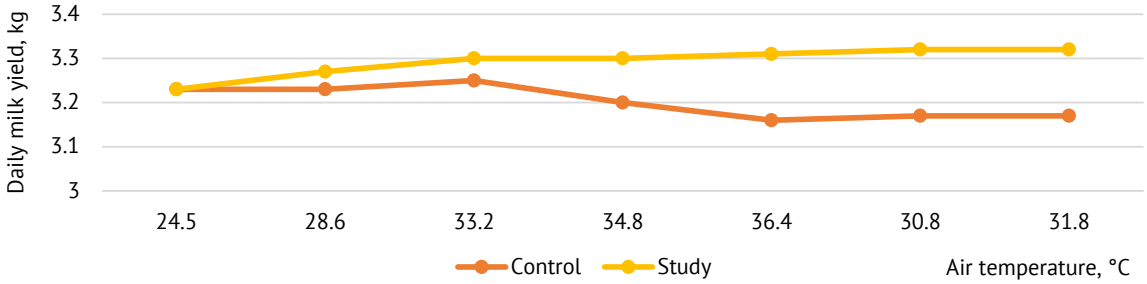

Figure 3. *Mass fraction of protein in milk, %*

Source: *compiled by the author*

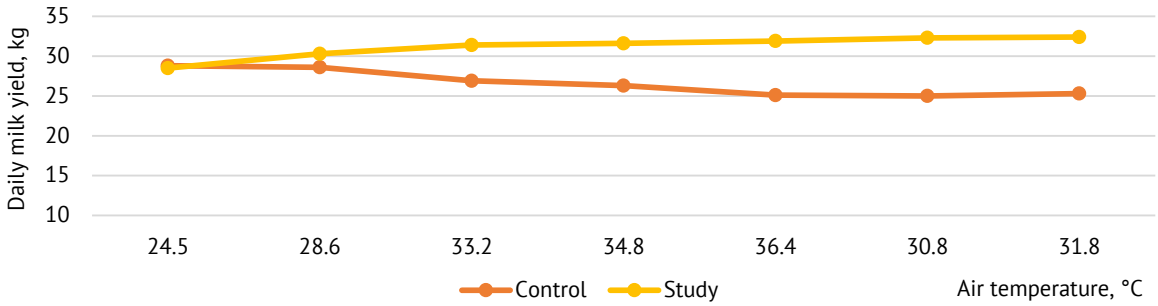

Figure 4. *Average daily milk yield of basic fat content*

Source: *compiled by the author*

The analysis of the results of the study by groups of cows shows that the amplitude of milk yield fluctuations in the control group animals was 9.2 kg (max-min), while in the experimental group animals it decreased to 6.0 kg. The amplitude of fat and protein fluctuations in the groups was almost the same. The obtained results prove that cows respond positively and significantly to changes in protein nutrition by increasing the level of non-degradable protein in the diet by adding TEP-mix to the basic diet.

The high difference in productivity between the control and experimental groups at the third stage of the study proved that the degree of resistance to heat stress also increases with increasing temperature. This fact can be explained by the stabilisation of metabolism in the body in general and a decrease in the intensity of protein fermentation in the rumen. During the stressful period of high ambient temperature, the organism of the experimental animals was better supplied not only with protein but also with energy. This is caused by the use of less energy for the digestion of non-digestible protein and, in addition, an increase in the flow of amino acids absorbed into the intestine contributes to the use of their share for energy purposes. That is why the energy supply of cows during heat stress with the use of TEP-mix in the diet was more significant, aimed at the synthesis of additional milk. At the same time, the increase in the amount of milk was not accompanied by losses in its quality (in terms of fat and protein mass fraction). Thus, it can be argued that the use of specific protein feed sources that regulate the level of non-degradable protein in the diet can be considered a factor in counteracting heat stress and increasing milk production of dairy cows throughout the physiological cycle of lactation, including in extreme conditions of high temperatures.

Stress caused in the body of ruminants by changes in climatic conditions hurts the general condition of the organism and, accordingly, the level of milk production and reproductive function. The solution to the problem of reducing the impact of heat stress on the body of animals requires the search for effective feed factors that can slow down the production of heat energy in the body. Thus, S. Gao *et al.* (2017) proved the effectiveness of using protein supplements of animal origin with a higher level of rumen-degradable protein fraction in the rearing of dairy calves. S. Salo (2018) confirms the importance of optimising both rumen-degradable and non-degradable protein content and synchronising protein with energy, which affects cow reproduction rates and environmental emissions. G. Conte *et al.* (2018) highlighted that feeding protected proteins reduces metabolic heat production and provides the right nutrient profile for high-yielding cows in early lactation.

Using experiments on dairy cows in climate-controlled chambers, S. Williams *et al.* (2021) showed that adding ingredients to the diet of lactating cows that increase energy density and reduce internal heat production can reduce the negative effects of hot weather on milk production. In recent years, an increasing number of studies have been devoted to the testing of feeds and feed additives with rumen-protected substances (Bypass products) in ruminant feeding, and they confirm our hypothesis that such products reduce the negative effects of heat stress on the animal body and contribute to increased milk production in the face of climate change.

For example, M. Lata & B. Mondal (2021) show that the use of feed additives with protected protein in feeding medium and high-performance dairy cattle increases dry matter intake and milk production by 10-15%, reduces milk cost and contributes to better live weight gain in young animals. J. Meneses *et al.* (2021) point out that the use of a rather expensive substance, protein, in the diet of dairy cows requires the calculation of the optimal ratio of degradable and non-degradable protein in the rumen. Based on the results of their research, they proposed the following ratio: 60% degradable protein and 40% non-degradable protein. They also suggest the use of supplements in the diet to improve rumen fermentation and digestibility.

Based on experiments on rationing cow diets with feeds with different rates of rumen decomposition, A. Shah *et al.* (2020) and J. Garner *et al.* (2022) argue that feeds with slowly decomposing starch, which reduces the amount of heat generated during digestion, reduce

the impact of heat stress. The most recent data on the use of Byrass products in dairy cow diets under heat stress was published by P. Roskopf *et al.* (2023). In their experiments on cows in mid-lactation, the replacement of fermentable energy (corn grain) in the rumen with non-fermentable energy (protected fat) improved milk yield and milk fat content, while the respiratory rate and body temperature of cows did not change. The authors emphasise the need to clarify the mechanism of increasing milk production in cows by using protected fat in feeding without improving heat balance under heat stress.

In the course of scientific and production testing, the high efficiency of using the high-protein additive TEP-Mix in dairy cattle feeding under conditions of climate change, in particular, critical air temperatures, has been proven, which has resulted in an increase in average daily milk production, fat and protein content in the milk of experimental cows in both cold and warm seasons.

CONCLUSIONS

The use of specific protein feed sources that regulate the level of non-degradable protein in the diet can be considered as a factor in counteracting heat stress and increasing milk production of dairy cows throughout the physiological cycle of lactation, including in extreme conditions of high temperatures. It has been proven that the inclusion of high-protein feed additives with a high degree of protection against rumen breakdown in the diet of cows can increase average daily milk production by 9.1% in the cold season (0-10°C) and by 28.0% in the summer (21-36.4°C). In addition, cows treated with the high-protein feed additive TEP-mix increased the fat and protein content in milk.

It has been determined that an increase of protein protected from rumen decomposition by 10.35% in the feed ration of dairy cattle helps to reduce the effect of heat stress on the animal body, which is reflected in an increase in milk production and improvement of milk quality. The high difference in productivity between the control and experimental groups at the third stage of the study proved that the degree of resistance to heat stress also increases with increasing temperature. This fact can be explained by the stabilisation of metabolism in the body in general and a decrease in the intensity of protein fermentation in the rumen.

During periods of high ambient temperature, when the body is exposed to heat stress, experimental animals fed a high-protein feed additive with a high degree of protection against rumen breakdown were more supplied with protein and energy. This is caused by lower energy consumption for digestion of non-digestible protein, and an increase in the flow of amino acids absorbed into the intestine helps to use a portion of them for energy purposes. That is why the energy supply of cows during heat stress with the use of TEP-mix in the diet was more significant and aimed at the synthesis of additional milk. The prospect of further research is to test feed additives with protected protein against a wide range of climatic indicators in different climatic zones of Ukraine, under different conditions of keeping dairy cattle of different breeds, and different levels of milk production throughout lactation.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

None.

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Особливості метаболізму високопродуктивних корів в умовах кліматичних змін за використання кормової добавки із захищеним від розщеплення в рубці протеїном

Ігор Євгенович Седюк
Кандидат сільськогосподарських наук, завідувач лабораторії
Інститут тваринництва Національної академії аграрних наук України
61026, вул. Тваринників, 1-А, м. Харків, Україна
https://orcid.org/0000-0003-1765-2868

Галина Леонідівна Прусова
Кандидат сільськогосподарських наук, старший науковий співробітник
Інститут тваринництва Національної академії аграрних наук України
61026, вул. Тваринників, 1-А, м. Харків, Україна
https://orcid.org/0000-0002-2604-5720

Анатолій Валерійович Ткачов
Молодший науковий співробітник
Інститут тваринництва Національної академії аграрних наук України
61026, вул. Тваринників, 1-А, м. Харків, Україна
https://orcid.org/0000-0002-6325-4724

Вікторія Станіславівна Петраш
Кандидат сільськогосподарських наук, провідний науковий співробітник
Інститут тваринництва Національної академії аграрних наук України
61026, вул. Тваринників, 1-А, м. Харків, Україна
https://orcid.org/0000-0001-9114-6117

Ірина Володимирівна Ткачова
Доктор сільськогосподарських наук, головний науковий співробітник
Інститут тваринництва Національної академії аграрних наук України
61026, вул. Тваринників, 1-А, м. Харків, Україна
https://orcid.org/0000-0002-4235-7257

Анотація. Актуальність досліджень полягає у вирішенні проблеми термічного навантаження на організм молочних корів за рахунок збільшення вмісту захищеного від розщеплення в рубці протеїну. Мета роботи – визначити метаболізм та рівень доставки в організм високопродуктивних корів різних форм протеїну та енергії в раціонах корів другої половини лактації в умовах кліматичних змін. Методи дослідження – зоотехнічні (оцінка споживання кормів, складання раціонів, підбір груп, організація досліду, визначення молочної продуктивності), біохімічні (визначення вмісту основних поживних речовин у кормах, показників якості молока), статистичні, математичні. Компенсацію протеїну у раціоні високопродуктивних корів в умовах кліматичних змін здійснювали за допомогою білкової кормової добавки ТЕП-мікс із захищеним від розщеплення в рубці протеїном. Доведено, що включення її до раціону корів дає змогу збільшити середньодобову молочну продуктивність на 9,1 % у холодний період року (0-10 °С) та на 28,0 % влітку (21-36,4 °С). Також у корів, що отримували високобілкову кормову добавку ТЕП-мікс, підвищувався вміст жиру і білка у молоці. Встановлено, що підвищення протеїнового живлення корів з 23,18 % до 33,53 % за використання кормової добавки ТЕП-мікс із захищеним від рубцевого розщеплення протеїном, забезпечує зменшення впливу кліматичних змін на метаболізм організму молочних корів. Таким чином, доведено, що використання специфічних білкових кормових джерел, що регулюють рівень нерозщеплюваного протеїну в раціоні, можна розглядати як фактор протидії тепловому стресу і підвищення молочної продуктивності дійних корів впродовж усього фізіологічного циклу лактації, у тому числі і в екстремальних умовах підвищених температур

Ключові слова: метаболізм; молочні корови; тепловий стрес; захищений протеїн

SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 61-69

UDC 639.517.043.2
DOI: 10.48077/scihor9.2023.61

Aquaculture indicators of young *Cherax Quadricarinatus* under various feeding plans

Valeriia Zharchynska*
Postgraduate Student
Bila Tserkva National Agrarian University
09111, 3a Heroes of Chernobyl Str., Bila Tserkva, Ukraine
https://orcid.org/0000-0002-5823-9095
Nataliia Hrynevych
Doctor of Veterinary Sciences, Professor
Bila Tserkva National Agrarian University
09111, 3a Heroes of Chernobyl Str., Bila Tserkva, Ukraine
https://orcid.org/0000-0001-7430-9498

Article's History:
Received: 30.04.2023
Revised: 20.08.2023
Accepted: 27.09.2023

Abstract. Aquaculture of crustaceans is one of the main sources of valuable food protein, which cannot be replaced by other animal or vegetable proteins due to its properties. The use of nutritious and balanced feeds in feeding crayfish helps to increase productivity and at the same time obtain ecologically clean and safe products. The research aims to determine the effect of feeding the developed Decapoda fodder. It has been established that feeding Decapoda food contributes to a more intense increase in the size of crayfish. In particular, the increase in length was 1.1±0.1 cm for the period from 90 to 120 days of cultivation and 1.2±0.1 cm for the period from 120 to 150 days. When using Decapoda food, the increase in length of crayfish during the first month of rearing was 1.8 times greater than that of crayfish fed Ancistrus menu. In the growing period from 120 to 150 days, the increase in the length of crayfish in the experimental group was 2.0 times greater, compared with crayfish in the control group for the same period. When crayfish were fed with Decapoda, the intensity of body weight gain was, on average, 1.4 times stronger, compared to control group crabs. At the same time, the survival rate in this group was 74%, which is a 20% higher number of live specimens compared to the control group of crayfish. The results of the study can be used in the development of a technological scheme for the reproduction and cultivation of crayfish

Keywords: Ancistrus menu; aquaculture; Decapoda food; length gain; survival; weight

Suggested Citation:
Zharchynska, V., & Hrynevych, N. (2023). Aquaculture indicators of young *Cherax Quadricarinatus* under various feeding plans. *Scientific Horizons*, 26(9), 61-69. doi: 10.48077/scihor9.2023.61.

*Corresponding author

INTRODUCTION

Aquaculture of crustaceans is one of the main sources of valuable food protein, which cannot be replaced by other animal or vegetable proteins due to its properties. Research on the use of fodder of various origins for economically profitable and intensive reproduction and cultivation of *Cherax quadricarinatus* is relevant.

Y. Cheng & S. Wu (2019), and D. Rigg *et al.* (2020), note that, following dietary norms, a person should consume from 60 to 120 g of complete protein daily. M. Kukhtyn *et al.* (2020), in their studies, note that crustacean aquaculture is a necessary source of valuable food protein, which cannot be replaced by other animal or vegetable proteins due to its properties. According to C. Jones and C. Valverde (2020), over the past twenty years, Australian red-clawed crayfish have been introduced to many countries and successful aquaculture sectors of this species have been created.

K. Crandall and S. Grave (2017) and Y. Sun *et al.* (2023) note that *Cherax quadricarinatus* is an optimal species for growing in aquaculture for several reasons. Scientists note its advantages, in particular, due to its relatively simple life cycle, unpretentiousness to the conditions of keeping, high resistance, and ability to consume a wide range of fodder. The results of a study (Shehata *et al.,* 2020), which examined the digestibility of different feeds in freshwater crayfish, show that Australian red claw crayfish digest plant ingredients more efficiently than animal ingredients.

Evaluating the influence of nutritional requirements, in particular protein, on spawning rate and quality of roe (Shehata *et al.,* 2022) found that the recommended content of crude protein in the feed ration for sexually mature females of *Cherax quadricarinatus* is at least 32%. It was also established (Shehata *et al.,* 2022) that the fatty acid composition of the hepatopancreas and muscles of *Cherax quadricarinatus* is significantly influenced by dietary supplements used for feeding, the content of vitamin E, Haematococcus pluvialis, and yeast extract. According to researchers (Díaz *et al.,* 2017), feed digestibility in *Cherax quadricarinatus* depends on many factors, including housing conditions, sex, body size, type of feed, season, and stage of development.

S. Shyamal *et al.* (2018) and Y. Sun *et al.* (2023) investigated the gender difference has a significant effect due to the difference in energy used for reproduction; females may invest 20-25% of their pre-breeding mass in gonads, while males invest only 3-9%. Several scientists (Nanda *et al.,* 2021; Vecchioni *et al.,* 2022), note that the composition of feeds used in the process of growing crustaceans includes such components as attractants, enzyme lysates (hydrolysates), growth stimulants, protectors from toxins, lipids, vitamins, amino acid preparations, minerals, pigments, antioxidants. In the feeding process, balanced feed mixtures are used (in terms of essential fatty acids, vitamins, and essential minerals).

P. Haubrock *et al.* (2021) and S. Hou *et al.* (2021) determined that with age, the need for red crab proteins decreases. Young need 31 to 34%, and individuals weighing more than 50 g need 25.6%. Lipids are also an important component of the diet that affects the growth, development, and health of crayfish. Unlike protein, the need for lipids does not change with age. Carbohydrates perform an energy function, participate in the formation of steroids and fatty acids, and also contribute to glycogen accumulation and chitin synthesis.

Y. Méndez-Martínez *et al.* (2021) prove that, as a delicacy, decapod crustaceans are traditionally valued on the domestic and world markets. N. Hrynevych *et al.* (2022) claim that intensive cultivation of Australian crayfish, Rosenberg's shrimp *Macrobrachium rosenbergii* in artificially created conditions is a promising direction of aquaculture development, which is at the stage of development in Ukraine.

Therefore, considering the value of the meat of Australian crayfish, a promising direction of aquaculture research is the development of feed mixtures, the consumption of which contributed to the maximum conversion of fodder. Furthermore, it increased the biological value of the obtained meat. The research aims to determine the effect of feeding the developed feed Decapodafood on crayfish in aquaculture.

MATERIALS AND METHODS

The research was carried out in the educational-scientific aquarium-basin complex of the Department of Ichthyology and Zoology of the Bila Tserkva National Agrarian University (Fig. 1). The object was the grown juveniles of the Australian red-clawed crayfish *Cherax quadricarinatus* (Von Martens, 1868) at the age of 90 days after hatching. The individuals were placed in three identical aquariums, technically equipped (aquarium pump Atman AT-203; filter element; thermoregulator Atman AT (300W); water thermometer Hailea HL-02F; shelter), with a volume of 200 dm^3. The experiment was conducted for 60 days at a planting density of 50 specimens/m^2. The water temperature was in the range of 27-28°C. The main hydrochemical indicators met the requirements for keeping and growing decapod crayfish (Decapoda) (Hrynevych *et al.,* 2022). Duration of the experiment – 60 days.

Figure 1. *Juveniles of the Australian red-clawed crayfish Cherax quadricarinatus in the aquarium of the Department of Ichthyology and Zoology*

For the experiment, 3 groups of crayfish were formed. Fifty individuals were planted in each experimental aquarium. Firstly, crayfish were fed the aquarium *Ancistrus menu* (control). The nutritional value per 100 g of feed is shown in Table 1, the composition of the second *Decapodafood* feed developed by authors is given in Table 2, the third contains *Ancistrus menu* feed and *Decapodafood* in a ratio of 50:50. The nutritional value per 100 g of *Ancistrus menu* fodder is presented in Table 1. The nutritional value per 100 g of *Decapodafood* is presented in Table 2.

Table 1. *Nutritional value per 100 g of Ancistrus menu fodder*

Indicator	Quantity, g
Raw protein	21.0
Raw fat	5.0
Cellulose	10.0
Ash (ash substances)	13.0
Calcium	3.0
Phosphorus	0.70

Source: *compiled by the authors*

Table 2. *Nutritional value per 100 g of Decapodafood*

Indicator	Quantity, g
White	37.0
Fats	23.5
Cellulose	10.0
Calcium	4.5
Phosphorus	0.8

Source: *compiled by the authors*

Crayfish were fed twice a day, at 08:00 and 20:00. Feed rationing was carried out depending on water temperature, average weight of individuals and total biomass in the tank. Before the experiment, each individual was weighed, and the total body length was measured. The obtained results were subjected to statistical processing using Statistica 10⩽ Edition programs. The difference was considered probable at $p \leqslant 0.05$.

The Ethical Committee approved the use of animals in this study of the Bila Tserkva National Agrarian University on the treatment of animals in research and the educational process (protocol No. 9 of October 1, 2020) following the Law of Ukraine "On the Protection of Animals from Cruelty" (2006) and Directive 2010/63/EC of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (2010).

RESULTS AND DISCUSSION

The obtained data confirm the importance of different types of feed for the feeding of Australian red-clawed crayfish. Figures 2-4 show the main fish breeding indicators of young Australian red-clawed crayfish *Cherax quadricarinatus* under the condition of feeding different fodder during 60 days of rearing. It was established (Fig. 2) that when feeding crayfish with control aquarium

food (Ancistrus menu) from 90 to 120 days, the length of crayfish increased to 6.8±0.2 cm, i.e., by 0.6 cm on average. During the next 30 days during cultivation, the length of crayfish increased to 7.4±0.2 cm, however, in the total increase, the increase in length was, as in the previous month, 0.6±0.1 cm.

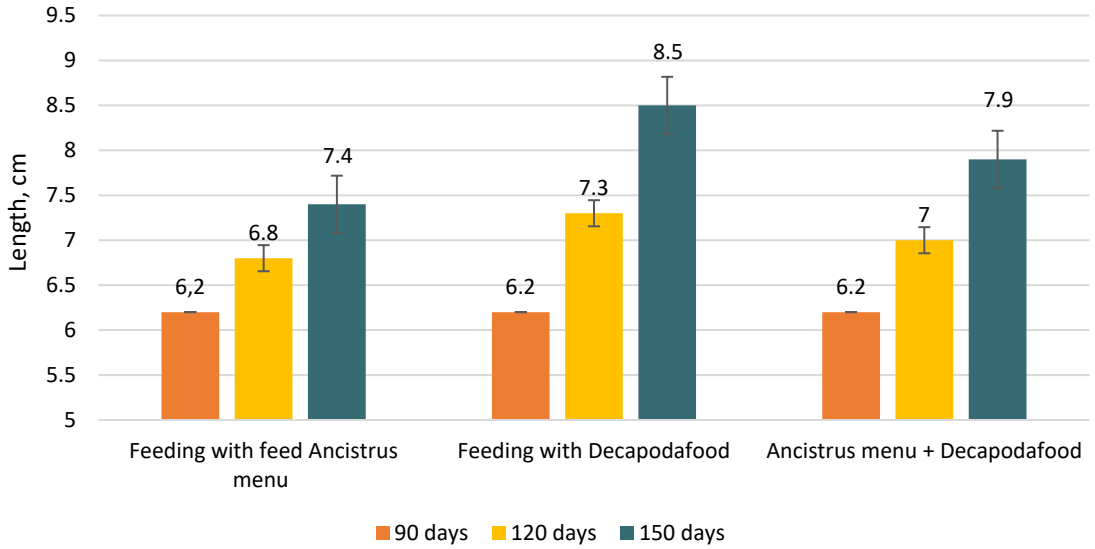

Figure 2. *Length of juveniles of the Australian red-clawed crayfish Cherax quadricarinatus during 60 days of rearing, n=150*

Figure 3. *Measurement of the growth in length of the Australian red claw crayfish*

In the second experimental group of crayfish fed the developed *Decapodafood*, was noted a more intensive growth of young Australian red claw crayfish compared to the control group of crayfish. In particular, during the first month of cultivation, the length of crayfish increased to 7.3±0.2 cm, and during the second – to 8.5±0.2 cm. Accordingly, the increase in length was 1.1±0.1 cm for the period from 90 to 120 days and 1.2±0.1 cm for the second period from 120 to 150 days. When feeding *Decapodafood*, the increase in length of crayfish was 1.8 times ($p \leq 0.05$) greater during the first month of rearing than in crayfish fed *Ancistrus menu*. In the growing period from 120 to 150 days, the increase in the length of crayfish in the experimental group was 2.0 times ($p \leq 0.05$) greater, compared with crayfish in the control group for the same period.

In the third experimental group, crayfish were fed *Ancistrus menu* and Decapoda food in a ratio of 50:50. The intensity of body length growth was lower than when feeding *Decapoda food* but higher than when feeding *Ancistrus menu*. In particular, the increase in length of crayfish during the first 30 days of feeding was 0.8±0.1 cm, and during the next 30 days 0.9±0.1 cm. Thus, the increase in the body length of crayfish in this experimental group was greater than in the control when crayfish were fed *Ancistrus menu* feed by 1.3 and 1.5 times ($p \leq 0.05$).

Thus, this study shows that feeding *Decapoda food*, which in its composition contains 1.8 times more protein substances and 4.6 times fatter, contributes to a more intense increase in the size of crayfish. In studies (Liu *et al.,* 2018) it is reported that for intensive

cultivation of crayfish, it is necessary to select a recipe composition of feed that will allow to use their biological growth potential as much as possible.

During the determination of the mass of the young of the Australian red-clawed crayfish *Cherax quadricarinatus* in the period from 90 to 150 days of cultivation (Fig. 4), similar changes were found, concerning body length. That is, the mass of crayfish that were fed *Decapodafood* was the most intensively gained. In particular, in the control group of crayfish (fed with *Ancistrus menu* feed), during the period from 90 to 120 days, body weight increased by an average of 1.0 g and amounted to 6.1±0.1 g, and during the period from 120 to 150 days, the weight increased, on average by 1.2 g.

When feeding crabs with *Decapoda food*, the intensity of body weight gain was, on average, 1.4 times ($p \leq 0.05$) stronger, compared to crabs of the control group. On the 150th day of cultivation in this experimental group of crayfish, the weight was 8.1±0.2 g, which is 0.8±0.1 g more than in the control group of crayfish. During the mixed feeding of Australian crayfish with *Ancistrus menu* and *Decapoda food*, the intensity of body weight gain was intermediate between the control group and the experimental group fed the developed feed. In particular, at the end of the experiment (150 days), the weight of the crayfish was 7.7±0.2 g, which is 0.4 g more than in the control and 0.4 g less, compared to the crayfish in the experiment, which were fed with *Decapoda food*.

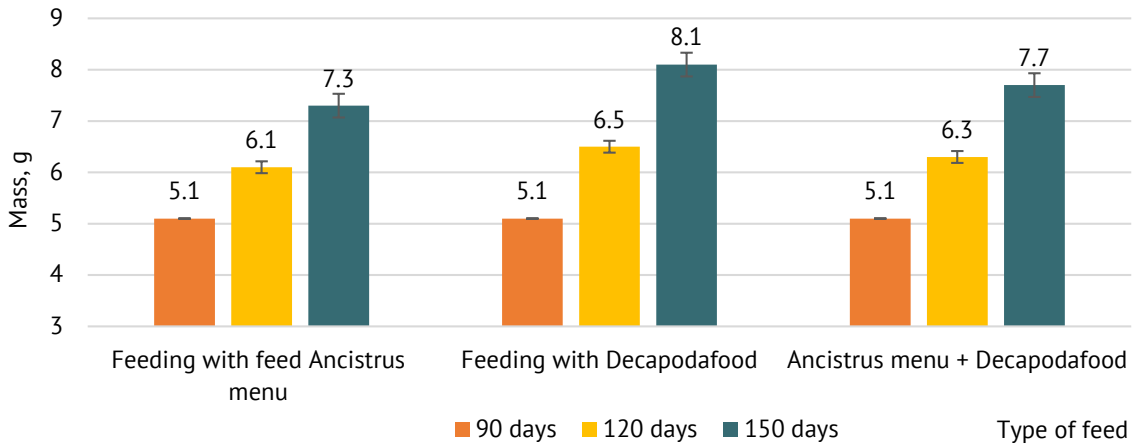

Figure 4. *Weight of juveniles of the Australian red-clawed crayfish Cherax quadricarinatus during 60 days of rearing, n=150*

Therefore, the study indicates that feeding the young crayfish *Cherax quadricarinatus* with *Decapoda food* leads to a faster metabolism since the intensity of weight gain is higher than when feeding the *Ancistrus menu*. This gives reason to believe that providing young crayfish with protein and biologically nutritious feed significantly intensifies the growth and development of Australian red-clawed crayfish in aquaculture conditions. An important indicator that affects the economic efficiency of crayfish cultivation in artificial conditions is survival. After all, researchers (Boyd *et al.*, 2022) indicate that cannibalism is intensively manifested among crayfish during cultivation in aquaculture conditions. Therefore, survival during the period of cultivation of Australian red claw crayfish was determined (Fig. 5).

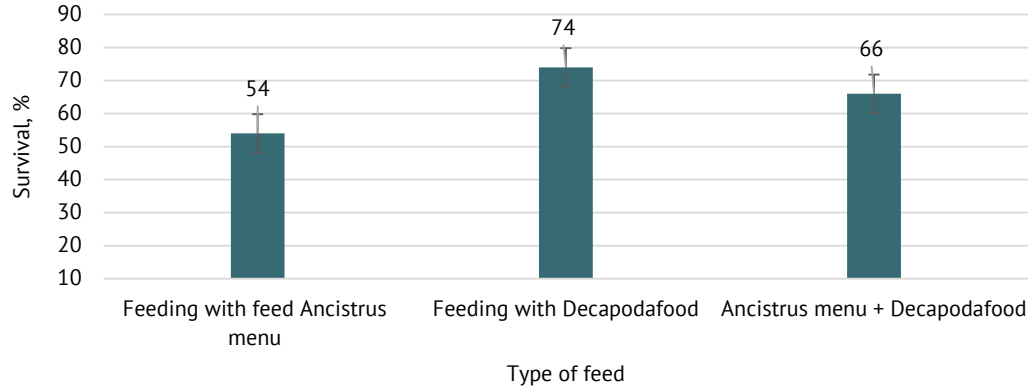

Figure 5. *Survival of juveniles of the Australian red-clawed crayfish Cherax quadricarinatus during 60 days of cultivation under different feeding, n=150*

The largest number of crayfish at the end of the experiment in the group fed with *Decapoda food* was observed. In particular, the survival rate in this group was 74%, which is 20% more live specimens compared to the control group of crayfish and 8% more than the experimental group fed mixed feed. The highest survival among crayfish fed *Decapoda food* can be explained by the presence of a significant amount of easily digestible proteins (37%) and mineral substances, in particular organic origin of calcium and phosphorus salts (5%). The manifestations of cannibalism among young crayfish are decreasing as this phenomenon most significantly reduces the survival of crayfish (Stumpf *et al.,* 2019) under the condition of protein starvation and an insufficient amount of minerals in the diet.

Table 3 and Figure 6 present the scoring scale developed for evaluating the colour of *Cherax quadricarinatus* when fed with different types of fodder.

The material of Table 3, simplified for visual perception, is presented in Figure 6

Table 3. *Cherax quadricarinatus colour grading scale for feeding different types of fodder*

Indicator		**Mark**	**Differentiation**	**Colour**
Background of maintenance (colour of pools, soil of the aquarium)	**Feeding**			
Light	Feeding with boiled cereals	**1**	A	
	Feeding with boiled cereals + and oak leaves (90:10)		B	
	Feeding with boiled cereals + Javanese moss (90:10)		C	
Light	Feed for aquarium fish Ancistrus menu	**2**	A	
50:50	Feed for aquarium fish Ancistrus menu + oak leaves (90:10)		B	
Dark	Feed for aquarium fish Ancistrus menu + Javanese moss (90:10)		C	
Dark	Feed for aquarium fish Ancistrus menu	**3**	A	
	Feed for aquarium fish and crustaceans (various manufacturers)		B	
	Feed for aquarium fish and crustaceans (various manufacturers) + oak leaves (90:10)		C	
Dark	Feed for aquarium fish and crustaceans (various manufacturers) + Javanese moss (90:10)	**4**	A	
	Ancistrus menu + *Decapoda food* (50:50)		B	
	Ancistrus menu + Decapoda food + oak leaves (40:50:10)		C	
Dark	*Decapoda food* + oak leaves *(90:10)*	**5**	A	
	Decapoda food		B	
	Decapoda food + live feed (90:10)		C	

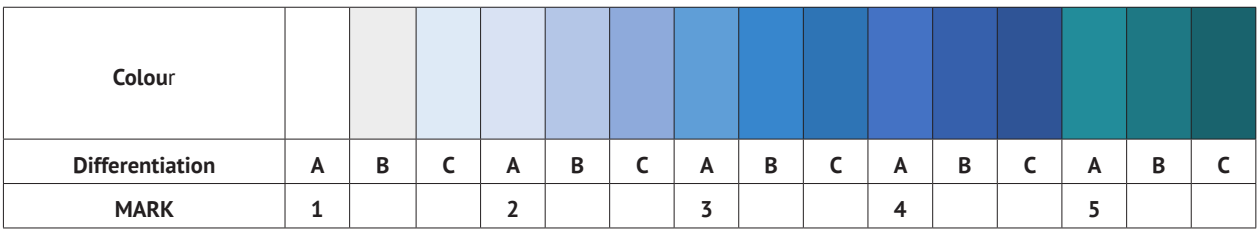

Figure 6. *Scoring scale for evaluating the colouration of Cherax quadricarinatus when feeding different types of feed (compiled by the authors)*

Table 4 shows the colour of the Australian red-clawed crayfish *Cherax quadricarinatus* (Von Martens, 1868), determined by the point scale for feeding food: *Ancistrus menu, Decapoda food, Decapoda food + Ancistrus menu.*

Table 4. *The colouration of the Australian red-clawed crayfish Cherax quadricarinatus was determined using a point scale*

Type of feed	Colour at the end of the experiment
Ancistrus menu	
Decapoda food	
Decapoda food + Ancistrus menu	

Source: *compiled by the authors*

Analysing the data of *Ancistrus menu* feeding of crayfish, the predominance of light colour of the exoskeleton is noted, which is caused by the lack of carotenoids in the composition of the feed and corresponds to point 3 of the differentiation scale – A. Feeding *Decapodafood* contributes to a rich green colour. According to the scoring scale developed by us, it corresponds to a score of 5, differentiation – B.

Feeding the *Decapoda food + Ancistrus menu* combination to the crayfish also gave a dark colour, corresponding to 4 points, differentiation – B. The combination of food is not effective since the two types during the experiment, the crayfish preferred *Decapoda food*.

Currently, Ukraine does not have a sufficient selection of high-quality and balanced fodder for feeding young Australian red-clawed crayfish. Manufacturers of commercial feed in most cases do not want to disclose its composition and nutritional value (Nightingale *et al.,* 2021). Therefore, the obtained data are consistent with the results (Méndez-Martínez *et al.,* 2021), which used feed based on protein components of plant-based protein components, obtained better results than when feeding conventional vegetable feed. Studies (Qian *et al.,* 2021) found that Australian crayfish were on diets containing five different sources of plant-based protein. The weight gain and specific growth rate were significantly reduced (6.7±0.1 g) compared to the author's studies.

CONCLUSIONS

In the conclusion, the authors indicated the efficiency of using Decapodafood feed. The results are an increase in body length and weight. When crayfish were fed with Decapodafood, the increase in length was 1.1±0.1 cm for the period from 90 to 120 days of cultivation and 1.2±0.1 cm for the period from 120 to 150 days. At the same time, the increase in length of crayfish was 1.8 times greater during the first month of cultivation than in crayfish fed with Ancistrus. In the period of cultivation from 120 to 150 days, the increase in length of crayfish was 2.0 times greater, compared with crayfish in the control group for the same period.

When crabs were fed Decapoda food, the intensity of body weight gain was, on average, 1.4 times stronger, compared to the control group crabs. On the 150th day of cultivation in the experimental group of crayfish, the weight was 8.1±0.2 g, which is 0.8±0.1 g more than in the control group of crayfish. At the same time, the survival rate in this group was 74%, which is 20% more than the number of live specimens compared to the control group of crayfish.

Therefore, studies indicate the possibility of rearing juvenile Australian red-clawed crayfish under artificial conditions with high growth rates, efficient use of provided feed, and low levels of cannibalism. The perspective of further research is to determine the physicochemical composition of the meat of the red-clawed crayfish Cherax quadricarinatus using Decapoda food.

ACKNOWLEDGEMENTS

Bila Tserkva National Agrarian University, represented by the rector, Professor Shust O.A.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Показники аквакультури молоді *Cherax Quadricarinatus* за різних схем годівлі

Валерія Сергіївна Жарчинська
Аспірант
Білоцерківський національний аграрний університет
09111, вул. Героїв Чорнобиля, 3а, м. Біла Церква, Україна
https://orcid.org/0000-0001-7430-9498

Наталія Євгеніївна Гриневич
Доктор ветеринарних наук, професор
Білоцерківський національний аграрний університет
09111, вул. Героїв Чорнобиля, 3а, м. Біла Церква, Україна
https://orcid.org/0000-0001-7430-9498

Анотація. Аквакультура ракоподібних є одним з основних джерел цінного харчового білка, який за своїми властивостями не може бути замінений іншими білками тваринного або рослинного походження. Використання поживних та збалансованих кормів у годівлі раків сприяє підвищенню продуктивності та отриманню екологічно чистої та безпечної продукції. Метою дослідження є визначення ефекту від згодовування розробленого комбікорму для декаподів. Встановлено, що згодовування корму Decapoda сприяє більш інтенсивному збільшенню розмірів раків. Зокрема, приріст довжини становив 1,1±0,1 см за період з 90 по 120 добу вирощування та 1,2±0,1 см за період з 120 по 150 добу. При використанні корму Decapoda приріст довжини рачків протягом першого місяця вирощування був у 1,8 рази більшим, ніж у рачків, яких годували кормом Ancistrus menu. У період вирощування від 120 до 150 днів приріст довжини раків у дослідній групі був у 2,0 рази більшим, порівняно з раками контрольної групи за той самий період. При згодовуванні ракам Декаподи інтенсивність приросту маси тіла була в середньому в 1,4 рази вищою, порівняно з крабами контрольної групи. При цьому виживання в цій групі становило 74 %, що на 20 % перевищує кількість живих особин порівняно з контрольною групою раків. Результати дослідження можуть бути використані при розробці технологічної схеми відтворення та вирощування раків

Ключові слова: Ancistrus menu; аквакультура; корм Decapoda; приріст довжини; виживання; маса

SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 70-80

UDC 591.598.26:612.1
DOI: 10.48077/scihor9.2023.70

Biochemical parameters of chicken blood under the influence of technological stimuli of various etiologies

Yulia Osadcha*
PhD in Agriculture, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0003-4126-2456
Alona Shuliar
PhD in Agriculture, Associate Professor
Polissia National University
10008, 7 Staryi Blvd., Zhytomyr, Ukraine
https://orcid.org/0000-0002-8462-2135
Olena Sydorenko
PhD in Agriculture, Senior Research Fellow
Institute of Animal Breeding and Genetics
named after M.V. Zubets of National Academy of Agrarian Science of Ukraine
08321, 1 P.L. Pogrebnyak Str., Chubinske vil., Ukraine
https://orcid.org/0000-0003-2429-9361
Pavlyna Dzhus
PhD in Biology, Senior Research Fellow
Institute of Animal Breeding and Genetics
named after M.V. Zubets of National Academy of Agrarian Science of Ukraine
08321, 1 P.L. Pogrebnyak Str., Chubinske vil., Ukraine
https://orcid.org/0000-0002-4808-0260
Alina Shuliar
PhD in Agriculture, Associate Professor
Polissia National University
10008, 7 Staryi Blvd., Zhytomyr, Ukraine
https://orcid.org/0000-0002-0823-6814

Article's History:
Received: 15.05.2023
Revised: 26.08.2023
Accepted: 27.09.2023

Abstract. Violation of the main technological parameters of keeping and feeding poultry is the main factor in reducing egg productivity and health in the industrial production system. Therefore, the research aims to determine biochemical markers in acute technological stress caused by factors of various etiologies. In this study, serum

Suggested Citation:
Osadcha, Yu., Shuliar, A., Sydorenko, O., Dzhus, P., & Shuliar, A. (2023). Biochemical parameters of chicken blood under the influence of technological stimuli of various etiologies. *Scientific Horizons*, 26(9), 70-80. doi: 10.48077/scihor9.2023.70.

*Corresponding author

biochemical parameters were evaluated for the effects of stimuli in egg-laying chickens. The study was conducted on several Hy-Line W-36 cross chickens in the conditions of a production line. Four groups of chickens (101 heads each) aged 52 weeks were formed to conduct the study. The control group was formed from 10 representatives of each of the groups. Further, each group was exposed to a corresponding technological stimulus. Birds of group 2 were not fed for 24 hours, those of group 3 were kept in a darkened place, and 60 additional birds were placed in a cage with the chickens of group 4. The highest effect on the dynamics of biochemical parameters was an increase in their concentration by 1 m^2 cage space. The studies identified a substantial increase in the concentration of glucose and creatinine in the blood serum of chickens during over-compaction – by 24-28% ($p<0.05$) compared to the control group, regardless of the cause of the stressful situation, all birds exposed to acute stress had an increase in these indicators in the blood serum. The main part of the examined enzymes, such as aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase, tended to increase their activity depending on the strength of the influence of the technological factor on the body of birds. Dynamic characteristics of glucose and creatinine levels were distinguished among the examined biochemical parameters by rapid and differentiated reactions within the physiological norm, and considering the simplicity of their determination, they may have prospects of becoming the main markers of early diagnosis of stress in industrial poultry farming

Keywords: glucose level; creatinine; aspartate aminotransferase; lactate dehydrogenase; stress; marker

INTRODUCTION

Poultry farming is one of the actively developing branches of animal husbandry not only in Ukraine but also around the world. However, according to K. El-Sabrout *et al.* (2022), the economic attractiveness of this area of animal husbandry is based on the short period of poultry rearing, rapid turnover of invested funds, and intensive development due to the variability of production. Such prerequisites contributed to the emergence of large, specialised poultry enterprises in Ukraine. Modern scientific findings in the field of animal husbandry and zoo hygiene have allowed egg poultry farming to a new level of production. Specialised hybrid chickens produce 300 or more eggs per year. Therewith, due to artificial light control and optimisation of feeding rations, it was possible to level out the seasonal decrease in egg productivity during moulting and get a constant egg laying throughout the year. This was made possible by the introduction of the results of studies into the main production processes: S. Molnár & L. Szőllősi (2020) on increasing the concentration of animals in the room through the use of high-tech cage equipment, Y. Ouchi *et al.* (2022) and M. Madkour *et al.* (2022) on the use of modern air conditioning and temperature control systems during maintenance, and W.L. Bryden *et al.* (2021) on ration feeding. However, industrial poultry farming, despite its high profitability, achieved by reducing logistics and labour costs, also remains the most vulnerable industry in terms of the large crowding of birds in a relatively small area and the impact of constant technological stress on them.

I.A. Fisenko *et al.* (2021) are convinced that the productivity of an adult bird is largely determined by the conditions of its rearing and keeping. Due to the targeted influence on the zootechnical parameters of the microclimate, which is artificially created in poultry houses where laying hens are kept, a substantial increase in egg productivity is achieved, compared to an extensive type of production, while maintaining the quality of commercial eggs. However, such substantial physical and feed loads on the bird's body correspondingly affect its physiological state. According to L. Yan *et al.* (2022), this is accompanied by stressful changes in the blood system and hormone balance and leads to a decrease in productivity and leads to substantial economic losses. Therefore, ensuring optimal microclimate parameters, comfortable conditions for keeping, and proper feeding of birds is the basis of industrial technology for the production of egg and meat products in poultry farming.

To date, according to K. Olejnik *et al.* (2022), monitoring the condition of animals in the conditions their productive use, with intensive egg production technology in Ukraine, is conducted by monitoring the behaviour of animals and constant observation of the level of productivity, feed consumption, bird safety, and their general condition, in particular, the development of aggression, cannibalism, or eating feathers. Such approaches allow stating the harmful effect of stress factors on animals only when the clinical picture develops from negative effects and forces the measures to eliminate the consequences of stressors. A more promising method of monitoring the condition of animals in an industrial system of keeping is laboratory methods that allow the identification of the negative technological impact of one or more factors on animals in advance of the manifestation of a decrease in productivity. This will allow the prevention of short-term losses and maintain production at a high level.

Based on the importance of monitoring the productive well-being of poultry in industrial egg production, the research aims to determine biochemical markers of poultry blood under acute technological stress caused by factors of various etiologies.

LITERATURE REVIEW

Stress is the response of the body to an internal or external stimulus that causes anatomical, physiological, and behavioural changes. Among the most common stresses during cage keeping, the primary stress source is temperature fluctuations, high humidity, lack of light, high crowding of animals, insufficient air exchange, noise, and fear (Bulent & Niyazi, 2018). According to A. Usturoi *et al.* (2023), high egg production in poultry balances on the edge of the physiological norm and the constant risk of stress. Therefore, industrial poultry farming is constantly looking for methods to control the occurrence of stress and prevent it.

According to most authors, the most common stress factors are heat stress (Ghoname *et al.*, 2022) and high crowding of caged birds (Osadcha *et al.,* 2022; Herbert *et al.*, 2021). According to J.A.C. Sáenz (2021), heat stress in the United States alone causes economic damage of 1.128-165 million annually and a 0.5-7.2% decline in egg production. M. Ghoname *et al.* (2022) determined acute heat stress to reduce feed intake in all egg-laying chicken crosses, a direct correlation between the intensity of temperature stress and quantitative indicators of egg productivity was identified. Losses from over-compaction of poultry in cages, calculated by G.T. Herbert *et al.* (2021) in the UK egg industry, amounted to 6.5 million pounds a year as were result of suffocation alone, excluding the effects of sublethal injuries such as bone fractures and stress from physical restriction. Birds kept in cages showed signs of fear, decreased faecal immunoglobulin A levels, and increased corticosterone concentrations in their feathers (Campbell *et al.*, 2022).

A study by E.E. Onbasilar & F.T. Aksoy (2005) confirms the conclusions of previous authors on the deterioration of both egg productivity and the condition of the birds themselves with an increase in their concentration. In addition, it was shown that the higher the ceiling level of the cage, the better the performance indicators. The difference in egg quality was substantially better in animals from higher levels compared to lower ones. Antibody titers were lowest on compacted planting. The optimal number of birds was 3 per cage. L. Hedlund and P. Jensen (2022) proved the sustained effect of stress on the subsequent productivity of chickens. Thus, productive poultry raised under stressful conditions substantially ($p<0.001$) had the worst productivity and egg quality. In addition, according to L. Yan *et al.* (2022), birds exposed to stress have impaired reproductive capacity. Corticosterone, which is actively produced during stress, inhibits the development of follicles and prevents the deposition of yolk in them.

Control of stress levels in birds is conducted by invasive and non-invasive methods. Non-invasive methods based on observation and analysis are most often used. The following parameters are monitored: egg production percentage, average egg weight, average feed intake per bird per day, average feed conversion expressed in grams of feed/egg, average chicken weight, cumulative mortality rates, and the number of suffocation cases that resulted in mortality. In addition, a mandatory element in determining stress is monitoring the bird's behavioural responses – a test for a stationary person, a new object, and feather damage. Among invasive methods, several biochemical parameters of blood serum are used (Nwaigwe *et al.*, 2020), which, unlike non-invasive ones, allow for determining in more detail the physiological state of the body, adaptive responses, and metabolic disorders.

MATERIALS AND METHODS

The study was conducted in the branch of one of the largest producers of chicken eggs in Ukraine on the number of chickens in the egg productivity area of the cross "Hy-Line W-36". All manipulations with controlled poultry were conducted following the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986). During the experiments, the company operated normally in compliance with all sanitary and hygienic, veterinary, and technological requirements. The study was conducted in the conditions of a technological line of an industrial-type poultry complex for the production of commercial eggs. The bird was kept in standard poultry houses with a controlled microclimate, equipped with cage equipment from the company "Salmet" (Germany). The microclimate parameters corresponded to the recommendations for breeding chickens of the commercial cross "Hy-Line W-36" (W-36 Commercial Layers…, 2020). Feeding of birds was conducted with complete mixed feeds using automatic cup feeders following the standards specified in the recommendations of breeders. 4 groups of chickens (101 heads each) aged 52 weeks were formed to conduct the study. Each group was kept in separate cage analogues by area and equipment (Table 1).

Table 1. *Scheme of conducting experiments to examine the effect of technological stimuli of various etiologies on the body of chickens*

Feature	Groups of egg-laying chickens			
	1	2	3	4
Irritant	**Control**	**Food stress**	**Lighting stress**	**Over-compaction**
Duration of exposure to the stimulus, h	24			
Number of heads in a cage	101			161

Table 1, Continued

Feature	Groups of egg-laying chickens			
	1	2	3	4
Irritant	**Control**	**Food stress**	**Lighting stress**	**Over-compaction**
Concentration, heads/m^2		24.9		39.7
Area availability, cm^2/head		401.4		251.8
Feeding front, cm		7.2		4.5

Source: *compiled by the authors*

The control group was formed from 10 representatives of each of the 4 groups before the start of the acute technological stress simulation. In the future, each group was exposed to a corresponding technological stimulus of different etiologies. Birds of group 2 were not fed for 24 hours, those of group 3 were kept in a darkened place, and 60 additional birds were placed in a cage for chickens of group 4 to ensure substantial over-compaction. Modelling of the effect of acute stimuli in chickens of groups 2 and 3 was achieved by turning off the corresponding systems in the poultry house – the feed distribution line and lighting. Exposure to the factor in all groups was 24 hours, after which the biochemical parameters and activity of enzymes in the blood serum of chickens were determined. Blood samples were taken from randomly selected 30 laying hens aged 52 weeks from each of the experimental groups. 1-1.5 ml of blood was taken from the axillary vein and stabilised with 2-3 drops of ethylenediaminetetraacetic acid solution (EDTA, trilon-B), according to the guidelines for collecting biopsies in veterinary practice for the diagnosis of small pets in the laboratory where biochemical studies were conducted (Guidelines for collecting..., 2021).

Biochemical parameters and enzyme activity in the blood serum of laying hens were determined using a BioChem FC-360 biochemical analyser (High Technology Inc., USA) in the laboratory of the Centre for Animal Diagnostics "Bald" (Kyiv). Biochemical parameters (glucose, total protein, albumin, creatinine, urea, cholesterol, phosphorus, and calcium) and the activity of enzyme complexes (Alanine aminotransferase (ALT), Aspartate transaminase (AST), Lactate dehydrogenase (LDH), Alkaline phosphatase (ALP), and Gamma-glutamyltransferase (GGT) were determined using commercial diagnostic kits from High Technology Inc. (USA) following the guidelines for their application. The results of the examination were subjected to mathematical analysis using the software product TIBCO Statistica v14.0.0.15 (USA). Indicators of descriptive statistics and the influence of technological factors on the biochemical parameters of laying hens' blood were determined using a one-factor analysis of variance. The probability of differences between the groups was estimated by determining the Student's t-criterion.

RESULTS

Technological stress is the main factor in reducing egg productivity in poultry at the enterprise, which is caused by many factors. Therefore, to reduce the factors not considered that could affect the results of research, poultry was fed with recommended diets. Table 2 shows the composition of complete mixed feeds, as their elements can also cause certain biochemical changes in blood parameters in the body.

Table 2. *Composition of mixed feed for laying hens during the productive period, %*

Component	Mixed feed				
	Guaranteed performance level, %				
	100	93	88	85	Molting period
Grain group					
Wheat	20.42	19.34	12	10.57	10
Corn	37.05	45.4	54.33	52.33	52.84
Sunflower meal	20.75	22.28	18.17	23.53	19.39
Soy meal	7	-	3	-	8.07
Dietary supplements					
Soy oil	0.96	0.66	-	0.5	-
Crushed feed shell	10.7	9.92	10.25	11.09	7.61
Salt	0.21	0.2	0.2	0.21	0.23
Monocalcium Phosphate	1.19	0.81	0.81	0.53	1.06
Sodium sulfate	0.16	0.12	0.12	0.09	0.16
Methionine	0.19	0.1	0.09	0.08	0.04

Table 2, Continued

Component	Mixed feed				
	Guaranteed performance level, %				
	100	93	88	85	Molting period
Lysine sulfate	0.64	0.59	0.52	0.58	0.18
Threonine	0.13	0.09	0.06	0.06	-
LOXIDAN TD 100 (preservative)	-	0.01	-	-	-
Millertime (enzyme preparation)	0.01	0.02	0.01	-	0.02
Globamax 1000 (microflora activator)	0.1	-	-	-	-
Proactive Poultry (microflora activator)	-	-	0.15	0.15	-
Enteronormin Detox (probiotic)	0.15	0.15	-	-	0.15
Mastersorb (mycotoxin inactivator)	0.15	0.13	0.13	-	-
Mycocide Pro (mycotoxin inactivator)	-	-	-	0.09	0.09
Choline Chloride	0.05	0.05	0.04	0.04	0.04
Kronozyme (enzymatic preparation)	-	-	-	0.01	-
Yellow aerophilous (dye)	0.003	0.003	0.003	0.003	-
Karofil red (dye)	0.003	0.003	0.003	0.003	-
Mineral complex	0.1	0.1	0.1	0.1	0.1
Vitamin complex	0.03	0.03	0.03	0.03	0.03
Total	100	100	100	100	100

Source: *compiled by the authors*

In these diets, the main part of the feed is represented by high-energy cereals and various biologically active additives, in particular, amino acids, vitamins, macro-and microelements, antioxidants and other useful elements that increase the digestibility and absorption of nutrients. In addition, the composition of mixed feeds, regardless of the level of productivity, did not include pharmacological preparations with adaptogenic properties that could reduce or distort the influence of the examined factors on the body's response. After considering most of the factors that could affect the results of the study in one way or another, a series of controlled stress stimuli of a technological nature was conducted on experimental groups of birds. Biochemical parameters of the blood of controlled laying hens after the action of technological factors are given in Table 3.

Table 3. *Biochemical parameters of chicken blood serum under the influence of technological stimuli of various etiologies, n=30/group*

Attributes and units of measurement	Group				Reference values*
	1	2	3	4	
Total protein, g/L	54.42±0.41^{a}	57.02±0.78^{b}	57.46±0.55^{b}	58.54±0.56^{b}	41-65
Albumin, g/L	18.5±0.19^{a}	18.61±0.22^{ab}	19.38±0.08^{c}	19.18±0.22^{c}	19-24
Glucose, mmol/l	15.17±0.21^{a}	16.31±0.11^{b}	16.82±0.16^{c}	18.12±0.13^{d}	11.6-275
Creatinine, mmol/L	21.86±0.2^{a}	22.82±0.24^{b}	25.22±0.35^{c}	27.02±0.28^{d}	20.3-28.6
Urea, mmol/L	1.1±0.05	1.04±0.06	1.02±0.07	1.12±0.01	0.31-0.73
Bilirubin, mmol/L					
- total	1.36±0.03^{a}	1.46±0.11^{a}	1.54±0.02^{b}	1.6±0.04^{b}	1.2-1.7
- direct	0.12±0.03^{a}	0.38±0.02^{b}	0.26±0.02^{c}	0.26±0.02^{c}	0.2-0.5
- indirect	1.24±0.05	1.08±0.1	1.28±0.11	1.34±0.06	0.8-1.4
Cholesterole, mmol/L	2.7±0.13	2.72±0.11	2.91±0.08	2.78±0.09	3.3-12.2
Phosphorus, mmol/L	1.43±0.05	1.46±0.04	1.59±0.08	1.58±0.07	1.3-2.2
Calcium, mmol/L	4.12±0.01^{a}	4.38±0.02^{b}	4.54±0.02^{c}	4.56±0.04^{c}	3.5-6.8
Calcium/phosphorus ratio	3.01±0.15	3.01±0.11	3.02±0.14	3.03±0.13	3-3.6:1

Note: *a, b, c – differences are reliable (p <0.05) within the indicator; * Reference values according to I.V. Nasonov et al. (2014)*
Source: *compiled by the authors*

As a result of the conducted studies, it was established that only indicators of glucose and creatinine in the blood serum had a prognostic value for the manifestation of acute stress on the bird's body, while other indicators in the first hours of exposure to technological stimuli almost did not change. The first, under the action of stress factors, are the biochemical parameters associated with carbohydrate and energy metabolism, which is a manifestation of the compensatory phase of the body's response to stimuli. Most often, this is manifested by an increase in the concentration of glucose and creatinine in the blood since these compounds are indicators of increased energy consumption by the body. Studies have identified a substantial increase in the concentration of glucose in the blood serum of chickens under the influence of various technological stimuli. In laying hens of group 2, underfeed stress, the glucose content increased by 15.1% ($p<0.05$) compared to birds of the control group. The highest stress factor for birds was a decrease in the area of keeping per chicken. It was in the birds of group 4 that were exposed to over-compaction that the glucose content was highest – 27.9% ($p<0.05$) higher compared to the control group, and by 11.1% ($p<0.05$) and 7.7% ($p<0.05$) with groups 2 and 3, respectively.

Similar dynamics were characteristic of the level of creatinine in the blood serum of birds, which corresponds to the activation of energy metabolism in the compensatory period to the effect of a technological stimulus. Regardless of the cause of the stressful situation, all birds exposed to acute stress had elevated serum creatinine levels. As was the case with glucose levels, its maximum increase was in laying hens of group 4, which, due to over-compaction, had creatinine content 23.6% ($p<0.05$) higher compared to the control group, and 18.4% ($p<0.05$) and 7.1% ($p<0.05$) compared to groups 2 and 3, respectively. In birds to which feed and light stressors were applied, the level of creatinine, although elevated, was still within the physiological norm, in particular, in chickens of group 2, the content of creatinine in the blood serum during fasting was higher by 4.4% ($p<0.05$), and in group 3 – by 10.5% ($p<0.05$) compared to animals that were not exposed to stress.

Under the influence of technological stimuli, an increase in the total protein content in the blood serum occurred within the physiological norm. Thus, in chickens of group 2, under food stress, the total protein content in the blood serum was higher by 4.8% ($p<0.05$), in chickens of group 3 – by 5.6% ($p<0.05$), and in chickens of group 4 – by 7.6% ($p<0.05$) compared to poultry of the control group. While differences in protein content between different groups of animals exposed to stress of different etiologies ranged from 0.8-2.7% and were not statistically confirmed. A similar pattern was observed in terms of albumin content. A notable increase in the level of albumin fractions of the protein under the action of various technological factors was not observed, all of them were within the reference intervals. Slightly higher was the increase in the number of albumins in the blood serum of chickens of the third group by 4.1-4.8% ($p<0.05$) and the fourth – 3.1-3.7% ($p<0.05$), which were kept in over-compacted cages and under the condition of darkening, compared with groups 1 and 2, respectively. There was no substantial difference in serum albumin content between the laying hens of the control and first experimental groups.

The highest effect on the dynamics of biochemical parameters of blood from technological stressors was an increase in their concentration by 1 m^2 cage spaces. It was this factor that caused the fastest and greatest reaction of the body to the action of the stress factor. In addition to the above-mentioned effect of the over-compaction factor on several blood serum parameters, an increase in such indicators as the content of total bilirubin and the level of calcium ions was also detected. Other technological factors also had a substantial impact on the bird's body, but their effect was substantially lower compared to the stress of over-compaction, even though the "Hy-Line W-36" cross is considered the most adapted to caged breeding. Thus, the content of total bilirubin in animals of group 4 exceeded the level by 3.9-9.6% of its concentration in animals of other groups that were subjected to technological exposure and 17.6% ($p<0.05$) of chickens of the control group. A slightly different picture was observed for other bilirubin fractions. Thus, the highest level of direct bilirubin was observed in chickens of group 2, which were exposed to feed stress, the content of direct bilirubin in them substantially exceeded the indicators in animals of other groups. This may be due to liver activity due to prolonged starvation of animals. The indirect bilirubin content was at the same level in chickens of all groups. Thus, the increase in total bilirubin under the influence of technological stimuli occurred due to direct bilirubin.

The calcium content in the blood serum of chickens of all groups was within the physiological norm, but its increase was observed under the influence of technological stimuli. In particular, in group 2, its content was higher by 6.3% ($p<0.05$) compared to group 1. The differences between groups 3 and 4 were only 0.4% and were not statistically confirmed. The effect of technological stimuli did not affect the content of cholesterol and phosphorus in the blood serum of chickens, which were at the same level in chickens of all groups and were within the physiological norm, which also affected the ratio of calcium and phosphorus. No fewer substantial markers of the action of acute stress in poultry may be the activity of certain enzymes. As part of the study, the activity of several serum enzyme systems after 24 hours of exposure to individual technological stressors was analysed (Table 4).

Table 4. *The activity of several enzymes in the blood serum of laying hens under the influence of technological factors, n=30/group*

Indicator, units/L	Group				Control value*
	1	2	3	4	
Alanine aminotransferase (ALT)	1.2±0.15	1.04±0.02	0.84±0.06	1.02±0.01	1.5-3.3
Aspartate transaminase (AST)	164.61±3.74[a]	211.4±5.84[b]	224.42±1.09[c]	259.8±2.31[d]	118-298
Gamma-glutamyltransferase (GGT)	22.19±1.11	22.42±1.03	22.24±0.49	22.64±1.07	13.9-27.6
Alkaline phosphatase (ALP)	654.86±11.85[a]	686.41±10.42[b]	717.62±12.4[bc]	732.02±16.26[c]	350-830
Lactate dehydrogenase (LDH)	1368.82±63.68[a]	1694.84±64.49[b]	2019.05±26.41[c]	2358.04±41.91[d]	1794-2372

Note: *a, b, c – differences are reliable (p <0.05) within the indicator; *Reference values according to I.V. Nasonov et al. (2014)*
Source: *compiled by the authors*

The main part of the examined enzymes, such as aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase, tended to increase their activity depending on the strength of the influence of the technological factor on the body of birds. An increase in the activity of these enzymes in the blood is associated with partial destruction of cages. Therefore, the study showed a proportional increase in the activity of enzymes to the strength of the stress factor. As in previous studies, the strongest stressor for birds, based on the dynamics of growth in enzyme activity, was the over-compaction of animals. Thus, for the main enzymes – AST, ALP, and LDH, the increase was from 13 to 73% and substantially exceeded the corresponding values in animals of the control group. Animals exposed to feed or lighting stress occupied an intermediate place and slightly differed both from each other (2-4.5%) and from animals of the control group (4.8-9.6%). GGT in animals of all groups, both experimental and control, was at the same level and was within the physiological interval, so as a marker of stress in poultry farming, it will not have practical importance. The results obtained regarding the decrease in ALT activity in animals after the negative effect of technological factors require a more in-depth study. Ultimately, even in animals of the control group, ALT activity was at the lower limit of the physiological norm, and after stress load, it generally decreased below the permissible interval, which is more similar to the genetic feature of the bird population of the cross "Hy-Line W-36".

Summarising the results obtained, it can be stated that individual biochemical parameters of blood serum and the activity of enzyme complexes can be used for early diagnosis of the negative impact of technological stimuli of various etiologies.

DISCUSSION

The biggest challenge of modern industrial poultry farming is the constant search for a compromise between high bird productivity and balancing on the edge of stress. Stress reduces the birds' feed intake, the intensity of their growth and productivity, reduces the immune response and function, which leads to the high susceptibility of laying hens to diseases. Therefore, the actual area of research in poultry farming is the search for informative systems for early diagnosis of stress development in laying hens, as a precursor to reduced productivity. Several cytological techniques (Lee *et al.*, 2022) and humoral approaches (Osadcha *et al.*, 2021) have already been used to diagnose stress. However, they have substantial drawbacks: cytological methods have a long-hidden period, caused by the accumulation of appropriate cellular elements in the blood, and humoral methods are rather expensive and resource-intensive. These shortcomings are devoid of methods for determining most of the biochemical characteristics of animal blood and, the main thing is that changes in the blood develop within a short time after the action of the stimulus, which allows consideration of these methods as markers of acute stress in poultry.

From the results of the study on the influence of some technological factors of various etiological nature on the biochemical parameters of the blood of productive laying hens, it was determined that such characteristics as an increase in the concentration of glucose and creatinine, and the activity of aspartate aminotransferase and lactate dehydrogenase are fast and reliable markers of stress in poultry. The changes in these biochemical parameters were manifested already in the first hours after the action of stressors, so they can become markers of the development of a pathological reaction of the bird body to the action of technological factors. Moreover, the methods for determining the levels of these indicators are simple, and the use of ready-made kits for conducting tests generally makes the duration of the study no longer than a few minutes.

Mechanisms of increasing the level of glucose and creatinine in the serum of animals exposed to stress are a consequence of the destruction of part of the body's cells, and the intensity of this process is directly proportional to the strength of the stressor. Similar conclusions were made by M.I. Sakhatsky & and Yu.V. Osadcha (2021), when analysing the influence of such a technological parameter as the height of cages on the

biochemical parameters of the blood of laying hens in an industrial egg production system. An increase in certain biochemical parameters of the blood is associated with the active work of the adrenal glands and the expression of corticosteroids. The latter, as proven in the study by Y. Wang *et al*. (2020), trigger the mechanism of activation of the enzymes creatine kinase and lactate dehydrogenase, which is observed in the results obtained in the framework of this study. An increase in blood glucose in animals exposed to stress occurs due to increased gluconeogenesis and glycogenolysis under the influence of epinephrine and norepinephrine, released during the development of the acute phase of the body's response to stimuli (Chu *et al.*, 2022).

A similar pattern was observed in the blood serum of experimental chickens. In particular, feeding stress resulted in only a slight increase in glucose levels within the physiological norm, 16.3 mmol/L. While due to other factors, its concentration increased substantially higher than the reference values for poultry. In addition, the concentration of glucose in the blood was directly proportional to the strength of the stimulus. In the conducted studies, the highest glucose level was observed in chickens exposed to technological stress of over-compaction – 18.2 mmol/L. Analysis of other marker indicators – creatinine levels and blood enzyme activity confirmed that this factor is the greatest stress for industrial birds involved in the study. Similar conclusions were obtained by Yu. Osadcha *et al*. (2022), identified that with an increase in the planting density of laying hens up to 26.7 head/m^2 resulted in a decrease in the safety of chickens by 8.9-9%, and egg production – by 4-5.8%. That is, in their opinion, clinical signs similar to those that occur under the influence of chronic stress were obtained. This is assumably why most farmers are considering replacing the conventional cage-keeping system with other, less stressful approaches.

Informative marker was the concentration of creatinine in the blood of experimental birds. Creatinine, like glucose, is a high-energy compound involved in the body's energy metabolism, namely, it provides energy to muscle tissue. Therefore, an increase in the level of creatinine in the blood serum of chickens indicates neuromuscular tension in their body, which is confirmed by the conducted studies. In particular, when the body of chickens was exposed to technological stresses, creatinine concentrations fluctuated from 21.9 mmol/L in the control group to 27.2 mmol/L in the group of chickens that were subjected to over-compaction. That is, this indicator had a wider range of fluctuations compared to glucose levels under appropriate conditions and also had a straight-line correlation with the intensity of stress. The main reason for its use as a future marker is that all creatinine values were within the physiological norm. That is, it allows determining the effect of the stress factor on the body more accurately, after minor analytical studies with a change in the intensity of one technological stimulus, without causing pathological changes or a decrease in productivity. S.C. Huang *et al*. (2018), confirmed the use of prognostic value of creatinine as a marker of the effect of temperature stress in chickens. Therefore, it is planned to examine in more detail the dynamics of changes in creatinine concentration depending on the intensity of the technological stimulus in the future, and this is the goal of subsequent studies.

As for other biochemical parameters, there were no substantial changes in their levels that could recommend making them markers of the effect of stress on the body. S. Tang *et al*. (2018) identified prognostic levels of urea in the blood of chickens after exposure to temperature stress, while in a study by C. Nwaigwe *et al*. (2020), this indicator was the dynamics of changes in albumin levels. The results of the analysis of biochemical parameters of blood performed in the framework of this study did not confirm these assumptions, which may have been due to the different periods of laboratory tests.

Changes in the activity of enzymes, in particular, aspartate aminotransferase and lactate dehydrogenase, were equally informative markers. The increased activity of these enzymes is due to the overexpression of corticosteroids and epinephrine (Wang *et al.*, 2020). Like other biochemical parameters that have been identified as potential markers, the activity of AST and LDH increases in proportion to the strength of the stress factor, and this can be used for prognostic purposes since the activity of both enzymes increased within the physiological norm. The results obtained are quite consistent with those of most researchers (Park *et al.*, 2018; Abo Ghanima *et al.*, 2020), which note that the increase in aspartate aminotransferase activity is the reaction of the bird's body to the action of technological stimuli. An increase in the level of the enzyme above the physiological norm is associated with damage to the function of internal organs, which is already a sign of a pathological condition and is not quite suitable as a clinical tool. Similar dynamics are characteristic of the enzyme lactate dehydrogenase. LDH is an enzyme involved in carbohydrate metabolism, catalysing energy reactions, so it undergoes similar changes along with other products of energy metabolism. According to R. Klein *et al*. (2020), due to the branched structure of LDH isoenzymes, they can become the most informative systems since they enter the serum during tissue damage. Therefore, when determining the model of LDH isoenzyme in serum, it is possible to determine which organ damage prevails, which is also quite an attractive area for future research, provided that the necessary equipment and reagents are available.

Even though the recommendations for breeding chickens of the commercial cross "Hy-Line W-36" did not indicate the biochemical profile of birds and individual studies that were conducted with animals of this

genotype were not informative enough, the results obtained in the framework of this study are of scientific and practical importance, which can be used when working in egg poultry farming.

CONCLUSIONS

From the results obtained in the framework of the conducted studies and based on the data obtained by researchers in previous papers on the investigation of the influence of technological factors of various etiologies on the biochemical parameters of blood in laying hens in the industrial egg production system, several conclusions can be drawn. In the conditions of industrial production of commercial eggs, the main factor in reducing the productivity of birds is the effect of technological factors that cause them stress. Therefore, the search for informative systems that can become early markers of this condition in the animal body will have scientific and practical importance. The strongest stress factor used in the study was the factor of over-compaction of animals, while others – such as the feed factor and the factor of changing the lighting mode had substantially less impact on the birds' body and were approximately at the same level. Among the biochemical parameters of blood serum, glucose and creatinine concentrations were a rapid and differentiated response to the action of technological factors of different intensities. The range of glucose fluctuations under stress was from 15.2 to 18.1 mmol/L and for creatinine-from 21.9 to 27.2 mmol/L. Given the simplicity of determining these indicators and fluctuations in their levels within the physiological norm, they may have prospects of becoming the main markers of early diagnosis of stress in industrial poultry farming. Among the enzyme complexes, such enzymes as LDH and AST, which are involved in carbohydrate metabolism, had similar qualities. A substantial range of changes in the activity of these enzymes depending on the intensity of the stress factor also allows them to be used as markers of the influence of technological factors on the body of birds. The following research aims to examine the effect of a stress factor of varying intensity on the level of creatinine concentration in the body of birds and develop a scale of the negative impact of the technological factor on the egg productivity of birds in industrial production based on this.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Біохімічні показники крові курей за дії технологічних подразників різної етіології

Юлія Василівна Осадча
Кандидат сільськогосподарських наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0003-4126-2456

Альона Леонідівна Шуляр
Кандидат сільськогосподарських наук, доцент
Поліський національний університет
10008, бульвар Старий, 7, м. Житомир, Україна
https://orcid.org/0000-0002-8462-2135

Олена Василівна Сидоренко
Кандидат сільськогосподарських наук, старший науковий співробітник
Інститут розведення і генетики тварин
імені М.В. Зубця Національної академії аграрних наук України
08321, вул. П.Л. Погребняка, 1, с. Чубинське, Україна
https://orcid.org/0000-0003-2429-9361

Павлина Петрівна Джус
Кандидат сільськогосподарських наук, старший науковий співробітник
Інститут розведення і генетики тварин
імені М.В. Зубця Національної академії аграрних наук України
08321, вул. П.Л. Погребняка, 1, с. Чубинське, Україна
https://orcid.org/0000-0002-4808-0260

Аліна Леонідівна Шуляр
Кандидат сільськогосподарських наук, доцент
Поліський національний університет
10008, бульвар Старий, 7, м. Житомир, Україна
https://orcid.org/0000-0002-0823-6814

Анотація. Порушення основних технологічних параметрів утримання та годівлі птиці є основним чинником зниження яєчної продуктивності та здоров'я птиці в системі промислового виробництва. Тому метою дослідження є визначення біохімічних маркерів при гострому технологічному стресі, спричиненому чинниками різної етіології. У цьому дослідженні оцінювали біохімічні показники сироватки крові за дії подразників у курей-несучок. Дослідження проводили на декількох курчатах кросу Ну-Line W-36 в умовах виробничої лінії. Для проведення дослідження було сформовано чотири групи курей (по 101 голові в кожній) віком 52 тижні. Контрольна група була сформована з 10 представників кожної з груп. Далі кожна група піддавалася впливу відповідного технологічного подразника. Птицю групи 2 не годували протягом 24 годин, птицю групи 3 утримували в затемненому місці, а ще 60 голів додатково помістили в клітку до курей групи 4. Найбільший вплив на динаміку біохімічних показників мало збільшення їх концентрації на 1 м^2 кліткового простору. Дослідженнями встановлено суттєве підвищення концентрації глюкози та креатиніну в сироватці крові курчат при переущільненні – на 24-28 % ($p<0,05$) порівняно з контрольною групою, незалежно від причини виникнення стресової ситуації, у всієї птиці, що зазнала впливу гострого стресу, спостерігалося підвищення цих показників у сироватці крові. Основна частина досліджуваних ферментів, таких як аспартатамінотрансфераза, лужна фосфатаза та лактатдегідрогеназа, мали тенденцію до підвищення своєї активності залежно від сили впливу технологічного чинника на організм птиці. Динамічні характеристики рівнів глюкози та креатиніну вирізнялися серед досліджуваних біохімічних показників швидкими та диференційованими реакціями в межах фізіологічної норми, а з огляду на простоту їх визначення, можуть мати перспективу стати основними маркерами ранньої діагностики стресу в промисловому птахівництві

Ключові слова: рівень глюкози; креатинін; аспартатамінотрансфераза; лактатдегідрогеназа; стрес; маркер

SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 81-91

UDC 633.861: [633.11"324": 631.53.01]
DOI: 10.48077/scihor9.2023.81

The influence of water-soluble secretions of saffron seed on the germination of seeds of soft winter wheat varieties

Vira Mykolaichuk
PhD in Biological Sciences, Associate Professor
Mykolaiv National Agrarian University
54000, 9 Georgiy Gongadze Str., Mykolaiv, Ukraine
https://orcid.org /0000-0003-0110-6539
Margaryta Korkhova*
PhD in Agricultural Sciences, Associate Professor
Mykolaiv National Agrarian University
54000, 9 Georgiy Gongadze Str., Mykolaiv, Ukraine
https://orcid.org/0000-0001-6713-5098

Article's History:
Received: 19.05.2023
Revised: 16.08.2023
Accepted: 27.09.2023

Abstract. Due to climate change, there is a need to introduce new crops into agricultural production in Ukraine, among which *Crocus sativus* L., a herbaceous perennial tuberous plant, whose allopathic properties have been insufficiently studied, can take a leading place. Therefore, the aim of the study was to determine the effect of water-soluble flower secretions and their individual elements on the initial stages of seed germination of two winter durum wheat varieties (Vidrada and Koshova). In the course of the study, generally accepted methods were used: laboratory research, analysis, synthesis and statistical. It was found that water-soluble extracts of flowers of different states and elements of *C. sativus* flowers do not have a significant effect on the morphometric and quality parameters of wheat kernels during germination. Water-soluble secretions of flowers of different states have an inhibitory effect on the development of the root system; secretions of the bud and the bloomed flower inhibit the growth of coleoptiles. The effect of water-soluble secretions of sowing saffron flowers elements has varietal peculiarities: for winter wheat seedlings of Vidrada variety, the petals have a stimulating effect on the total length of roots and their weight; for seedlings of Koshova variety, the opposite effect is observed. The water-soluble secretions of petals and stamens have a stimulating effect on the coleoptile of Vidrada plants, and the water-soluble secretions of petals have a stimulating effect on Koshova seedlings. The correlation between the length of roots and coleoptile under the influence of water-soluble secretions of flowers of different states and their elements, as well as the index of allelopathy have varietal characteristics. The obtained scientific results will contribute to the development of agrotechnical measures for the cultivation of saffron in Ukraine, selection of winter wheat varieties for joint cultivation and use of plant residues as biological stimulants

Keywords: *Crocus sativum; Triticum aestivum*; allopathic properties; seedlings; grains; allopathic index

Suggested Citation:
Mykolaichuk, V., & Korkhova, M. (2023). The influence of water-soluble secretions of saffron seed on the germination of seeds of soft winter wheat varieties. *Scientific Horizons*, 26(9), 81-91. doi: 10.48077/scihor9.2023.81.

*Corresponding author

INTRODUCTION

Crocus sativus L., or sowing saffron, is a rare crop that is a source of the world's most expensive spices and valuable compounds used in the official pharmacopoeia. Due to the biological and ecological characteristics of the plants and their economic importance, the crop is promising for cultivation in Ukrainian agrocenoses. To this end, it is necessary to study the safety of *C. sativus* L. plant isolates on other crops and their role in soil toxin formation.

Demand for saffron is growing globally due to its use in cooking, medicine, and cosmetics, as well as new health benefits (Cardone *et al.*, 2020; Sharma *et al.*, 2020)**.** According to the National Agricultural Statistics Service (2017), in Iran, about 10-12% of the total saffron area is cultivated under intercropping systems (including cumin, sunflower, and wheat), while the remaining 88-90% of the *C. sativus* area is grown in monoculture. Over the past decade, *C. sativus* L. (perianth petals) largely has been studied for its by-products (perianth petals) in agriculture to increase yields (Lahmass *et al.*, 2018*).* According to L. Menghini *et al.* (2018)*,* about 90.0-92.6%, or 53 kg, of the production of one kilogram of spices is accounted for by perianth petals and 13.4% by stamens, which is equivalent to 150000-200000 flowers.

The perianth petals of the saffron flower contain several bioactive compounds, such as minerals, anthocyanins, monoterpenoids, carotenoids, flavonoids and flavonols (kaempferol) (Mykhailenko *et al.*, 2019; Belyagoubi-Benhammou *et al.*, 2023). Therefore, it is promising in the world to isolate biologically active compounds from saffron by-products (petals) for their use in the food, perfumery, and pharmaceutical industries (Mir *et al.*, 2022; Serda-bernad *et al.*, 2023). The study of the allopathic properties of plants is a promising area of research worldwide (Kheirabadi *et al.*, 2020; Janusauskaite, 2023). Considering the biochemical composition of various organs of *C. sativus* L., its allopathic activity has been studied for a long time. According to the results of M. Kheirabadi *et al.* (2020), it is known that saffron can be grown in one place for up to 12 years, but starting from 5-6 years, the yield of bracts decreases. I. El Aymani *et al.* (2019) found that the duration of cultivation of *C. sativus* L. also negatively affects the number of endomycorrhizal fungal species.

A minor inhibitory effect, which depended on the concentration of the filtrate, was found by Fallahi *et al.* (2018) in the effect of aqueous extracts of leaves and corms of *C. sativus* L. on seedlings of fenugreek (*Trigonella foenum-graecum* L.), chickpea (*Cicer arietinum* L.) and arugula (*Eruca sativa* Mill.) as promising related crops. According to the results of a study by M. Abbasi & A. Sepaskhah's (2022) research on combined winter wheat and saffron crops during 2013-2017 in Iran, the benefits of growing intercrops in production were found to be 74% higher, with a land equivalent ratio of 1.74. Farmers are recommended to grow saffron in wide-row crops with winter wheat to increase income and soil use efficiency.

In Ukraine, the effect of water-soluble secretions from flowers of *C. sativus* L. of different states on the germination of winter wheat has not been studied. In connection with the possible expansion of the area under *C. sativus* L., there is a need to study the interaction of a new promising crop with traditional ones. Ukraine is one of the largest exporters of wheat grain, so for agricultural production, the study of the possibility of growing saffron and winter wheat in joint crops and establishing a place in the crop rotation, considering the allopathic activity of *C. sativus* L., is an important direction of crop introduction. In addition, by-products (petals, flowers, pistils) from saffron production, such as spices, can become a biological stimulant for the germination of winter wheat seeds. The research aims to determine the peculiarities of the influence of water-soluble secretions of *C. sativus* flowers and their elements on the germination of seeds of two varieties of winter wheat.

MATERIALS AND METHODS

To establish the allelopathic activity of water-soluble extracts of *C. sativus* L. flowers grown in the Northern Black Sea region of Ukraine at the initial stages of ontogeny of plants of different varieties of winter wheat, field studies were conducted in November 2021 at the experimental field of Mykolaiv National Agrarian University, and laboratory studies were conducted in the laboratories of the Department of Plant Production and Gardening.

The determination of the allopathic activity of water-soluble secretions of individual parts of *C. sativus* L. flower on the growth of roots and coleoptiles of seedlings of Ukrainian winter wheat varieties Vidrada and Koshova was carried out according to the method of A. Grodzinskiy *et al.* (1979). Seeds of the studied soft winter wheat varieties were sown in sterile Petri dishes on filter paper moistened with distilled water and placed in a thermostat at +23-24°C for 24 hours. To determine the allopathic activity of *C. sativus* L. flower elements, freshly harvested flowers with fertile and infertile parts were used (experimental variants: "petals", "petals + stamens", "petals + pistil"). Stamens weighed 0.025±0.003 g, pistils 0.035±0.002 g, petals 0.376±0.013 and flowers 0.435±0.016 g. They were placed in distilled water in a ratio of 1 g per 100 ml (0.01%) and kept for 24 hours at a temperature of +23-24°C. The choice of concentration was based on tests conducted previously on watercress seeds (Mykolaichuk *et al.,* 2021). The solution was filtered through filter paper No. 2. A day later, 25 wheat seeds with signs of primary roots were transferred to filter paper in Petri dishes and 4 ml of filtrate was added.

To determine the allelopathic activity of water-soluble secretions of flowers of different states, buds, a blooming flower, and a flower that bloomed 1 day ago were used. The flowers were kept for a day in 100 ml of distilled water. Further development of the seedlings took place in a Petri dish in a thermostat at 20°C. Seven days later, at the beginning of the seedling development phase, the metric and qualitative parameters of seeds were determined, and the maximum and total root length, the number of adventitious roots, the length of the coleoptile and the root weight were measured. To determine the effect of water-soluble secretions of *C. sativus* L. flowers on the process of grain swelling, metric (length, width, thickness, mm) and qualitative (weight, g) parameters were measured before the experiment and on day 7. The metric parameters were measured with a calliper with an accuracy of 0.01 mm, the mass was determined using a balance with an accuracy of 0.001 g. The experiment was carried out in five replications.

Statistical processing of the results was carried out using Microsoft Excel and Statistica 7.0. Graphs were created using Excel 7.0. To quantify the allopathic effect, the allopathic activity index (AI) was used, which was determined by the formula:

$$RI=1-C/T,$$

where C – control values, T – test values. If RI>0, then there is a stimulation effect, RI<0 indicates inhibition (Williamson *et al.*, 1988).

RESULTS AND DISCUSSION

As a result of laboratory studies, an increase in the morphometric parameters of the grain: length by 1.87%, and width and thickness – on the contrary – a decrease by 5.0 and 1.1% compared to the initial values – was found during the period of germination of wheat seeds of Vidrada variety in plants of the control variant. The increase in kernel weight was 25.0% compared to the initial weight. The same peculiarity was observed for the Koshova grains, but the length increased by 6.4%, and the width and thickness also decreased by 32.1 and 18.0%. The increase in kernel weight was 33.3% compared to the initial weight.

Under the influence of water-soluble secretions of *C. sativus* L. bud, the increase in the length of the grain of Vidrada variety to the initial value was 4.0%, and to the control 2.1%. For the width, a slight increase was observed compared to the initial value, but more compared to the control (1.0 and 6.6%, respectively). The thickness, on the contrary, decreased by 2.5 and 1.5%, respectively. At the same time, the weight of the kernel increased by 50% compared to the initial and by 20% compared to the control (Table 1).

Table 1. *Influence of water-soluble secretions of C. sativus L. flowers of different states on morphometric and quality parameters of winter wheat grains of different varieties during germination*

Indicators	Water extracts from flowers in different states (Factor B)				
	Initial variable	Control	Bud	The blooming flower	The bloomed flower
	M±m min-max	M±m min-max	M±m min-max	M±m min-max	M±m min-max
	Varieties (Factor A)				
Vidrada					
Length, mm	6.94±0.05 5.90-7.85	7.07±0.14 6.31-8.05	7.22±0.20 5.34-8.45	7.07±0.15 5.76-8.40	6.93±0.14 5.56-7.78
Width, mm	2.89±0.3 2.30-3.35	2.74±0.14 1.76-4.14	2.92±0.13 1.92-3.90	2.77±0.09 2.01-3.30	2.69±0.13 1.38-3.62
Thickness, mm	2.79±0.03 2.30-3.12	2.76±0.11 1.77-3.66	2.72±0.09 1.96-3.25	2.78±0.09 1.57-3.51	2.63±0.09 1.74-3.07
Mass, g	0.04±0.00 0.02-0.04	0.05±0.00 0.03-0.08	0.06±0.00 0.03-0.09	0.05±0.00 0.03-0.08	0.05±0.00 0.02-0.07
Koshova					
Length, mm	6.88±0.06 5.94-7.51	7.32±0.17 5.60-8.63	7.19±0.15 6.19-8.32	6.87±0.11 5.95-7.79	6.91±0.16 5.68-8.53
Width, mm	2.87±0.04 2.04-3.44	1.95±0.11 1.00-3.04	2.55±0.12 1.70-3.41	2.46±0.11 1.69-3.66	2.29±0.10 1.56-3.05
Thickness, mm	2.67±0.17 2.36-3.03	2.19±0.11 1.25-2.97	2.55±0.11 1.39-3.25	2.27±0.09 1.71-2.87	2.28±0.08 1.34-3.05
Mass, g	0.03±0.00 0.03-0.04	0.04±0.00 0.02-0.06	0.05±0.00 0.03-0.07	0.04±0.00 0.01-0.07	0.04±0.00 0.02-0.05

Source: *compiled by the authors*

Koshova wheat grain was characterised by an increase in length of 4.5% compared to the initial one, but a decrease in this indicator by 1.8% compared to the control. There was also a decrease in width by 11.2%

compared to the original, but an increase of 30.8% compared to the control. The same trend is observed for thickness: a decrease of 4.5% compared to the initial and an increase of 16.4% compared to the control. An increase in kernel weight was recorded by 66.7% compared to the initial and 25% compared to the control.

The water-soluble secretions of the blossoming flower of *C. sativus* L. affect the morphometric parameters of the grain of the Vidrada variety: compared to the initial length, it increased slightly by 1.87%, compared to the control, there was no increase. The width and thickness are characterised by a decrease of 4.2 and 0.4% compared to the initial values. Compared to the control, there was a slight increase of 1.1 and 1.5%, respectively. Compared to the initial weight of the grain, there was an increase of 25%, but compared to the control, the weight did not increase. For the Koshova variety, there was a slight decrease in all morphometric parameters compared to the initial ones: length – by 0.2, width – by 14.3, thickness – by 15.0%. Compared to the control, the length of the grain decreased by 6.2%, and other morphometric parameters increased: width – by 26.2, thickness – by 3.7%.

The water-soluble secretions of the blossoming flower of *C. sativus* L. affected all morphometric parameters of Vidrada grain and were lower than the initial ones: length – by 0.2%, width – by 6.9%, thickness – by 5.7%, respectively. Compared to the control, a slight decrease in all morphometric parameters was observed: length – by 2.0, width – by 1.0, and thickness – by 4.7%, respectively. For Koshova grains, the length slightly increased by 0.4% compared to the initial one. Under the influence of water-soluble secretions, the weight increased by 33.3% compared to the initial weight. At the same time, width and thickness decreased by 20.2 and 14.6%, respectively. Compared to the control, the length of the kernel is 5.6% shorter, and the width and thickness are 17.4 and 4.1% larger, respectively. The grains of both varieties are characterised by a 25% increase in weight, which, in our opinion, is associated with the process of swelling during germination.

The analysis of the effect of water-soluble secretions of *C. sativus* L. flower elements on the germination of wheat kernels of the Vidrada variety shows the following: compared to the initial indicators, a slight increase in the length of the control variant kernels by 1.9% was observed, while a slight decrease in width and thickness (5.2 and 1.1%, respectively) was observed, while the weight increased by 33.3% (Table 2).

Table 2. *Influence of water-soluble secretions of C. sativus L. flower elements on morphometric and qualitative parameters of winter wheat kernels of different varieties during germination*

Indicators	Water extracts from flowers in different states (Factor B)				
	Initial variable	Control	Petals	Petals + stamens	Petals + Stamens
	M±m min-max	M±m min-max	M±m min-max	M±m min-max	M±m min-max
	Varieties (Factor A)				
	Vidrada				
Length, mm	6.94±0.05 5.90-7.85	7.07±0.14 6.31-8.05	7.00±0.18 5.67-8.65	7.02±0.15 5.66-8.35	7.09±0.16 5.78-8.26
Width, mm	2.79±0.03 2.30-3.12	2.76±0.11 1.77-3.66	2.73±0.10 2.14-3.65	2.57±0.08 1.96-3.37	2.64±0.06 2.20-3.08
Thickness, mm	0.04±0.00 0.02-0.04	0.05±0.00 0.03-0.08	0.05±0.00 0.03-0.08	0.05±0.00 0.03-0.07	0.06±0.00 0.03-0.08
Mass, g	6.94±0.05 5.90-7.85	7.07±0.14 6.31-8.05	7.00±0.18 5.67-8.65	7.02±0.15 5.66-8.35	7.09±0.16 5.78-8.26
	Koshova				
Length, mm	6.88±0.06 5.94-7.51	7.32±0.17 5.60-8.63	7.02±0.13 6.12-8.27	7.18±0.12 5.955-7.91	7.32±0.11 6.62-8.55
Width, mm	2.87±0.04 2.04-3.44	1.95±0.11 1.00-3.04	2.23±0.11 1.34-2.93	2.17±0.09 1.28-2.75	2.23±0.11 1.34-2.93
Thickness, mm	2.67±0.17 2.36-3.03	2.19±0.11 1.25-2.97	2.24±0.12 1.12-2.99	2.29±0.08 1.62-2.85	2.24±0.12 1.12-2.99
Mass, g	0.03±0.00 0.03-0.04	0.04±0.00 0.02-0.06	0.04±0.00 0.02-0.06	0.04±0.00 0.03-0.07	0.04±0.00 0.02-0.06

Source: *compiled by the authors*

Koshova grains are characterised by a 6.4% increase in length in the control variant compared to the original, but other indicators decrease in width by 32.1% and thickness by 18.0%, respectively, while weight increases by 33.3%, indicating intensive germination processes. The water-soluble secretions of *C. sativus* L. petals slightly affected the length of the grain of wheat variety Vidrada, the increase of this indicator

was 0.9% compared to the initial one, other morphometric parameters were characterised by a slight decrease: width – by 1.04, thickness – by 2.2%. Compared to the control, there was a slight decrease in length and thickness by 1.0 and 1.1%, respectively, and an increase in width by 4.4%. The weight of the grain increased by 25% compared to the initial weight, and no increase was observed compared to the control. Koshova grains were also characterised by a slight increase in length by 2.0%, but a decrease in width and thickness (by 22.3 and 16.1%, respectively). The increase in weight compared to the initial weight was 33.3%, while no increase was observed compared to the control variant.

Water-soluble secretions of petals and stamens of *C. sativus* L. slightly affected the length and width of the kernels of Vidrada variety compared to the initial ones (1.2 and 0.7%, respectively), but the kernel thickness decreased by 7.9%. Compared to the control, there was a slight decrease in length and thickness by 0.7 and 6.9%, but an increase in width by 6.2%. Under the influence of water-soluble secretions of petals with stamens, the weight of the grain increased by 25% compared to the initial one, but there was no increase compared to the control. Kernels of the Koshova variety were characterised by an increase in length only, by 4.4% compared to the initial one, while other parameters showed a decrease in width by 24.4 and thickness by 14.2%.

Thus, no significant effect of water-soluble secretions of flowers of *C. sativus* L. of different states and elements of flowers grown in the Northern Black Sea region of Ukraine on the morphometric and qualitative parameters of wheat grains of the two studied varieties was found. The study of the effect of water-soluble secretions of *C. sativus* L. flower elements on the development of the root system of seedlings of the studied varieties indicates that it has varietal characteristics of the reaction (Table 3).

Table 3. *Influence of water-soluble secretions of C. sativus L. flowers of different states on morphometric and qualitative parameters of winter wheat roots of different varieties*

Water extracts from saffron flower elements (Factor B)	Varieties (factor A)			
	Vidrada			
	Max length, mm	Overall length, mm	Number of roots	Mass, g
Control	91.15±2.65 75.00-114.00	233.35±6.37 166.00-296.00	3.45±0.15 3.00-5.00	0.05±0.00 0.03-0.08
Flower state				
Bud	81.65±7.23 39.00-141.00	196.25±21.61 94.00-392.00	3.35±0.14 3.00-5.00	0.04±0.00 0.01-0.08
The blooming flower	92.89±7.15 22.00-134.00	254.32±27.82 46.00-437.00	3.89±0.19 3.00-5.00	0.05±0.01 0.01-0.11
The bloomed flower	106.25±5.76 50.00-135.00	284.48±18.98 108.00-405.00	3.40±0.11 3.00-4.00	0.04±0.00 0.02-0.08
Flower elements				
Petals	104.20±7.23 35.00-142.00	277.65±21.64 90.00-410.00	3.55±0.15 3.00-5.00	0.05±0.01 0.01-0.10
Petals + stamens	124.30±10.88 73.00-321.00	309.75±11.79 172.00-415.00	3.75±0.10 3.00-4.00	0.04±0.00 0.02-0.06
Petals + stamens	99.10±7.58 25.00-145.00	266.25±22.51 70.00-404.00	3.70±0.18 3.00-5.00	0.04±0.00 0.02-0.08
Koshova				
Control	121.75±3.63 84.00-145.00	311.25±9.23 226.00-375.00	3.25±0.12 3.00-5.00	0.05±0.00 0.02-0.08
Flower state				
Bud	75.89±8.94 15.00-130.00	174.79±25.67 17.00-388.00	3.21±0.12 3.00-5.00	0.03+0.00 0.01-0.07
The blooming flower	119.35±5.00 68.00-150.00	298.10±14.63 148.00-410.00	3.20±0.09 3.00-4.00	0.04±0.00 0.02-0.06
The bloomed flower	124.65±3.22 97.00-144.00	302.95±6.49 233.00-370.00	3.15±0.08 3.00-4.00	0.04±0.00 0.03-0.07
Flower elements				
Petals	114.80±6.17 55.00-153.00	281.60±16.34 130.00-362.00	3.40±0.13 3.00-5.00	0.05+0.00 0.02-0.08

Table 3, Continued

Water extracts from saffron flower elements (Factor B)	Varieties (factor A)			
	Vidrada			
	Max length, mm	Overall length, mm	Number of roots	Mass, g
Petals + stamens	99.55±9.00 68.00-150.00	239.75±26.13 35.00-393.00	3.10±0.07 3.00-4.00	0.04±0.00 0.02-0.06
Petals + stamens	112.80±8.05 38.00-153.00	281.15±20.82 102.00-419.00	3.15±0.08 3.00-4.00	0.04±0.00 0.02-0.08

Source: *compiled by the authors*

The water-soluble bud secretions harmed the root indices of Vidrada seedlings, especially on the total root length (-15.9%) and the number of roots (-2.9%). The negative effect on the seedlings of the Koshova variety was greater – -43.8% for the total length of roots and -1.2% for their number. The influence of water-soluble secretions of the blossoming flower on the parameters of roots of seedlings of the Vidrada plant variety has a slight stimulating effect, with the highest values also characteristic of the total length of roots, and the lowest – for the maximum length (9.0 and 1.9%, respectively). Seedlings of the Koshova variety are characterised by a negative effect on all root parameters compared to the control, with the greatest effect on the total length and a lesser effect on the number of roots (-7.1 and -1.5%, respectively). The water-soluble secretions of the faded flower stimulate the development of roots, especially the total length, with a smaller effect on their number (21.9 and 1.5%, respectively). A positive effect on the maximum length of roots (2.4%) was found for seedlings of the Koshova variety, but a slightly negative effect on other indicators (-2.7% for the total length of roots and -1.5% for the number of roots). The weight of roots of seedlings of Vidrada variety is characterised by a negative effect of water-soluble secretions of flowers of different states on the growth compared to the control: bud and bloomed flower – by 20%, and secretions of the bloomed flower have no effect.

The water-soluble secretions of *C. sativus* L. flowers of different states negatively affect the root weight of seedlings of the Koshova variety compared to the control, with a greater effect on the bud (-40%), and a negative effect on the root weight (-20%) for the bloomed and faded flowers. The water-soluble secretions of *C. sativus* L. flower elements have a positive effect on the growth of maximum and total root length (14.3 and 19.0%, respectively), as well as on the number of roots (2.9%) of seedlings of Vidrada plants. Root weight did not differ from the control. For seedlings of plants of the Koshova variety, water-soluble petal secretions negatively affect the maximum and total length of roots compared to the control (-5.7 and -9.5%, respectively), but positively – the number of roots (4.6%), no effect on root weight was found.

Water-soluble petal and stamen secretions also have a positive effect on the growth of the maximum and total root length of seedlings of the Vidrada variety (36.4 and 32.7%, respectively), and the number of roots (8.7%), but the root weight was 20% less. Water-soluble secretions of petals and stamens negatively affect all indicators of roots of seedlings of the Koshova variety: the greatest effect is observed on the total length of roots (23.0%), the least – on the number of roots (4.6%). The water-soluble secretions of petals and pistils have a positive effect on the growth of roots of seedlings of Vidrada plants compared to the control. The highest values are characteristic of the total length (14.1%), the lowest – for the number of roots (7.3%). In seedlings of the Koshova variety, a negative effect on the total length of roots (-9.4%) and the number of roots (-3.1%) was observed. The root weight of seedlings of both varieties is characterised by a negative effect of -20%. The effect of water-soluble secretions of the blossoming flower of *C. sativus* L. on the maximum and total length of roots of both wheat varieties is significant, the effect on the number of roots is insignificant. The water-soluble secretions of *C. sativus* L. flowers of different states negatively affect the length of the coleoptile of plants of the studied winter wheat varieties (Table 4).

Table 4. *Influence of water-soluble secretions of C. sativus L. flower of different state and flower elements on morphometric and qualitative parameters of collyoptile of winter wheat seedlings*

Water extracts from saffron flower elements (Factor B)		Varieties (Factor A)			
		Vidrada		Koshova	
		length, mm	mass, g	length, mm	mass, g
		M±m min-max	M±m min-max	M±m min-max	M±m min-max
Control		55.15±1.43 45.00-67.00	0.06±0.00 0.04-0.08	58.50±1.81 50.00-74	0.06±0.00 0.04-0.08
Flower state	Bud	42.50±4.52 13.00-75.00	0.04±0.00 0.02-0.09	39.47±5.88 3.00-82.00	0.04±0.01 0.01-0.09

Table 4, Continued

Water extracts from saffron flower elements (Factor B)		Varieties (Factor A)			
		Vidrada		Koshova	
		length, mm	mass, g	length, mm	mass, g
		M±m min-max	M±m min-max	M±m min-max	M±m min-max
Flower state	The blooming flower	51.84±4.62 12.00-76.00	0.05±0.00 0.01-0.08	53.05±2.81 25.00-69.00	0.05±0.00 002-0.07
	The bloomed flower	59.20±2.88 35.00-81.00	0.05±0.00 0.03-0.08	63.95±1.86 44.00-75.00	0.05±0.00 0.04-0.07
Flower elements	Petals	54.05±3.27 18.00-78.00	0.06±0.00 0.02-0.10	61.00±3.56 20.00-79.00	0.06±0.00 0.02-0.08-
	Petals + stamens	58.05±2.67 26.00-71.00	0.06±0.00 0.03-0.07	52.15±5.15 6.00-79.00	0.05±0.00 0.01-0.08
	Petals + stamens	53.80±3.60 15.00-77.00	0.06±0.00 0.02-0.08	56.90±14.02 18.00-75.00	0.05±0.00 0.03-0.07

Source: *compiled by the authors*

The Vidrada variety is characterised by an intensive decrease in the length of the coleoptiles under the influence of water-soluble secretions of the bud and the bloomed flower by -22.9 and -6.0%, respectively, compared to the control, while the water-soluble secretions of the bloomed flower have a slight positive effect of 7.3% compared to the control. There is also a negative effect on the weight of coleoptiles, ranging from -33.3% for bud secretions to -16.7% for bloomed and faded flowers. For coleoptiles of seedlings of the Koshova variety, the corresponding indicators range from -32.5%, which is typical for bud secretions, to -9.3%, which is typical for a bloomed flower, but the secretions of a bloomed flower have a slight positive effect compared to the control (9.3%). There is also a negative effect of water-soluble secretions on the weight of the coleoptile compared to the control by -33.3% for the bud secretions and by -16.7% for other variants.

Water-soluble petal secretions stimulate the growth of coleoptiles of Vidrada plants by 4.3%, and water-soluble secretions of petals and stamens and pistils inhibit growth compared to the control by 10.8 and 2.7%. For plants of the Koshova variety, a slight inhibition of growth under the influence of water-soluble petal secretions by -2.0% and stimulation by water-soluble secretions of petals with stamens and pistils compared to the control by 5.3 and 1.5%, respectively, is characteristic. There was no negative effect of water-soluble secretions of *C. sativus* L. flower elements on the weight of coleoptiles in seedlings of the Koshova variety, in contrast to the weight of coleoptiles of seedlings of the Vidrada variety, which is characterised by the absence of influence of water-soluble petal secretions and the inhibitory effect of water-soluble petal secretions and stamens and pistils (-16.7%).

The effect of water-soluble secretions on the length and weight of coleoptile of seedlings of the studied wheat varieties is significant. A high correlation ($p<0.05$) between the total length of the roots and the length of the coleoptile is characteristic of seedlings of the Vidrada variety in variants with the influence of water-soluble secretions of flowers of all states and elements of the flower (petals and petals with pistil). For seedlings of the Koshova variety, there is also a relationship between the total length of the roots and the length of the coleoptile under the influence of water-soluble secretions of buds and a blooming flower. The correlation between these parameters under the influence of water-soluble secretions of petals stamens and pistils is not very high and inverse (Table 5).

Table 5. *Correlation between root length and coleoptile length of winter wheat seedlings of different varieties under the influence of water-soluble secretions of C. sativus L. flowers of different states and their elements*

Aqueous extracts of saffron flower elements (Factor B)		Varieties (Factor A)	
		Vidrada	Koshova
Control		-0.0209	0.2794
Flower state	Bud	0.9276	0.9612
	The blooming flower	0.9298	0.3151
	The bloomed flower	0.7981	0.6933
Flower elements	Petals	0.7803	0.2863
	Petals + stamens	0.0195	-0.2672
Flower elements	Petals + stamens	0.7677	-0.0585

Source: *compiled by the authors*

For a complete assessment of the allelopathic activity of water-soluble secretions of *C. sativus* L. flowers of different states and flower elements on winter wheat, the index of allelopathic activity was used. The main indicators are the effect of *C. sativus* L. flower extracts on the maximum and total length of roots and colioptile of seedlings of the studied varieties. It was found that there is a negative effect of water-soluble secretions of *C. sativus* flower buds on the total and maximum root length of seedlings of the Vidrada variety and the total root length of seedlings of the Koshova variety (Table 6).

Table 6. *Index of allelopathy of water-soluble secretions of C. sativus L. flowers of different states and flower elements on the metric parameters of roots of plants of different winter wheat varieties*

Aqueous extracts of saffron flower elements (Factor B)		Varieties (Factor A)			
		Vidrada		Koshova	
		Max length, mm	Overall length, mm	Max length, mm	Overall length, mm
		M±m min-max	M±m min-max	M±m min-max	M±m min-max
Flower state	Bud	-0.12	-0.19	0.6	-0.78
	The blooming flower	0.02	0.1	-0.02	-0.04
	The bloomed flower	0.14	0.18	0.02	-0.03
Flower elements	Petals	0.13	0.16	-0.06	-0.11
	Petals + stamens	0.27	0.25	-0.22	-0.3
	Petals + stamens	0.08	0.12	0.08	-0.11

Source: *compiled by the authors*

The water-soluble secretions of the bloomed and faded flowers have a stimulating effect on these indicators of seedlings of the Vidrada variety. For the roots of seedlings of the Koshova variety, the inhibitory effect of water-soluble secretions of the bloomed flower and the opened flower, but the stimulating effect of secretions of the opened flower was established. The study of the influence of water-soluble secretions of *C. sativus* flowers and their elements on the coleoptile length of the studied winter wheat varieties shows certain peculiarities: the secretions of buds and bloomed flowers have an inhibitory effect on the coleoptile of seedlings of both varieties and the bloomed flower, on the contrary, has a stimulating effect (Table 7).

Table 7. *Index of allelopathy of water-soluble secretions of C. sativus L. flowers of different states and their elements on the development of coleoptiles of plants of different winter wheat varieties*

Aqueous extracts of saffron flower elements (Factor B)		Varieties (Factor A)			
		Vidrada		Koshova	
		Max length, mm	Overall length, mm	Max length, mm	Overall length, mm
		M±m min-max	M±m min-max	M±m min-max	M±m min-max
Flower state	Bud	-0.30	-0.48	-0.30	-0.48
	The blooming flower	-0.06	-0.10	-0.06	-0.10
	The bloomed flower	0.07	0.09	0.07	0.09
Flower elements	Petals	-0.30	0.48	-0.30	0.48
	Petals + stamens	-0.06	0.10	-0.06	0.10
	Petals + stamens	0.07	0.09	0.07	0.09

Source: *compiled by the authors*

Water-soluble extracts of petals and petals with stamens of *C. sativus* L. flowers have an inhibitory effect on the length of the coleoptiles of Vidrada plants, but petals with pistils have a stimulating effect. Water-soluble extracts of all elements of *C. sativus* L. flowers have a stimulating effect on the length of coleoptiles of seedlings of the Koshova variety. As a result of the studies, both stimulating and inhibitory effects were found from the treatment of seeds of different varieties of winter wheat with water-soluble secretions of the flower and parts of the flower of saffron seed. Other scientists (Murimwa *et al.,* 2019) focused their research on the identification and development of plant growth inhibitors as an alternative to the use of chemicals.

Studies (Godlewska, 2019) have shown that extracts of organs of other plants, in particular saffron

flowers, can be used in agriculture as natural biostimulants. Thus, A. Khoulati *et al.* (2020) found a stimulating effect of the aqueous extract of the perianth (petals) of *C. sativus* L. at a dose of 2 mg/ml on the growth of eggplant seedlings. The improvement in plant height in this study suggests that perianth extract may act as a plant growth promoter. Higher levels of these antioxidant compounds tend to disrupt the physiological balance of the recipient plants, resulting in a corresponding inhibition of growth.

Y. Tkachova *et al.* (2022) studied the effect of the allopathic activity of aqueous extracts of hyssop leaves, stems and flowers on the growth of watercress roots, and determined the optimal concentration of aqueous extracts of the culture with a stimulating and inhibitory effect. It was found that the highest positive allopathic activity was observed in the variant with the use of hyssop flowers of the second year of vegetation at a concentration of 1:10. Thus, aqueous extracts of flowers of essential oil and medicinal crops can have both stimulating and inhibitory effects on other plants. However, the scientific literature contains almost no results of studies on the effect of water-soluble flower secretions, their state (bud, bloomed and faded flower) and parts of flowers (petals, petals + stamens, petals + pistil) of saffron on the germination of winter wheat seeds. Therefore, these studies were conducted for the first time.

Earlier studies by V. Mykolaichuk *et al.* (2022) determined that saffron flowers of different flowering stages had both inhibitory and stimulating effects on the growth of watercress roots. The highest stimulatory indicators were recorded with water-soluble secretions of the newly bloomed flower and stamens, and the lowest – from the pistils. In the experiments with the winter wheat test crop, the aqueous extract from the blooming saffron flower had a greater stimulating effect, the length of roots and colloid of seedlings of the studied varieties increased by 2.4-19.9% and 7.3-9.3%, respectively. M. Alam *et al.* (2018) suggest that different rice varieties grown in the Tanjung Karang region of Malaysia contain allelochemicals that differ in type and concentration, which is why the productivity of rice varieties varied significantly. M. Korkhova and V. Mykolaichuk (2021) determined the varietal response of water extracts from the rhizosphere of the soil of different winter wheat varieties to watercress seed germination.

The study of the influence of water-soluble extracts of *C. sativus* L. flowers on the development of the root system of winter wheat seedlings confirms the different reactions of the studied varieties. It was determined that the aqueous extract from petals and stamens of sowing saffron flowers stimulated root growth of winter wheat seedlings of the Vidrada variety by 36.4%, but inhibited root growth of the Koshova variety by 18.2%. This study is unique, as there is no scientific data on the reaction of water-soluble secretions from different states of saffron flower and its parts to the germination of seeds of different winter wheat varieties.

CONCLUSIONS

The results of studies of the effect of water-soluble secretions of *C. sativum* flowers of different states and elements of flowers grown in the Northern Black Sea region of Ukraine do not significantly affect the morphometric and qualitative parameters of Vidrada and Kosova grains during germination. No significant effect of water-soluble secretions of *C. sativum* flowers and their elements on the number of roots of seedlings of both varieties was found.

The maximum and total length of roots of soft winter wheat seedlings is influenced by varietal characteristics: water-soluble secretions of the bud inhibit the development of roots of both varieties, and the bloomed flower stimulates the development of roots of seedlings of the Vidrada variety but inhibits the development of roots of seedlings of Koshova variety; water-soluble secretions of the bloomed flower stimulate the development of roots of seedlings of Vidrada variety, and in the roots of seedlings of Koshova variety stimulates only the maximum length. The water-soluble secretions of flowers of all *C. sativus* states negatively affect the weight of the crowns of seedlings of the Koshova variety, while the secretions of the bud and flower have such an effect on seedlings of the Vidrada variety.

The water-soluble secretions of *C. sativus* flower petals have a stimulating effect on the parameters of the roots of seedlings of the Vidrada variety but inhibit the parameters of the roots of the Koshova variety. Water-soluble secretions of petals and stamens, as well as petals and pistils also have a positive effect on the roots of seedlings of Vidrada plants and a negative effect on the roots of Koshova plants. Seedlings of the Vidrada variety are characterised by a high correlation between root length and coleoptile in most variants, except for the influence of water-soluble secretions of petals and stamens. The coleoptile and root length of seedlings of the Koshova variety are characterised by a high correlation under the influence of water-soluble secretions of the bud and the blooming flower, for other variants it is low, direct, or inverse.

The allelopathy index of the effect of water-soluble secretions of buds and bloomed flowers on the length of the coleoptile for plants of both varieties has an inhibitory effect, and the secretion of the bloomed flower – a stimulating effect. There is a difference in the index of allelopathy of water-soluble secretions of flower elements: for seedlings of the Vidrada variety, the secretion of petals and petals with stamens has a stimulating effect, and petals and pistils have an inhibitory effect; for coleoptiles of seedlings of Koshova variety, it has a stimulating effect. Thus, seedlings of the Vidrada variety compared to seedlings of the Koshova

variety are more resistant to the effects of water-soluble flower secretions of *C. sativus* L. The results obtained indicate that it is necessary to carefully select winter wheat varieties for compatible sowings with saffron and consider their reactions to water-soluble flower secretions. Further research will be aimed at studying the influence of weather conditions on the formation of allopathic activity of vegetative and generative organs of *C. sativus* in the Northern Black Sea region of Ukraine.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

None.

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Вплив водорозчинних виділень шафрану насіннєвого на проростання насіння сортів пшениці м'якої озимої

Віра Георгіївна Миколайчук
Кандидат біологічних наук, доцент
Миколаївський національний аграрний університет
54000, вул. Георгія Гонгадзе, 9, м. Миколаїв, Україна
https://orcid.org /0000-0003-0110-6539

Маргарита Михайлівна Корхова
Кандидат сільськогосподарських наук, доцент
Миколаївський національний аграрний університет
54000, вул. Георгія Гонгадзе, 9, м. Миколаїв, Україна
https://orcid.org/0000-0001-6713-5098

Анотація. У зв'язку зі зміною клімату постає необхідність у залученні до сільськогосподарського виробництва України нових культур, серед яких чільне місце може зайняти *Crocus sativus* L. – трав'яниста багаторічна бульбоцибулинна рослина, алелопатичні властивості якої вивчено недостатньо. Тому, метою досліджень було встановлення впливу водорозчинних виділень квіток та окремих їх елементів на початкові етапи проростання насіння двох сортів пшениці м'якої озимої (Відрада та Кошова). У ході дослідження були використані загальноприйняті методи: лабораторне дослідження, аналіз, синтез та статистичний. Було встановлено, що водорозчинні виділення квіток різного стану та елементів квіток *C. sativus* не мають істотного впливу на морфометричні та якісні показники зернівки пшениці при проростанні. Водорозчинні виділення квіток різного стану мають інгібуючу дію на розвиток кореневої системи; виділення бутону та розквітлої квітки пригнічують ріст колеоптиле. Вплив водорозчинних виділень елементів квіток шафрану посівного має сортові особливості: для проростків пшениці озимої сорту Відрада характерна стимулююча дія пелюсток на загальну довжину коренів та їх масу; для проростків сорту Кошова спостерігається зворотній вплив. Водорозчинні виділення пелюсток і тичинок мають стимулюючу дію на колеоптиле рослин сорту Відрада, а для проростків сорту Кошова стимулюючу дію мають водорозчинні виділення пелюсток. Кореляція між довжиною коренів та колеоптиле за впливу водорозчинних виділень квіток різного стану та їх елементів, а також індекс алелопатії мають сортові особливості. Отримані наукові результати сприятимуть розробці агротехнічних заходів щодо вирощування шафрану посівного в Україні, підборі сортів пшениці озимої для сумісного вирощування та використання рослинних залишків, як біологічних стимуляторів

Ключові слова: *Crocus sativum; Triticum aestivum;* алелопатичні властивості; проростки; зернівки; алелопатичний індекс

SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 92-109

UDC 634.1/7
DOI: 10.48077/scihor9.2023.92

Ecological and biological bases of creating source material of sea buckthorn (*Hippophae rhamnoides* L.) on adaptability and productivity for further breeding

Tetiana Moskalets*
Doctor of Biological Sciences, Professor
Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine
03027, 23 Sadova Str., Novosilky vil., Ukraine
https://orcid.org/0000-0003-4373-4648
Ivanna Rozhko
PhD in Agricultural Sciences, Associate Professor
Lviv National Environmental University
80381, 1 Volodymyra Velykoho Str., Dubliany, Ukraine
https://orcid.org/0000-0001-5450-0906
Bohdan Hulko
PhD in Agricultural Sciences, Associate Professor
Lviv National Environmental University
80381, 1 Volodymyra Velykoho Str., Dubliany, Ukraine
https://orcid.org/0000-0002-5915-9564
Tetiana Datsko
PhD in Agricultural Sciences, Associate Professor
Lviv National Environmental University
80381, 1 Volodymyra Velykoho Str., Dubliany, Ukraine
https://orcid.org/0000-0002-2957-1822
Ihor Rozhko
PhD in Geographical Sciences, Associate Professor
Ivan Franko National University of Lviv, Ukraine
10002, 7 Universytetska, Lviv, Ukraine
https://orcid.org/0000-0003-2263-9828

Article's History:
Received: 24.04.2023
Revised: 26.08.2023
Accepted: 27.09.2023

Abstract. The research relevance is determined by the constant striving to improve and enhance sea buckthorn genotypes by using different genetic resources and breeding methods to achieve better adaptability, higher productivity, and fruit quality. The research aims to expand the genetic diversity of sea buckthorn, which combines high productivity and quality for further breeding. Phenology, morphological studies, and evaluation of breeding material for economically valuable traits were carried out

Suggested Citation:
Moskalets, T., Rozhko, I., Hulko, B., Datsko, T., & Rozhko, I. (2023). Ecological and biological bases of creating source material of sea buckthorn (*Hippophae rhamnoides* L.) on adaptability and productivity for further breeding. *Scientific Horizons*, 26(9), 92-109. doi: 10.48077/scihor9.2023.92.

*Corresponding author

according to the methodology for the examination of plant varieties, and molecular genetic studies – according to the polymerase chain reaction method, which is based on multiple copies of a specific DNA region. The formative process in sea buckthorn breeding for adaptability, productivity and quality was expanded by using the gene pool of related forms, which is one of the ways to solve the problems of creating source material for further breeding and is the basis of the research, which established a wide range of formative process by morphological traits and biological properties as a result of the hybridisation of biotypes, which allowed to select valuable hybrids for a set of breeding and, in particular, new forms of Soborna and Adaptyvna Improved were identified, characterised by high winter and drought resistance, productivity and fruit quality, absence of thorns and dry fruit separation, and submitted to the State Variety Testing. The obtained genotypes of sea buckthorn plants combine resistance to high and low environmental temperatures with a complex of other economic traits: disease resistance, low or no thorniness, fruit quality and their suitability for processing and production of products for healthy nutrition. The practical value of the study is based on the fact that the results of the research expand the information on the use of sea buckthorn adaptability, productivity, and quality in breeding, selected and studied in the natural and fallow ecosystems of Polissia and Forest-Steppe of Ukraine. As a result of the study, the best samples were selected and included in the Genetic Bank of Plants of Ukraine as material with valuable horticultural traits and involved in breeding work at the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine

Keywords: *Hippophae rhamnoides* L.; selection; new forms; evaluation results; prospects for further breeding

INTRODUCTION

The development of new genotypes of sea buckthorn with high adaptability to changing climatic conditions, high productivity and exquisite fruit quality is an extremely important task to meet the needs of the population in food resources. Resistant to adverse biotic and abiotic environmental factors, highly productive varieties of agrobioproducts fully solve the problems of energy and resource conservation, protection of the biosphere from pollution by pollutants and management of sustainable development of agroecosystems. In works (Moskalets *et al.*, 2020; Todd, 2022), it is described that sea buckthorn is dioecious (staminate flower formula: $*K_{(2)}C_0A_4G_0$, pistillate flower – $*K_{(2)}C_0A_0G_1$), is a diploid (2n=24), wind-pollinated, facultative parthenogenesis, perennial shrub or tree with thorny or nonthorny branches, an ancient crop with modern significance and is important among the cohort of rarely cultivated fruit and berry plants, as its fruits are a source of biologically active substances.

Sea buckthorn is a typical plant of the Eurasian continent and is distributed between 27° and 69° north latitude and 7° west to 122° east longitude. Most of the native populations of sea buckthorn species and subspecies occupy natural and semi-natural ecosystems in Europe and Asia. Over time, large areas of North America and northeastern Eurasia have been occupied by introduced sea buckthorn species. the new taxonomy of the genus *Hippophae* includes seven species and eleven subspecies, as indicated in the Genetic Plasmid Information and Resource Network (GRIN): 1) *Hippophae goniocarpa*, 2) *Hippophae gyantsensis*, 3) *Hippophae litangensis*, 4) *Hippophae neurocarpa* та 2 підвиди subsp. *neurocarpa* і subsp. *stellatopilosa*, 5) *Hippophae. salicifolia*, 6) *Hippophae. tibetana*, 7) *Hippophae rhamnoides* and 8 subspecies subsp. *carpatica*, subsp. *caucasica*, subsp. *fluviatilis*, subsp. *mongolica*, subsp. *rhamnoides*, subsp. *sinensis*, subsp. *turkestanica*, subsp. *wolongensis*i subsp. *yunnanensis*. (Moskalets *et al.*, 2020).

Z. Ciesarová *et al.* (2020) pointed out that the autochthonous populations of *Hippophaer hamnoides* L. are more widespread in Eurasia than other species and, depending on the physical and geographical area and place of growth, plants of this species can be in the form of a bush (mostly in the Western European part, up to 10 m high) or a tree (Sichuan, China; Åland Islands, archipelago in the Baltic region) up to 10-18 m high. The average life span of sea buckthorn plants is 30-40 years, with a maximum of 80 years. Studies (Husain *et al.*, 2018) indicate that sea buckthorn was favoured by climate, soil, topography, and other environmental factors that led to the formation of a rich germplasm resource in the ecosystems of China, India, Pakistan, Siberia, Germany, Denmark, Romania, Hungary, Tajikistan, etc. However, the agronomic potential of many Hippophae species remains underutilised or undiscovered. J. Zhao *et al.* (2023) described that sea buckthorn in Ukraine is a source of vitamins, an ingredient in food combinatorics for the production of healthy foods and can take an important place in the structure of an orchard. According to Serhii Ostapets, CFO of Sady Donbasu (2022), producers should stop creating new apple orchards and start focusing on other crops, including sea buckthorn.

The authors (Stobdan *et al.*, 2022) noted that the high level of sea buckthorn diversity offers excellent opportunities for the genetic improvement of plants. The main objectives of most sea buckthorn breeding programmes are yield, fruit size, thornlessness, fruit size, oil content, ripening time, frost resistance, resistance to desiccation disease, fruit taste, pollen, and ornamental value. The first sea buckthorn breeding programmes began with massive selection from natural populations. This method is still an established

practice, but it is gradually being replaced by mutation and hybridisation.

Studies of genetic resources of the genus Avena sativa (Bartish & Thakur, 2022) have accumulated valuable information on the history of evolution, biogeography, genetic diversity in populations, population structure, and genes with putative specific adaptive functions in different species and taxa. Attempts to disseminate foreign varieties of sea buckthorn in production have been unsuccessful due to their low adaptability to significant fluctuations in weather conditions throughout the year (Höhn *et al.*, 2019). First of all, plants of foreign varieties cannot withstand low temperatures in winter, are not sufficiently drought-resistant, are affected by pathogens and specific pests, and are characterised by low productivity and fruit quality. According to L. Dienaite *et al.* (2021), the low level of genetic variation in sea buckthorn populations requires work to develop source material of different ecological and geographical origins. In China, the main emphasis was placed on local forms, given their high adaptability and wide ecological valence, and introduced varieties that were used in hybridisation with local ones.

The use of the diversity of natural and grassland ecosystems in the breeding process, given the narrowing of sea buckthorn variability, will expand its genetic base for further breeding for adaptability, productivity, and quality to overcome food problems, including providing the processing industry with healthy raw materials for the production of products for healthy eating. The research aims to develop new forms of sea buckthorn that combine high adaptability and productivity for further breeding.

MATERIALS AND METHODS

The research was carried out during 2017-2022 at the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine (NAAS). Based on the designed model of the variety, a programme was developed, which consistently substantiated the ways to create new sea buckthorn source material. The first stage of research was carried out in 2015-2016, which involved studying the populations of fallow land in the Polissia and Forest-Steppe zones and selecting samples with desirable economic traits. Locations of sea buckthorn fallow land populations were examined and analysed according to the methodology of S. Tkachyk *et al.* (2016). The second stage of the breeding involved the introduction of selected sea buckthorn forms into the hospital of the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine for further study and involvement of the best ones in further breeding. The soil type of the site is dark grey podzolised, medium loamy on loess loam, characterised by the following agrochemical features: humus content – 2.3%, easily hydrolysable nitrogen – 8.72 mg/100 g of soil, mobile phosphorus, and exchangeable potassium – 27.5 and 24.8 mg/100 g of soil, respectively, pH – 6.8 (Puzniak *et al.*, 2022). Planting scheme of sea buckthorn plants 4×2 m and 4×1.5 m.

In collaboration with the Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine, the following reagents were used to extract plant DNA from sea buckthorn to construct a molecular genetic profile: lysis DTT buffer with PVP (20 g/l DTT, 1.4 M NaCl, 20 mM EDTA, 100 mMTris-HClpH 8.0, 20 g/l PVP, 40.5 mM ascorbic acid, 4.0 mM DIECA); – chloroform mixture: single-atom isoamyl alcohol (24:1); absolute isopropanol; 70% ethyl alcohol; Tris+EDTA (TE) buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). The 20 µl polymerase chain reaction (PCR) reaction mix with the primers used contained 0.50 units of FIREPol®DNAPolymerase (SolisBioDyne), a thermostable polymerase enzyme, 0.50 µM primers, 2 µL of 10×ReactionbufferB PCR buffer (SolisBioDyne), 0.2 mM of each deoxyribonucleotide-3-phosphate (ThermoFisher Sci.), 50.0-100.0 ng of total deoxyribonucleic acid (DNA), deionised water Milli-Q.

Before the polymerase chain reaction, it was necessary to defrost, mix and centrifuge the special solution to the main components of the polymerase chain reaction (except for the DNA polymerase enzyme involved in DNA replication) and total DNA preparations. The process of DNA fragment fractionation involved the use of the following reagents: UltraClean™ agarose (MOBIO); LB buffer (10 mM LiOH, H_3BO_3 to pH 8.5); ethidium bromide 10 mg/ml concentration; GeneRuler™ DNA LadderMix molecular weight marker (ThermoScientific), distilled water, total DNA preparations.

An agarose gel was produced at a concentration of 1.2% in 1×LB buffer by weighing the required amount of agarose and placing it in a Simax thermobarrel to the desired volume of buffer. The mixture of agarose and buffer was then brought to complete dissolution in a microwave oven. Next, the heat-resistant jar was cooled to 60°C under a constant stream of tap water, while stirring the mixture continuously. Ethidium bromide was used as a dye at a concentration of 0.50 µg/ml. The plates were sealed with rubber spacers and the combs were placed on the required number of wells. The plates were filled, and the agarose was allowed to solidify for 30-60 min under a fume hood. Before inserting the plate into the chamber, the combs were removed. The gel plate was placed in the chamber so that it was completely covered with the buffer solution. In each well, 8 µl of sample (for PCR products) or 5 µl (for total DNA) and 1 µl of 6× application buffer was carefully added.

Next, the GeneRuler™ DNALadderMix molecular weight marker (ThermoScientific) was used (plycon sizes, bp: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1500, 2000, 2500, 3000, 3500, 4000, 5000, 6000, 8000, 10000) to determine the size of the amplification products. For this purpose, the electrode voltage was set to 3 to 7 V/cm, depending on the size

of the desired amplicons. The electrophoresis time is 2 to 4 hours. To obtain electrophoregrams, UV light was used in conjunction with a Canon camera and the GIMP graphic editor. The choice of DNA markers and primer design and their characterisation of sea buckthorn SSR primer marker loci are presented in Table 1.

Table 1. *Amplification profile of sea buckthorn EST-SSR polymorphism markers*

Locus name	Primer sequence (5'→3')	Repeat	Location	Annealing temperature (°C)	Numberofalleles
HrMS003	F:GCTCTCATC CGA TTT GAT CC R:GTC GCA GTC TTC TTG GGT TC	{TCA}6	C D S	56.0	2
HrMS012	F:CTC CAT CTC AAT CAT CAC TGC R:TTA GGG ATC CGG ATG AAG	{CTT}11	C D S	58.0	3
HrMS014	F:ATA CCT AGC TCG GCA ACA AG R: ACG ACC CAT GGC ATA ATA GTA C	{TG}6 {TA}8	3'-U TR	57.0	1
HrMS025	F:GTA CTG TGA CCA CGC TGC R: GGG TTC AAA GTA ATG GCA AG	{AG}8	3'- U TR	53.0	4
HrMS026	F:ATG ATG ACG ACG ACA ACG R: AGT GGT GGT GAC GAT AGT ATC	{CAC}5	5'-UTR	53.0	4

Source: *compiled by the authors*

Total DNA was extracted from the plant material by the RT-PCR method (cetyltrimethylammonium bromide). Then, 0.030-0.040 g of the sample was taken and placed in a 1.5 ml Eppendorf microcentrifuge tube. Next, 600 µl of DTAB buffer (2% DTAB; 1.42 M NaCl; 20 mM EDTA; 100 mM Tris-ClpH 8.0; 2% PVP; 5 mM ascorbic acid; 4.0 mM Diethyldithiocarbamic acid (DIECA)) was added to the sample and heated to 70°C. The prepared tubes were stirred using an Eppendorf Thermomixer comfort for 1 minute. The resulting mixture was added 500 µl of chloroform and isoamyl alcohol as a 24:1 mixture. All of this was stirred at 500 rpm for 5 minutes on an orbital shaker (ML-1). Next, centrifugation was performed for 5 minutes on an Eppendorf Centrifuge 5418 at 12,000 rpm, after which 200 µl of the upper liquid phase containing DNA was taken and placed in a sterile tube with 140 µl of isopropanol, stirred and left for 5 minutes at 18-20°C. After that, the sample was precipitated in a microcentrifuge at 12,000 rpm for 20 min, and then the supernatant was decanted and 800.0 µl of 70% ethanol was added. This was followed by resuspension and centrifugation for 5 minutes at 12,000 rpm. Subsequently, the supernatant was decanted again, precipitated for 2 minutes, and the ethanol residue was removed using a pipette. The precipitate was dried at 55°C and then DNA was dissolved in 100 µL TE at pH 8.0 (10 mM Tris-ClpH 8.0, 1 mM EDTA). After that, an electrophoregram was generated (Fig. 1).

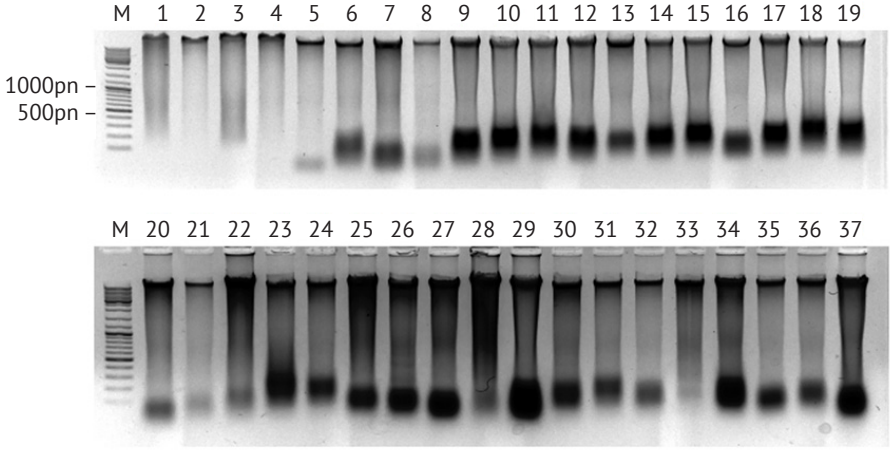

Figure 1. *Electrophoregram of total plant DNA before RNase treatment: M – molecular weight marker, 1-37 – samples of sea buckthorn: 1 – Elizaveta, 2 – Eva (standard), 3 – Karotynna (F 1-15-9), 4 – Rankova (F 1-15-4), 5 – Pyamatka (F 1-15-3), 6 – F 5- 17-144, 7 – Aboryhen (1-15-6h), 8 – Olyana (1-10-11), 9 – Nosivchanka (F 1-15-1), 10 – Apelsynova (F 1-15-6), 11 – Mitsna (F 1-15-8C), 12 – F 1-15-8D, 13 – F 4-17-213, 14 – Nosivchanka 2 (F 1-15-2b), 15 – Sonyachne syayvo (F 1-15-8B), – Obil'na (standard), 17 – Moskvichka, 18 – Nivelena, 19 – Adam, 20 – Adaptyvna (F 1-15-5), 21 – Sribnolysta 5a (F 1-15-5a), 22– Vladna (F 1-15-11), 23 – Nadiyna (F 2-15-50), 24 – Hergo, 25 – Pahorbova (F 2-15-233), 26 – Vdala (F 1-15-12), 27 – Morkvyana (F 2-15-173), 28 – F 4-17-279, 29 – Solodka zhinka, 30 – F 3-15-17, 31 – Syurpryz Baltyky, 32 – Kyyivs'kyy yantar, 33 – Chuyska, 34 – Oranzheva rannya (F 2-15-174), 35 – F 8-18-33, 36 – Lybid', 37 – F 8-18-32*

Spectrophotometric measurement of total plant DNA was then performed. Sample dilutions and controls were prepared. The sample was prepared as follows: 95 µl of deionised Milli-Q water (Millipore) and 5 µl of total DNA preparation were poured into a separate tube and mixed thoroughly. The control was treated as follows: 95 µl of water was poured into the tube, followed by 5 µl of TE buffer (pH 8). The concentration of the samples was then measured using a BioPhotometer (Eppendorf) v.1.35. The total DNA samples were then normalised to 30 ng/µL to measure their DNA concentration on a spectrophotometer. The results obtained made it possible to generate the following tabular data (Table 2).

Table 2. *Results of measuring DNA concentration on a spectrophotometer*

Sample No.	Variety	C, ng/µl	A260/280	A260/230
1	Elizaveta	564	1.48	0.59
2	Eva	366	1.75	0.68
3	Karotynna (F 1-15-9)	323	1.63	0.55
4	Rankova (F 1-15-4)	259	2.00	0.76
5	Pamiatka (F 1-15-3)	241	2.23	1.40
6	Kulykivska (F 5-17-144)	264	2.19	1.56
7	Aboryhen (F 1-15-6c)	231	2.37	1.41
8	Olyana (F 1-10-11)	136	2.68	1.33
9	Nosivchanka (F 1-15-1)	271	2.24	1.68
10	Apelsynova (F 1-15-6),	401	2.12	1.51
11	Mitsna (F 1-15-8C),	207	2.55	1.36
12	F 1-15-8D	244	2.54	1.58
13	F 4-17-213	125	3.27	1.20
14	Nosivchanka 2 (F 1-15-26)	280	2.35	1.51
15	Soniachne siaivo (Sunbeam) (F 1-15-8B)	504	1.91	1.39
16	Obilna	316	2.32	1.67
17	Moskvichka	359	2.26	1.63
18	Nivelena	360	2.00	1.29
19	Adam	596	1.93	1.47
20	Adaptyvna (F 1-15-5)	295	2.43	1.56
21	Sribnolysta 5a (F 1-15-5a)	198	2.80	1.43
22	Vladna (F 1-15-11)	235	2.39	1.38
23	Nadiina (F 2-15-50)	442	1.83	1.25
24	Hergo	240	2.22	1.35
25	Pahorbova (F 2-15-233)	302	2.10	1.42
26	Vdala (F 1-15-12)	535	1.94	1.46
27	Morkviana (F 2-15-173)	454	2.17	1.38
28	F 4-17-279	289	2.20	1.40
29	Solodka zhinka	747	2.04	1.78
30	F 3-15-17	408	2.21	1.68
31	Siurpryz Baltyky	272	2.11	1.39
32	Kyivskyi yantar	239	2.56	1.60
33	Chuiska	177	2.06	1.11
34	Oranzheva rannia (F 2-15-174)	434	2.21	1.59
35	F 8-18-33	452	2.22	1.84
36	Lybid	273	2.34	1.51
37	F 8-18-32	520	2.14	1.83

Source: *compiled by the authors*

Then sea buckthorn DNA was diluted in TE buffer, which was a solution of 4 μg of DNA sample + 8 μg of PCR buffer with the HrMS014F/HrMS014R primer system. Then, electrophoresis was performed in a 2% agarose gel (1×LB buffer + ethidium bromide), and the electrophoregram showed the following image (Fig. 2). The field data were processed using Excel 2007 and Statistica 5.5.

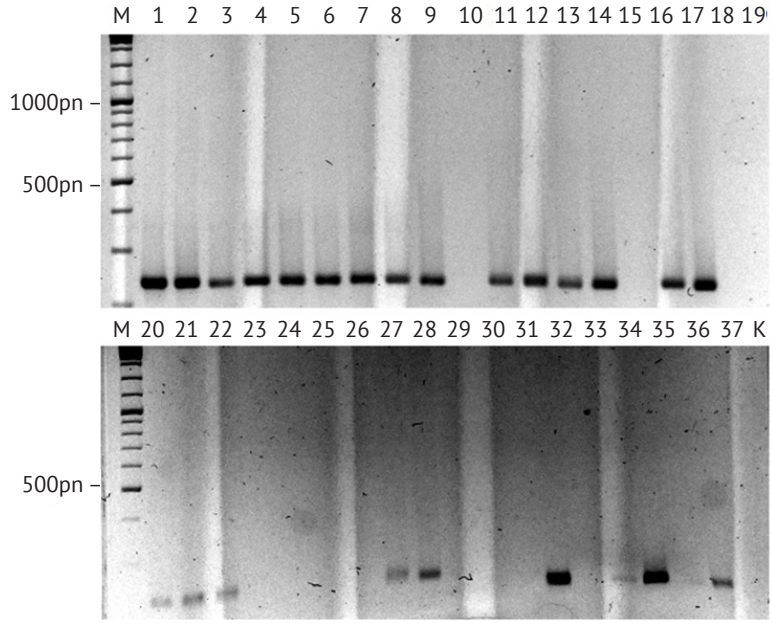

***Figure 2**. Electrophoregram of amplification products of HrMS014F/HrMS014R system after dilution of DNA samples in TE buffer: M – molecular weight marker, 1-37 – sea buckthorn samples (see Table 2) diluted 3-fold in TE buffer, K – negative control without DNA*

***Source:** compiled by the authors*

RESULTS

The research carried out at the first stage of the study allowed to identify the forms of *Hippophae rhamnoides* L., most of which belong to bushy forms (Adaptyvna, Osoblyva, Mitsna, Sonyachne syayvo, etc.), the rest – to tree-like forms (F 1-10-11, F 1-2-500, F 2-12-4, F 8-18-32), as well as to distinguish forms by growth vigour and height, depending on the selection zone. It is worth noting that the bush forms (Morkvyana, Apel'synova, Adaptyvna, Nosivchanka) and some tree-like forms (F 1-10-11, F 2-12-4, F 1-2-500) are characterised by increased annual growth rates of about 0.90-1.10 and 0.70-1.30 m, which is an important feature for nursery production. Osoblyva, Lymonna, and Sonyachne syayvo forms have a lower growth force, which allows for a reduction in the cost of manual labour for plant care when growing them in a collection nursery.

In the course of the research, the following forms of *Hippophae rhamnoides* L. were selected and studied: Nosivchanka, Mitsna, Aboryhen 6/11, Karotynna, Pam'yatka, Sonyachne syayvo, Lymonna, Morkvyana, Adaptyvna, Osoblyva, Apel'synova, F 6A/11, Sribnolysta 5a are characterised by increased winter and drought resistance (9 points), as well as resistance to vascular wilt, fruit endomycosis, fruit fly (9 points), compared to the best foreign varieties Pollmix and Slovan. It was found that forms F 1-10-11 and Morkvyana have an increased fruit weight (about 0.5-0.7 g) and fruit yield per plant (more than 10 kg/plant).

Forms Adaptyvna, F 1-2-500, and F 1-10-11 are characterised by dry detachment, annual fruiting, and, like forms Osoblyva and Nosivchanka, by the optimal ratio of polyphenolic compounds, organic acids, and sugars in fruits, which indicates their suitability for the production of healthy food. The sea buckthorn samples were registered as a working collection by the National Centre of Plant Genetic Resources of Ukraine for priority areas in breeding and genetic research (certificate No. 00292 dated 23.10.2020). Based on the breeding results, the best forms for economic traits were formed and identified, described, and systematised, taking into account the origin, morphological traits, biological characteristics, and biochemical properties.

The results of phenological observations allowed to find out that, in general, in the northern part of the Forest-Steppe of Ukraine, the beginning of the vegetation of sea buckthorn forms selected in the fallow ecosystems falls on the third decade of March – first decade of April, in Polissia – on the first decade of April. During the budding phase, the swelling and blooming of vegetative and generative buds are observed, which lasts on average 18 days (for Polissia conditions – about 21 days). However, the weather conditions of April 2022

led to the passage of certain stages of organogenesis in a shorter time, in particular, it was noted that the period from the beginning of bud development to the beginning of flowering lasted only 14 days, 7-8 days shorter than in 2019, 2020 and 2021. The deficit of precipitation in 2020 and 2021 during fruit filling caused a decrease in yield and fruit weight compared to 2019. The most stable forms by years in terms of productivity were F 1-2-500, Lymonna, Apelsynova, and less stable – Morkviana, F 1-10-11, Osoblyva (Fig. 3). The forms Adaptyvna, Osoblyva, F 1-10-11 and Lymonna were stable in terms of fruit weight (Fig. 4).

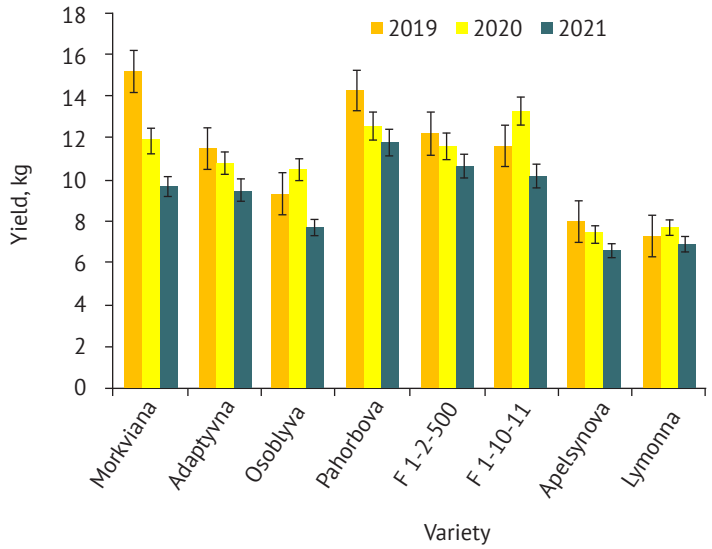

Figure 3. *Productivity of varieties and forms of Hippophae rhamnoides L. by years*
Source: *compiled by the authors*

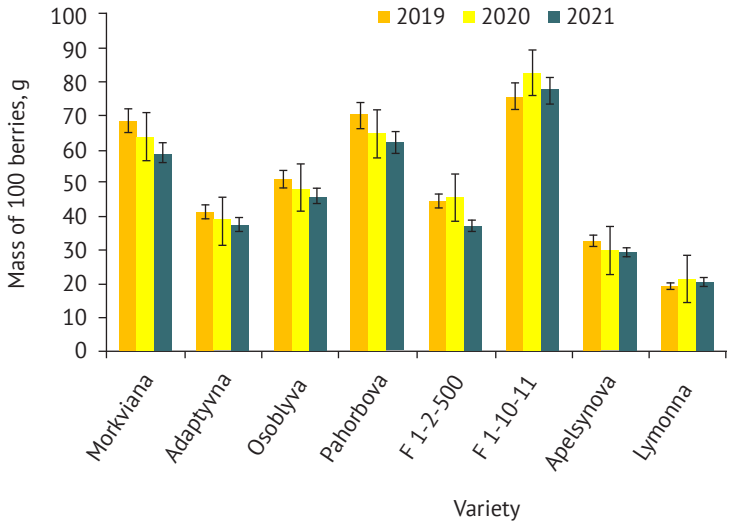

Figure 4. *Berry mass of varieties and forms of Hippophae rhamnoides L. by years*
Source: *compiled by the authors*

Genetic and epigenetic differences between the forms of *Hippophae rhamnoides* L. are inherited, which causes gene expression under the influence of environmental factors, resulting in the manifestation of economically valuable traits, the identification of which is the basis for further breeding work. Sea buckthorn plants are characterised by low environmental resistance, in particular, when introduced to unfavourable soil and climatic conditions (high air temperatures, moisture deficit, heavy soils by mechanical composition, etc.), which causes the plants to become relatively weak in development and are affected by pathogens, damaged by pests, and often fall out, in particular, male forms. It should be noted that underestimation of these facts leads to a decrease in the productivity of female plants. The PCR analysis was used to trace the difference between the forms against the background of varieties to establish the range of relatedness and to identify the genetic polymorphism of the sea buckthorn genome under study. In the process of applying 5 DNA

markers for molecular genetic characterisation of the studied culture, HrMS025 and HrMS026 were the most polymorphic, the others were also manifested in the studied samples, but mostly monomorphic (Fig. 5).

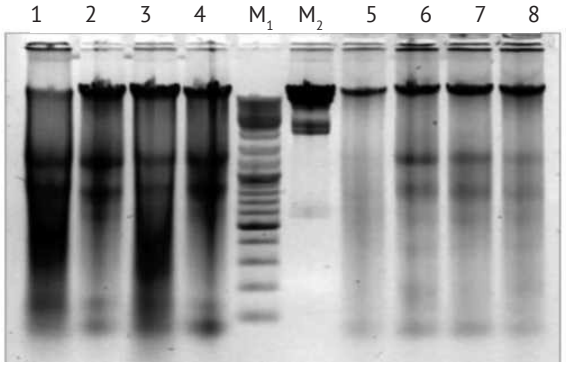

Figure 5. *Electrophoregram of total DNA of Hippophae rhamnoides L. forms in a 1% agarose gel with 1xLB electrode buffer: 1 – total DNA of the form F 6-18-01; 2 – F 4-17-279; 3 - F 3-15-17; 4 - F 5-17-144; lanes 5-8 are total DNA of Triticum aestivum L. (control); M_1 – LadderMix DNA molecular weight marker; M_2 – DNA A/HindIII molecular weight marker*

Source: *compiled by the authors*

Electrophoregrams depicting fragments of nucleotide sequences with 500 and 1000 base pairs were obtained by electrophoresis in a 2.5% agarose gel (1×LB buffer + ethidium bromide) (Fig. 6).

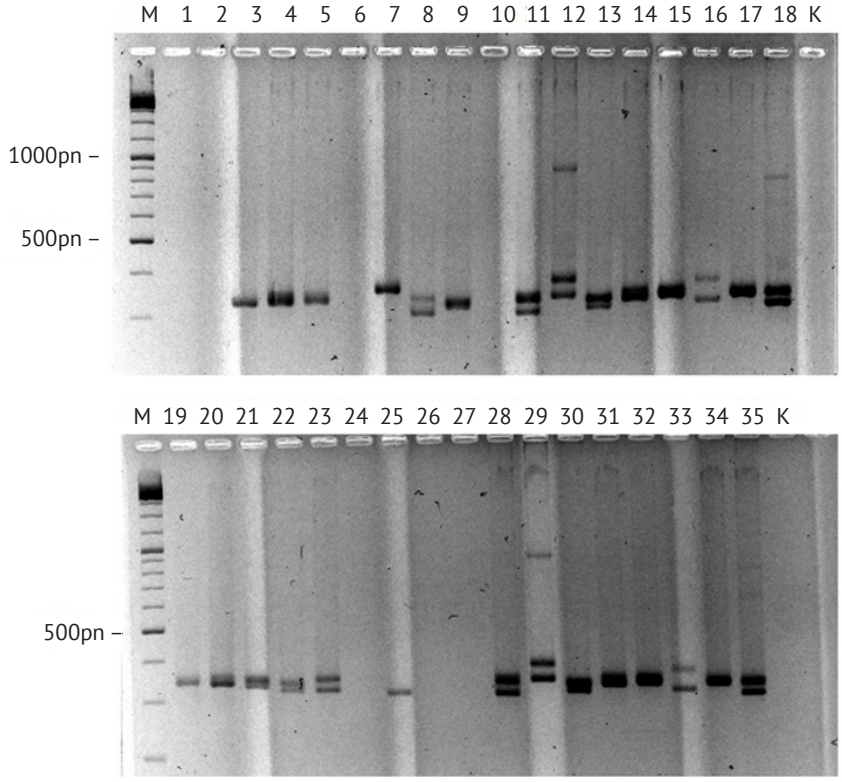

Figure 6. *Amplification products of the HrMS025F/HrMS025R system in the form of an electrophoregram, where: M – molecule mass marker, 1 – F 2-15-174, 2 – F 8-18-33, 3 – Yeva, 4– Nivelena, 5 – Moskvichka, 6 – Lybid', 7 – Yelyzaveta, 8 – Adam, 9 – Hergo, 10 – Solodka zhinka, 11, 28 – Olyana (F 6-18-01/1-10-11), 12 – F 4-17-279, 13, 30 – Kulykivs'ka (F 5-17-144), 14, 31 – Aboryhen 6/11 (F 1-15-6ch), 15, 32 – Lymonna (F 1-15-1), 16 – F 2-15-233, 17, 34 – Adaptyvna (F 1-15-5), 18, 35 – Apel'synova (F 1-15-6), 19 – Obil'na, 20 – Karotynna (F 1-15-9), 21 –1-15-8d, 22 – Pam'yatka (F 1-15-3), 23 – Nosivchanka (F 1-15-1), 24 – F 8-18-32, 25 – Syurpryz Baltyky, 26 – Kyyivs'kyy yantar, 27 – Chuyska, 29 – F 4-17-279, 33 – F 2-15-233; K – negative control without introduction of DNA*

Source: *compiled by the authors*

It was found that the use of ISSR marker A17898 for sea buckthorn samples allows for qualitative determination of polymorphism and development of genetic passports (Fig. 7, Table 3).

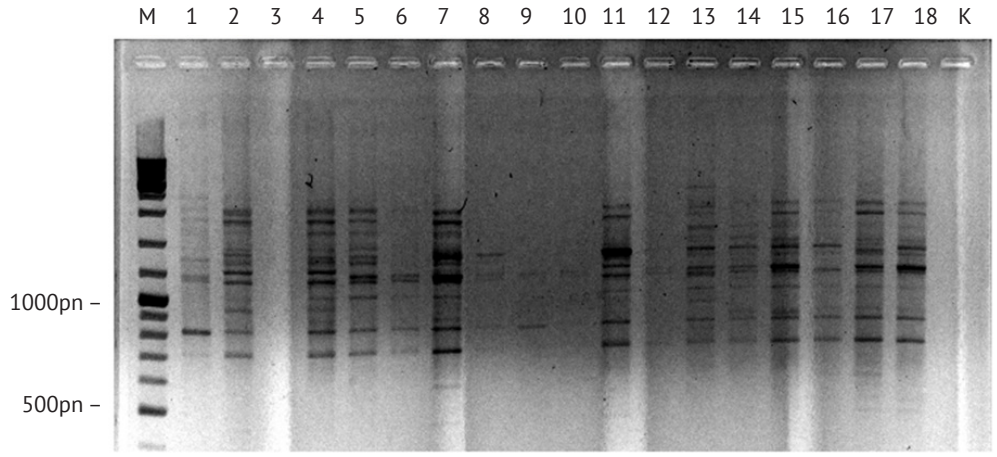

Figure 7. *Electrophoregram: the effect of using primer A17898 in the separation of amplification products: M – molecular mass marker, 1 – F 2-15-174, 2 – F 8-18-33, 3 – Yeva, 4– Nivelena, 5 – Moskvychka, 6 – Lybid', 7 – Yelyzaveta, 8 – Adam, 9 – Hergo, 10 – Solodka zhinka, 11 – Olyana (6-18-01 (1-10-11), 12 – F 4-17-279, 13 – Kulykivs'ka (5-17-144), 14 – Aboryhen 6/11 (1-15-6ch), 15 – Nosivchanka (1-15-1), 16 – 2-15-233, 17 – Adaptyvna (1-15-5), 18 – Apel'synova (1-15-6), K – negative control without introduction of DNA*

Source: *compiled by the authors*

Table 3. *Certification of sea buckthorn genotypes*

No.	Name of variety, form (breeding number)	Genetic passport formula
1	Olyana (F 6-18-01, 1-10-11)	$A_{189}B_{214+271}C_0D_{312+339}E_0F_{343}$
2	F 4-17-279	$A_0\ B_0\ C_{238}D_0\ E_{339+391}$
3	Aboryhen 3/17 (F 3-15-17)	A_{172}
4	Kulykivska (F 5-17-144)	$A_{172}\ B_{214}\ C_0\ D_{312+339}\ E_{343+365}$
5	Aboryhen 6/11 (F 1-15-6ч)	$A_0\ B_{214}\ C_0\ D_{330+339}\ E_0\ F_{343}$
6	Rankova (F 1-15-4)	$A_{172}\ B_{214}\ C_0\ D_{330+339}\ E_0\ F_{343+365}$
7	Vdala (F 1-15-11)	$A_0\ B_{214+238}\ C_0\ D_0\ E_{339}\ F_{343}$
8	Nosivska krupna (F 1-15-12)	$A_0\ B_0\ C_{238}\ D_{330+339}\ E_0$
9	Morkviana (F 2-15-173)	$A_0\ B_0\ C_{238}\ D_0\ E_{339}$
10	Pavilionna (F 2-15-174)	nd
11	Pamiati Zakharovoi (F 2-15-233)	$A_0B_0C_0D_0E_{339+391}$
12	Mikki 2 (F 4-17-213)	$A_0\ B_0\ C_{238}\ D_{330+339}\ E_0\ F_0\ G_{365}$
13	Nadiyna (F 2-15-500)	$A_0\ B_0\ C_{259}\ D_0\ E_{339}\ F_{343}$
14	Soniachne siaivo (Sunbeam) (F 1-15-8в)	$A_0\ B_0\ C_{238}\ D_{312+330}\ E_0\ F_{343+365}$
15	Karotynna (F 1-15-9)	$A_{172}\ B_{214}\ C_0\ D_{+339}\ E_0\ F_{343+365}$
16	Osoblyva (F 1-15-6c)	$A_0\ B_0\ C_{238}\ D_{312+330}\ E_0\ F_{343+365}$
17	Sribnolysta (F 1-15-5a)	$A_0\ B_{238+271}\ C_0\ D_0\ E_{339}\ F_{343}$
18	F 1-15-8d	$A_{172}B_{238+259}C_0D_0E_{339}F_{343}$
19	Pamiatka (F 1-15-3)	$A_{172}B_{214}C_0D_{312+339}E_0F_{343}$
20	Adaptyvna (F 1-15-5)	$A_0\ B_0\ C_{238+271}\ D_0\ E_{339}\ F_{343}$
21	Mitsna (F 1-15-8c)	$A_{172}\ B_{238+259}\ C_0\ D_{312}\ E_0\ F_{343}$
22	Apelsynova (F 1-15-6)	$A_{172}\ B_{238+259}\ C_0\ D_{312+339}\ E_0\ F_0\ G_{365}$
23	Nosivchanka (F 1-15-1)	$A_{172}B_{238+259}C_0D_{312+339}E_0F_{343+365}$

Table 3, Continued

No.	Name of variety, form (breeding number)	Genetic passport formula
24	F 8-18-33	nv
25	F 8-18-32	nd
26	Siurpryz Baltyky (F 5-6-17)	$A_0\ B_0\ C_0\ D_{312}$
27	Eva (F 6-2-17)	$A_{161+172}\ B_{214+271}\ C_0\ D_0\ E_{339}$
28	Nivelena (F 5-44-17)	$A_{161}\ B_{238+259}\ C_0\ D_0\ E_{339}$
29	Moskvichka (F 6-44-17)	$A_{161}\ B_{238+259}\ C_0\ D_0\ E_{339}$
30	Lybid (F 4-41-17)	nd
31	Kyivskyi yantar (F 5-41-17)	$A_0\ B_0\ C_{259}$
32	Elizabet (F 6-47-17)	$A_{172}\ B_{214+271}\ C_0\ D_0\ E_0\ F_0\ G_0\ H_{391}$
33	Chuiska (F 5-42-17)	$A_0\ B_0\ C_0\ D_0\ E_0\ F_0\ G_{365}$
34	Adam (F 8-42-17)	$A_{161+172}\ B_{238}\ C_0\ D_{312+339}$
35	Hergo (F 6-42-17)	$A_0\ B_0\ C_0\ D_0\ E_{339}$
36	Solodka zhinka (F 8-2-17)	A_{172}
37	Obylna (F 5-45-17)	$A_{161}\ B_{238+259}\ C_0\ D_0\ E_{339}$

Note: **- nd – not defined*
Source: *compiled by the authors*

PCR analysis allowed to identify the polymorphism and genetic identity of the studied sea buckthorn forms in parallel with the use of the MEGA7 programme and the UPGMA method, which were differentiated into five groups by their relationship: Group 1 – F 8-8-32, F 8-18-33, Lybid, F 2-15-174; Group 2 – Aborigine 3/17 (F 3-15-17), Sweet Woman; Group 3 – Silver-leaved 5a (F 1-15-5a), Adaptyvna (F 1-15-5); Group 4 – Nivelena, Moskvichka, Obilna; Group 5 – Nosivchanka (F 1-15-1) and Nosivchanka 2 (F 1-15-1b). The rest of the forms from the general list turned out to be heterogeneous and individual in terms of genetic components (Fig. 8).

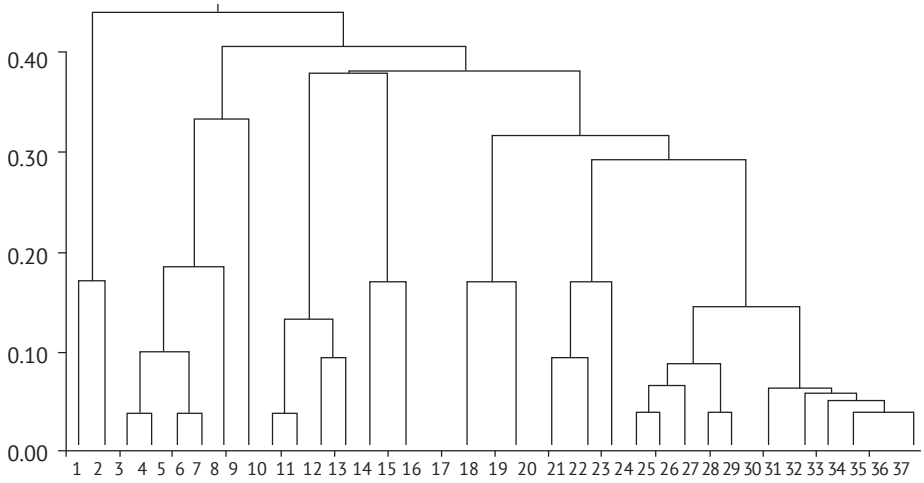

Figure 8. *The relationship of new forms of sea buckthorn in the form of a phylogenetic tree based on the results of PCR analysis: 1 – Yelyzaveta, 2 – Yeva, 3 – Karotynna (1-15-9), 4 – Rankova (1-15-4), 5 – Pam'yatka (1-15-3), 6 – 5-17-144, 7 – Aboryhen (1-15-6ch), 8 – Olyana (1-10-11), 9 – Nosivchanka (1-15-1), 10 – Apel'synova (1-15-6), 11 – Mitsna (1-15-8C), 12 – 1-15-8D, 13 –F 4-17-213, 14 – Nosivchanka 2 (1-15-2b), 15 – Sonyachne syayvo (1-15-8V), 16 – Obil'na, 17 – Moskvichka, 18 – Nivelena, 19 – Adam, 20 – Adaptyvna (1-15-5), 21 – Sribnolysta 5a (1-15-5a), 22 – Vladna (1-15-11), 23 – Nadiyna (2-15-50), 24 – Hergo, 25 – Pahorbova (2-15-233), 26 – Vdala (1-15-12), 27 – Morkvyana (2-15-173), 28 – 4-17-279, 29 – Solodka zhinka, 30 – F 3-15-17, 31 – Syurpryz Baltyky, 32 – Kyyivs'kyy yantar, 33 – Chuyska, 34 – Oranzheva rannya (2-15-174), 35 – F 8-18-33, 36 – Lybid', 37 – F 8-18-32*
Source: *compiled by the authors*

This information is important for promising directions of sea buckthorn breeding. Thus, the sea buckthorn forms studied were heterogeneous and individual. The information obtained was important for further breeding. The fruits of the varieties Nadiyna (F 2-15-50), Adaptyvna, Osoblyva, and Karotynna were also found

to be characterised by a high content of antioxidants and an inherent flavour-forming complex, which makes them suitable for the production of healthy food products. It should be added that several forms also have a high dry matter content, the share of which among other biochemical parameters varies between 18-25% (in particular, for forms F 1-15-6 and F 1-5-3). Fruits of forms F 1-15-8c, F 1-15-1, F 1-15-5a and F 1-15-2 contain higher than average soluble solids (>13%).

Based on the aforementioned, the combination of phenological observations, evaluation of breeding material for morphological traits, fruit biochemistry, and PCR analysis allowed us to select sea buckthorn forms with a set of valuable economic traits. This allowed to add new forms to the existing source material (Dublianska Osin, Prydorozhna, Pahorbova, Yaruzhna, Soborna, Adaptyvna Improved, Osinnya Krasunya, Rapsodiya), which were registered at the National Centre for Plant Genetic Resources of Ukraine, and the best ones (Soborna, Adaptyvna Improved, Osinnya Krasunya) received a certificate of registration from the General Bank of Ukraine. Sea buckthorn breeding samples are characterised by the following novelty elements, in particular, the breeding form Yaruzhna, which is a seedling from free pollination of the Adaptyvna variety, has a dry fruit detachment, no or a small number of thorns, high resistance to fruit flies (9 b), high winter and drought resistance (9 b) and low shoot formation capacity. The breeding form Dublianska Osin, whose pedigree is a seedling from free pollination of the Adaptyvna variety, is characterised by dry fruit detachment, annual fruiting, late maturity, high resistance to fruit fly (9 b), high winter and drought resistance (9 b), high vitamin C content in fruits (70 mg/100 g), and high transportability (9 b).

The breeding form Prydorozhnia, which is a seedling from free pollination of Morkvyana variety, is characterised by high growth vigour (over 1.5 m/vegetation), productivity (total number of fruits per inflorescence – 10 pcs.), high fruit yield (11 t/ha), annual abundant fruiting, medium early maturity, high winter hardiness (8 b), drought resistance (8.5 b), high content of vitamin C (72 mg/100 g), oil (3.8%), and has a universal type of fruit use. The breeding form Pahorbova is also a seedling from free pollination of the variety Morkvyana, characterised by high growth vigour (over 1.5 m/vegetation), productivity (number of fruits per inflorescence – 10 pcs.), fruit yield (11 t/ha), annual fruiting, medium early maturity, high winter and drought resistance (9 b.), high content of vitaminC (83 mg/100 g) and carotene (11.5 mg/100 g), and has a universal type of fruit used.

The breeding form Adaptyvna improved, the pedigree of which is a seedling from free pollination of the Adaptyvna variety, has the following elements of novelty: moderate or medium growth vigour (up to 0.7 m/vegetation), high productivity (increased number of fruits per inflorescence – 10 pcs.), dry fruit detachment, absence or small number of thorns, early age of fruiting (5 years), annual fruiting, late maturity, high resistance to fruit fly (9 b), high winter and drought resistance (9 b), high vitamin C content (70 mg/100 g), high transportability and fruit tasting evaluation (9 b). The breeding form Rapsodiya is a seedling from free pollination of the variety Morkvyana and has the following novelty elements: high productivity (increased number of fruits per inflorescence – ⩾10 pcs.), high level of self-fruitfulness (⩾75%), dry fruit detachment, low thorniness, high fruit yield (⩾15 t/ha), annual fruiting, high vitamin C content (120 mg/100 g), universal type of fruit use, high suitability of fruits for blended cider production.

The Soborna breeding form is a seedling from free pollination of the Nadiina variety and is characterised by high productivity (number of fruits per inflorescence – 10 pcs.), dry fruit detachment, increased fruit yield (15.6 t/ha), annual fruiting, thornlessness, high winter and drought resistance (9 b.), high vitamin C content (70 mg/100 g) and has a universal type of fruit used. The breeding forms Osinnia krasunia is a seedling from free pollination of the Morkvyana variety and is characterised by increased fruit yield (11 t/ha), dry fruit detachment, high resistance to fruit endomycosis (9 b), resistance to sea buckthorn fly (9 b), annual fruiting, late maturity, high winter and drought resistance (9 b) and fruit transportability (9 b).

Most of the new genotypes of female sea buckthorn have a tree life form (Dublianska Osin, Osinnya Krasunya, Soborna, Rapsodiya, Pahorbova, Pryrodorozhna, Yaruzhna), and one – Adaptyvna Improved – has a bush life form. Some forms (Adaptyvna Improved, Osinnya Krasunya, Soborna, Rapsodiya, Prydorozhnia) have an average growth force that does not exceed 1 m during the growing season, while the rest – Dublianskaia Osin, Yaruzhna and Pahorobova – have a growth force of about 1.5 m. All new forms have a semi-vertical position of branches, moderate crown density, average thickness of the annual shoot and the location of inflorescences on two-year-old and older shoots. It was found that Adaptyvna Improved and Soborna plants have no thorns, while the rest – Rapsodiya, Prydorozhna and Yaruzhna – have an average number of thorns. The leaf blade for all samples is narrow to broadly elliptical in shape, green with a faint silver bloom. Sea buckthorn samples differ in fruit shape and skin colour. For sea buckthorn, Dublianska Osin, Soborna, and Pryrozhna, the fruit shape is elongated-oval, Osnova Krasunya is broadly oval, Adaptyvna Improved is barrel-shaped, Rapsodiya, Pohorobova is elongated-barrel-shaped and for Yaruzhna it is rounded-barrel-shaped. The largest range of dark yellow, yellow-orange and dark orange shades is observed in Rapsodiya, Osinnya Krasunya, Pahorbova, and Pryrodozhna, while the rest (Dublianska Osin, Adaptyvna improved, Soborna, Yaruzhna) have light yellow colour (Fig. 9, Table 4).

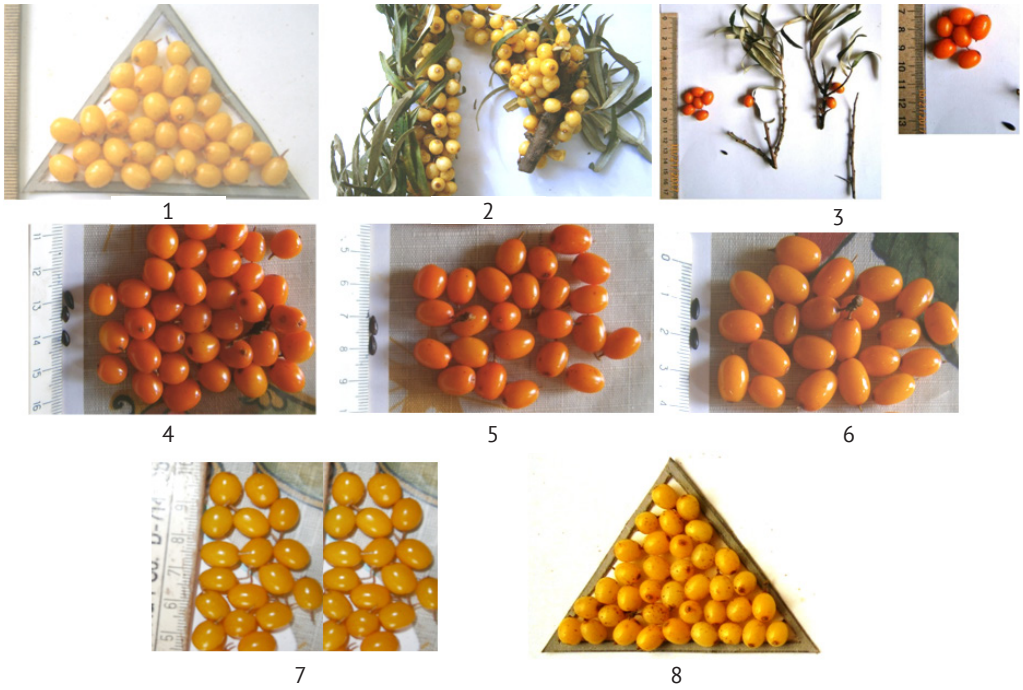

***Figure 9**. Fruits of buckthorn breeding forms: 1 – Adaptyvna Improved, 2 – Dublianska Osin, 3 – Osinnya Krasunya, 4 – Pahorbova, 5 – Prydorozhna, 6 – Rapsodiya, 7 – Soborna, 8 – Yaruzhna*

***Source:** photographed by the authors*

In particular, to describe female forms of sea buckthorn, the system of ranking morphological parameters by the following features was improved (Table 4).

***Table 4.** Improved system for ranking morphological parameters of sea buckthorn by economically valuable traits*

No.	Trait name	Trait description	Name of the variety or form that has the trait
1.	Plant lifeform	bush	2
		tree	1, 3, 4, 5, 6, 7, 8
2.	Growth strength	weak (< 0,6)	-
		average (0,6-1,0)	2, 3, 4, 5, 7
		strong (1.5 and more)	1, 6, 8
3.	Plant height	very low (up to 2 м)	-
		low (2.1-2.5 m)	-
		average (2.6-3 m)	2
		high (3.1-2.5 m)	4, 5
		very high (3.6-5 m and higher)	1, 3, 6, 7, 8
4.	Crown density	loose	
		moderate density	2, 3, 4, 5, 6, 8
		dense	1
5.	Crown form	oval	6, 7
		egg-shaped	5
		oval-bushy	2
		extensive	4, 8
6.	Branch formation	arched	-
		horizontal	-
		half-vertical	1, 2, 3, 4, 5, 6, 7, 8
		vertical	-
7.	Number of thorns (from the middle part to the top), pcs./10 cm of shoot length	No or few (1-3)	2, 4, 5, 7, 8
		average (up to 5)	1, 3, 6
		high (more than 5)	-
8.	Thorn by length, cm	none	2, 4, 8
		short (up to 1)	3, 5, 6, 7

Table 4, Continued

No.	Trait name	Trait description	Name of the variety or form that has the trait
8.	Thorn by length, cm	average (1.1-3.0)	1
		long (3.1- 5.0 and longer)	-
9.	Location of inflorescences	on annual shoots	-
		on annual and older shoots	-
		on two-year and older shoots	1, 2, 3, 4, 5, 6, 7, 8
10.	Annual shoot by thickness, cm	thin (1.5 or less)	-
		average (1.7-1.9-2.2)	1, 2, 3, 4, 5, 6, 7, 8
		thick (more than 2.5)	-
11.	The shape of the leaf blade	very narrowly elliptical	9
		narrowly elliptical	1, 2, 3, 8
		elliptical	3, 5, 7
		wide elliptical	6
12.	Leaf blade size, cm	small (< 5 x 0.45)	-
		average (more than 5.0 x 0.5)	1, 2, 3, 4, 5, 6, 7, 8
		big (> 9 x 0.9)	-
14.	Berry form	sharp	-
		rounded	-
		barrel-shaped	2
		elongated-oval	1, 4, 7
		broadly oval	3
		elongated barrel-shaped	5, 6
		rounded-barrel-shaped	8
		cylindrical	-
		egg-shaped	-
		inverse ovate	-
15.	Number of berries per flower bud	low	8
		average	3, 4
		high	1, 2, 5, 6, 7
16.	Berry skin colour	bright yellow	1, 2, 4, 8
		yellow	-
		dark-yellow	5
		orange	3
		dark-orange	6, 7
		red	-
17.	Fetal pubescence	present	-
		absent	1, 2, 3, 4, 5, 6, 7, 8
18.	Fruit stalk by length, mm	short (<3)	-
		average (from 3 to 5)	1, 2, 3, 4, 5, 6, 7, 8
		long (more than 5)	-
19.	Berry mass, g	low (up to 0.5)	1, 2, 8
		average (0.5-0.7)	3, 5, 6, 7
		high (more than 0.7)	4
20.	Vitamin C content, mg%	low (up to 50)	-
		average (51-100)	1, 2, 3, 4, 5, 6, 7, 8
		high (more than 100)	5
21.	Berry sugar content, % by fresh weight	low (up to 2.5)	-
		average (2.6-5.9)	1, 2, 3, 4, 5, 6, 7, 8
		high (>6)	-
22.	Carotene content, mg%	low (up to 10)	1, 4, 5, 7
		average (10-13.9)	2, 6, 8
		high (>14)	3
23.	Berry taste	Sour	1
		Moderately sour	5, 6, 8
		Moderately sour, pleasant	2, 3, 4, 7
24.	Time for the start of berry ripening	very early (third decade of July - first decade of August)	-
		early (second decade of August)	7

Table 4, Continued

No.	Trait name	Trait description	Name of the variety or form that has the trait
24.	Time for the start of berry ripening	average (third decade of August)	4, 5, 6
		late (I decade of September)	2, 3, 8
		very late (second decade of September)	1
25.	Yield per plant at the age of 6-7 years, kg	low (up to 6.6)	8
		average (6.7-9.9)	1
		high (more than 10)	2, 3, 4, 5, 6, 7

Note: *for all forms, the flowering time is late; the content of polyphenolic compounds in fruits is average (301-500 mg/100 g of fresh weight); the content of titratable organic acids in fruits is average (4-5 mg/weight); the content of dry soluble matter in fruits is average (10-15 mg/weight), only for Adaptyvna Improved is it high (over 15 mg/weight); fruit transportability – high (8.0-9 points); fruit tasting score – average (6.5-8.0 points); frost, winter and drought resistance – high, resistance to Fusarium wilt, fruit endomycosis, sea buckthorn fly – high (7.0-9 points); fruiting cycle – annual. Code for sea buckthorn forms: Dublianska Osin – 1, Adaptyvna Improved – 2, Osinnya Krasunya – 3, Soborna – 4, Rapsodiya – 5, Pahorbova – 6, Prydorozhnia – 7, Yaruzhna – 8, Lymonna – 9*

Source: *compiled by the authors*

Almost all of the new varieties have a medium-long stalk, which makes them suitable for easier harvesting. Adaptyvna Improved, Yaruzhna and Dublianska Osin have a low fruit weight (up to 0.5 g), while Soborna has a high fruit weight. It is worth noting that most of the samples are characterised by an average content of ascorbic acid in the fruit, only the Rapsodiya sample has a high vitamin C content (over 100 mg%). The fruits of the Adaptyvna improved form have a high content of dry soluble matter compared to the others, for which this indicator is average. Adaptyvna improved, Pahorbova, Yaruzhna and Osinnya Krasunya have medium to high carotene content. The taste of the fruit in all forms is moderately sour or moderately sour and pleasant. All samples form medium and high yields and are characterised by high winter and drought resistance (9 points), high resistance to sea buckthorn fly, Fusarium wilt, and fruit endomitosis. Genotypes differ in ripeness, in particular, the time of the beginning of fruit ripening for Pridorozhna is early, Soborna, Rapsodiya and Pahorobova are medium, Adaptyvna Improved, Osinnya Krasunya and Yaruzhna are late, and Dublianska osin is very late, which is important for both breeding and production, in particular for staggered harvesting.

The new sea buckthorn varieties have proven to be productive and adaptive, in particular, the Adaptyvna Improved (UN3700088), Osinnya Krasunya (UN3700093), Soborna (UN3700090) and Rapsodiya (UN3700091) forms have been included in the Plant Genetic Resources of Ukraine and are promising source material, of which Adaptyvna Improved and Soborna will be submitted to the State Variety Testing in 2021.

DISCUSSION

The research and development of new varieties of sea buckthorn with high adaptability to changing climatic conditions, high productivity and exquisite fruit quality is an extremely important task to ensure stable yields, increase productivity and meet the needs of the population in food resources. Sea buckthorn culture is popular not only in Europe and Asia but also on other continents (Hakeem *et al.*, 2018), due to the medicinal properties of the fruit. Therefore, the relevance of sea buckthorn culture obliges producers to grow varieties that meet modern requirements – characterised by high environmental stability, productivity, and fruit quality. In turn, such issues require the breeder to work hard and responsibly to develop appropriate source material for further breeding.

Sea buckthorn is believed to be a multipurpose medical crop (Gâtlan & Gutt, 2021). In particular, the anti-cancer activity of sea buckthorn raw materials has been proven. Much attention was also devoted to the antioxidant properties of polyphenols, flavonoids, and polysaccharides of sea buckthorn leaf and fruit extracts (Ma *et al.*, 2019; Zhuo *et al.*, 2019), including their processed products (juices, extracts, oils) (Ficzek *et al.*, 2019). Today, many Ukrainians enjoy products made from sea buckthorn fruit and consider them a great food since the products of sea buckthorn processing do not lose their beneficial properties (Olas, 2018; Tkacz *et al.*, 2020). Therefore, practitioners focus on the quality of the fruit, in particular the content of total sugars, organic acids (including malic acid), tannins, amino acids, trace elements, a complex of water- and fat-soluble vitamins, in particular ascorbic acid, and oil, as they are not sufficiently studied (Janceva *et al.*, 2022).

Breeding for quality, in particular for high oil content, is important (Olas *et al.*, 2018), as sea buckthorn oil has a positive effect on cell regeneration (Barkhuu *et al.*, 2021), has a positive effect on oral health (Smida *et al.*, 2019), is characterised by high antioxidant properties, is a source of bioavailable xanthophylls (Tudor *et al*, 2019), reduces blood cholesterol and changes the intestinal microbiota (Hao *et al.*, 2019), and is noted for its cardioprotective, anti-atherogenic, antibacterial, antiviral and anti-inflammatory properties (Ulanowska *et al.*, 2018).

According to experts (Leonov, 2022), sea buckthorn is just starting to be included in the ratings of crops popular with Ukrainian gardeners, but the global

market for berries is growing every year (Rypan, 2023). Several Ukrainian farmers have assessed the prospects for sea buckthorn cultivation. At Rovy Agro (Kyiv region), sea buckthorn plants do not require large investments in protection and nutrition (AgroTimes, 2022). To maximise the biopotential of a sea buckthorn variety, proper environmental conditions are required to determine the productivity, composition, and quality of the fruit (Pintea & Magdas, 2022).

In breeding, methods of vegetative propagation, molecular breeding genetic transformants and invitro culture are used for mass reproduction of genotypes with economically valuable traits and preservation of genetic purity of hybrids (Bansal *et al.*, 2018). Developments in sea buckthorn genomic resources have led to the creation of a variety of molecular markers, and DNA barcodes, which are important for targeted sea buckthorn breeding (Liu *et al.*, 2022). In particular, the data today shows an increase in the resources of microsatellite markers.

Summarising the aforesaid, it can be stated that scientists are concerned with the impoverishment of sea buckthorn genetic diversity, therefore, in many countries, measures are underway to create genetic banks to preserve different genotypes belonging to subspecies subsp. *caucasica*, subsp. *rhamnoides*, *carpatica*, subsp. subsp. *sinensis*, subsp. *turkestanica*, and subsp. *yunnanensis*., subsp. *mongolica*, subsp. *fluviatilis*, subsp. *wolongensis* well adapted to specific conditions to involve them in the breeding process and obtain forms resistant to adverse environmental factors with high yield and fruit quality.

CONCLUSIONS

According to the results of the first stage of analytical selection, new shrub and tree forms of sea buckthorn were selected and studied in the wetland ecosystems of Chernihiv, Kyiv, Zhytomyr, Rivne, Khmelnytsky, Lviv regions of Ukraine, such as (Aboryhen 3/17 (F 3-15-17); Kulykivskyi (F 5-17-144); Aboryhen 6/11 (F 1-15-6ch; Rankova (F 1-15-4); Vdala (F 1-15-11); Nosivska krupna (F 1-15-12); Soniachne siaivo (F 1-15-8v); Karotynna (F 1-15-9); Osoblyva (F 1-15-8b); Sribnolysta (F 1-15-5a); F 1-15-8d; Pamiatka (F 1-15-3); Adaptyvna (F 1-15-5); Mitsna (F 1-15-8s); Apelsynova (F 1-15-6); Nosivchanka (F 1-15-1); Morkviana (F 2-15-173); F 8-18-33; F 8-18-32; Nadiina (F 2-15-50) etc. The new sea buckthorn forms were found to be characterised by increased drought and winter hardiness (9 points), resistance to vascular wilt, fruit flies, and fruit endomycosis (9 points) against the background of high annual yield and fruit quality. This allowed to creation of a 1.5-hectare collection nursery at the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine in compliance with sea buckthorn cultivation technology.

The results of a detailed study of sea buckthorn samples by morphological, physiological, and biochemical parameters of the fruit allowed to improve the methodology for systematising the methods of their description both during the route and stationary study, taking into account the origin, morphological characteristics, biological features, biochemical properties of the fruit. It has been shown that the best samples of Adaptyvna improved, Osinnya Krasunya, Soborna, Rapsodiya, Pahorbova, Prydorozhnia, etc. were transferred to the National Centre of Plant Genetic Resources of Ukraine for study, and due to high yield and fruit quality, Adaptyvna, Morkviana, Osoblyva and Nadiyna were transferred to the State Variety Testing, which resulted in certificates of registration of plant samples of the gene pool of Ukraine, plant genetic collection and certificates of State Registration of plant varieties suitable for distribution in Ukraine. For the first time in Ukraine, new sea buckthorn samples were genotyped in comparison with the best varieties, and the corresponding genetic passports were developed, which indicate genetic identity, which is important for breeding.

The second stage of research involved the involvement of sea buckthorn varieties Adaptyvna, Morkviana, Osoblyva, Nadiyna, etc. in breeding and the formation of 1.5-2 thousand hybrid nursery plants under controlled and uncontrolled pollination. The breeding work allowed us to study and select forms that are valuable in terms of a set of economic traits, such as Dublianska osin, Prydorozhna, Pahorbova, Yaruzhna, Osinnya Krasunya, Rapsodiya, etc. All samples were studied at the National Centre of Plant Genetic Resources of Ukraine, and the last four samples received a certificate of registration from the Ukrainian Plant Genebank. In 2021, the varieties Soborna and Adaptyvna Improved were submitted to the State Variety Testing. New high-yielding varieties of female sea buckthorn Adaptyvna, Osoblyva, Morkviana, Nadiina, Soborna, Adaptyvna Improved and pollinator varieties Obrii, Aboryhen adapted to the conditions of Polissia and Forest-Steppe of Ukraine have been proposed for production.

ACKNOWLEDGEMENTS

We would like to take this opportunity to express our sincere gratitude to Vitalii Moskalets, a researcher at the Nosivka breeding and research station of the V.M. Remeslo Myronivka Institute of Wheat National Academy of Agrarian Sciences of Ukraine of the National Agrarian Academy of Sciences of Ukraine for his assistance in the study of fallow sea buckthorn plantations in the ecosystems of Chernihiv region, as well as scientific experts in horticulture Ivan Matlay, Zakhar Shusteruk and Vyacheslav Frantsko for their professional advice on biology, Bohdan Morgun, Doctor of Biological Sciences, Deputy Director for Scientific Work of the Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine, for his assistance in the genetic certification of sea buckthorn forms under the bilateral agreement on scientific cooperation,

to the staff of the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine for assistance in conducting agrotechnical activities in sea buckthorn nurseries, conducting biochemical analyses to determine the quality of the studied crop and processing of fruits to determine their suitability for the production of healthy food, as well as to the candidates of biological sciences Viktor Ryabchun and Olga Zadorozhna, head and leading presearcher of the National Centre of Plant Genetic Resources of Ukraine of the Plant Production Institute named after V.Ya. Yuriev of National Agrarian Academy of Sciences of Ukraine, for their work on thorough examination of applications and descriptions for sea buckthorn accessions, selection of the most valuable ones in the breeding and economic sense and their registration as accessions of the Genetic Bank of Ukraine.

CONFLICT OF INTEREST

None.

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Еколого-біологічні основи створення вихідного матеріалу обліпихи крушиноподібної (*Hippophae rhamnoides* L.) на адаптивність і продуктивність для подальшої селекції

Тетяна Захарівна Москалець
Доктор біологічних наук, професор
Інститут садівництва Національної аграрної академії наук України
03027, вул. Садова, 23, с. Новосілки, Україна
https://orcid.org/0000-0003-4373-4648

Іванна Семенівна Рожко
Кандидат сільськогосподарських наук, доцент
Львівський національний університет природокористування
80381, вул. Володимира Великого, 1, м. Дубляни, Україна
https://orcid.org/0000-0001-5450-0906

Богдан Іванович Гулько
Кандидат сільськогосподарських наук, доцент
Львівський національний університет природокористування
80381, вул. Володимира Великого, 1, м. Дубляни, Україна
https://orcid.org/0000-0002-5915-9564

Тетяна Миколаївна Дацко
Кандидат сільськогосподарських наук, доцент
Львівський національний університет природокористування
80381, вул. Володимира Великого, 1, м. Дубляни, Україна
https://orcid.org/0000-0002-2957-1822

Ігор Михайлович Рожко
Кандидат географічних наук, доцент
Львівський національний університет ім. Івана Франка
79000, вул. Університетська, 1, м. Львів, Україна
https://orcid.org/0000-0003-2263-9828

Анотація. Актуальність досліджень полягає у постійному прагненні до поповнення і покращення українського генофонду обліпихи крушиноподібної шляхом використання методів аналітичної та синтетичної селекції для формування генотипів з високою адаптивністю, продуктивністю та якістю плодів. Мета роботи полягала в створенні і вивченні цінного за господарськими ознаками генетичного різноманіття обліпихи крушиноподібної для подальшої селекції. Фенологію, морфологічні дослідження, оцінку селекційного матеріалу за господарсько-цінними ознаками проводили згідно методики проведення експертизи сортів рослин, молекулярно-генетичні дослідження – згідно методу полімеразної ланцюгової реакції, що заснований на багаторазовому копіюванні певної ділянки ДНК. У результаті виконання досліджень розширено формотворчий процес у селекції обліпихи крушиноподібної на адаптивність, продуктивність і якість шляхом використання генофонду споріднених форм, що є одним із способів вирішення проблем зі створення вихідного матеріалу для подальшої селекції й на чому базується суть досліджень, в результаті яких встановлено широкий спектр формотворчого процесу за морфологічними ознаками та біологічними властивостями в результаті гібридизації біотипів, що дозволило відібрати цінні гібриди за комплексом селекційних і господарських ознак. Зокрема, виділено нові форми Соборна і Адаптивна поліпшена, що відзначаються високою зимо- і посухостійкістю, продуктивністю і якістю плодів, характеризуюься відсутністю колючок і сухим відривом плодів та передані на Державне сортовипробування. Інші отримані генотипи рослин обліпихи поєднують стійкість до високих і низьких температур довкілля з комплексом інших господарських ознак: стійкістю до хвороб, мало- або безколючковістю, якістю плодів і їх придатністю до переробки і виготовлення продуктів для здорового харчування. Практична цінність наукової статті ґрунтується на тому, що результати дослідження розширюють відомості про використання в селекції обліпихи крушиноподібноїна адаптивність, продуктивність і якість форм, відібраних і вивчених в умовах природних і перелогових екосистем Полісся і Лісостепу України. В результаті їх вивчення відібрано кращі зразки, які увійшли до Генетичного банку рослин України, як матеріал з цінними господарськими ознаками та які залучено до селекційної роботи в Інституті садівництва Національної академії аграрних наук України

Ключові слова: *Hippophae rhamnoides* L.; добір; нові форми; результати оцінювання; перспективи для подальшої селекції

SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 110-119

UDC: 631.527.5: 633.11.5: 633.11.2
DOI: 10.48077/scihor9.2023.110

Creation and analysis of the starting material obtained by hybridisation of *Triticum spelta* L. × *Triticum compactum* Host.

Iryna Diordiieva*
PhD in Agricultural Scienses, Associate Professor
Uman National University of Horticulture
20300, 1 Institytska Str., Uman, Ukraine
https://orcid.org/0000-0002-8534-5838
Valentin Kochmarskyi
Doctor of Agricultural Scienses, Director of State Enterprise "Experimental Farm "Elite"
Mironovskyi Institute of Wheat named after V.M. Remeslo NAAS of Ukraine
08853, 68 Central Str., Central vil. Ukraine
https://orcid.org/0009-0005-9786-4685
Liudmila Riabovol
Doctor of Agricultural Scienses, Proffesor
Uman National University of Horticulture
20300, 1 Institytska Str., Uman, Ukraine
https://orcid.org/0000-0001-8988-4874
Iaroslav Riabovol
Doctor of Agricultural Scienses, Associate Professor
Uman National University of Horticulture
20300, 1 Institytska Str., Uman, Ukraine
https://orcid.org/0000-0003-4325-5313
Svitlana Karychkovska
PhD in Pedagogical Scienses, Associate Professor
Uman National University of Horticulture
20300, 1 Institytska Str., Uman, Ukraine
https://orcid.org/0000-0002-0620-4392

Article's History:
Received: 26.04.2023
Revised: 20.08.2023
Accepted: 27.09.2023

Abstract. In the conducted studies, as a result of the hybridisation of *Triticum spelta* L. × *Triticum compactum* Host. several new forms were obtained that differ in morphobiological and economically valuable traits. The research aims to expand the genetic diversity of spelt wheat and to obtain new introgressive forms with a high level of manifestation of economically valuable traits. The following methods were used**:** field, laboratory, hydrological analysis, and statistical analysis. As a result of

Suggested Citation:
Diordiieva, I., Kochmarskyi, V., Riabovol, L., Riabovol, Ia., & Karychkovska, S. (2023). Creation and analysis of the starting material obtained by hybridisation of *Triticum spelta* L. × *Triticum compactum* Host. *Scientific Horizons*, 26(9), 110-119. doi: 10.48077/scihor9.2023.110.

*Corresponding author

the research, new source material was obtained and valuable genotypes with a unique combination of genetic material of the source species were identified. An intermediate type of inheritance of plant morphology traits (plant height, ear length and density, grain weight per ear) in F_1 hybrids was established. In F_2 hybrids, a split of 3:1, indicates the dominant monogenic nature of inheritance. The shape of the ear is inherited monogenically by the type of incomplete dominance. The analysis of transgressive variability in F_{3-4} hybrids *Triticum spelta* L. × *Triticum compactum* Host. shows that the highest proportion of positive transgressions was recorded for the number of spikelets (28.1-28.5%) and grains (23.4-51.0%) in the ear and grain weight per spikelet (20.3-32.1%). The analysis of stability and adaptability indices allowed us to distinguish line 201, characterised by high stability (S^2_{di}=0.027), homeostasis (Hom=193.3) and breeding value (Sc=3.9), and line 206, combining high yield (5.11 t/ha) with high homeostasis (Hom=182.3) and breeding value (Sc=3.9). The introgressive hybridisation with *Triticum compactum* Host. identified new genetic sources characterised by the presence of deficient traits for breeding and of great practical importance for further breeding and genetic improvement of spelt, as they can enrich the existing gene pool of the crop

Keywords: spelt wheat; inheritance; cleavage; transgression; yield; adaptability

INTRODUCTION

Although the demand for spelt has been growing recently, it remains a widespread species that requires breeding improvement. Leading research institutions are engaged in spelt breeding in Ukraine, but only three varieties are available to farmers, and the source material for breeding is limited to local breeding. This leads to a narrow distribution area of spelt in agricultural production and low demand for its grain. Therefore, the creation and analysis of new source material with a broad genetic basis is an urgent task for crop breeding. The hybridisation of *Triticum spelta* L. × *Triticum compactum* Host. can produce new promising genotypes with improved quantitative traits due to the introduction of genetic material T *Triticum compactum* Host. into the *Triticum spelta* L. genome.

Intensive breeding to increase productivity has led to a significant narrowing of the wheat gene pool. This necessitated the search for natural sources of valuable traits for its breeding improvement (Voss-Fels *et al.,* 2019). The further development of farming and consumption culture has led to an awareness of the negative impact of genetic erosion and has attracted the attention of scientists and the intraspecific diversity of the genus *Triticum* L. and related taxa. L. Babenko *et al.* (2018) showed a positive effect of hybridisation of spelt with durum wheat, in particular, a significant increase in genetic diversity and the development of new transgressive forms. The same conclusion was reached by other scientists involved in the breeding improvement of spelt, in particular, R. Yakymchuk *et al.* (2020), E. Suchowilska *et al.* (2020), and J. Alvarez & C. Guzmán (2018). However, according to Rybalka *et al.* (2018), it is not advisable to limit ourselves to durum wheat, so other tetra-, octa- and diploid wheat species should be included in the hybridisation system, which will allow us to obtain starting material with a wide range of reaction rates to different soil and climatic conditions and improved grain quality. H. Akman (2021) notes the prospects of involving representatives of the closely related genus *Aegilops* L., as well as species of distant genera – *Hordeum, Secale, Agropyron*, etc. in breeding programmes.

In previous studies (Diordiieva *et al.*, 2019; Diordiieva *et al.*, 2021), the gene pool of less common hexaploid and tetraploid wheat species has been successfully used as a source of economically valuable traits for breeding and genetic improvement of wheat and triticale. I. Yadav *et al.* (2022) note that less common wheat species can serve as source material for the search for new mutant variants for genetic analysis and breeding. P. Shewry (2018) and M. Lacko-Bartošová *et al.* (2022) emphasise that ancient wheat species have several advantages over modern varieties, including valuable genes and gene blocks that determine high grain quality and adaptive potential.

One of these species could be compact or dwarf wheat (*Triticum compactum* Host.), an ancient, rare species that in the past occupied large areas in Eurasia, but nowadays is rarely found in pure cultivation. It is a hexaploid species with the genomic formula BA^uD (Wen *et al.*, 2022). Research by scientists at the V. Yuriev Institute of Plant Industry under the leadership of V. Kyrychenko *et al.* (2018) has shown that hybridisation of durum wheat with *Triticum compactum* Host. in the progeny increases sedimentation, protein, and gluten content in grain, and inherits genes for resistance to major fungal diseases. Therefore, the involvement of this species in interspecific hybridisation allows for enriching the wheat gene pool with new traits and gene blocks.

No data on hybridisation of *Triticum spelta* L. × *Triticum compactum* Host. species were found in the scientific literature. Crossing these species will allow the transfer of valuable genes that control economically valuable traits, in particular, plant height and spike density, to spelt. The research aimed to expand the genetic diversity of spelt wheat and to obtain new introgressive forms with a high level of manifestation of economically valuable traits. For this purpose, the

hybridisation of *Triticum spelta* L. and *Triticum compactum* Host species was carried out.

MATERIALS AND METHODS

The research was conducted during 2016-2021 at Uman National University of Horticulture. As a source material for hybridisation, the winter spelt wheat variety Zorya Ukrayiny (maternal form) and the sample of *Triticum compactum* Host. provided for research by the National Centre for Plant Genetic Resources of Ukraine were used. Hybridisation was carried out by manual castration of flowers of the maternal form and subsequently forced pollination with pollen of the paternal form by the limited free method under a parchment insulator. Spelt wheat was used as a mother form and *Triticum compactum* Host. was used as a pollinator. Harvesting and accounting of yield structure elements were performed in the phase of full ripeness.

The nature of inheritance of quantitative traits in F_1 hybrids was determined by the degree of dominance (hp) according to formula (1) of Griffing (1950):

$$hp = (F_1 - MP)/(P_{max} - MP), \quad (1)$$

where he is the degree of dominance; F_1 is the average value of F_1 hybrids; P_{max} is the average value of the best parental shape; MP is the average value of the two original forms.

The ranking of the created materials by the degree of dominance was carried out according to the gradation of Beyl & Atkins (1965): 1) hp>1 – positive dominance (positive heterosis); 2) 0.5<hp≤1 – positive dominance; 3) -0.5≤hp≤0.5 – intermediate inheritance; 4) -1≤hp< -0.5 – negative dominance; 5) hp< -1 – negative dominance (depression).

The proportion of hypothetical (2) and true (3) heterosis was calculated according to H. Daskalev *et al.* (1967):

Hypothetical heterosis:

$$X = F_1 \times 100/MP, \quad (2)$$

where F_1 is the level of manifestation of the indicator in the hybrid; MP is the average value of the two original forms. Real heterosis:

$$X = (F_1 - P_{max}) \times 100/P_{max}, \quad (3)$$

where F_1 is the level of manifestation of the indicator in the hybrid; P_{max} is the highest value of the indicator in one of the output forms;

The correspondence of cleavage in hybrid F_2 combinations to the theoretically expected one was assessed using $\chi 2$ (Yeshchenko *et al.,* 2014). All records and observations were carried out according to the methodology for the examination of plant varieties of the group of cereals, cereals, and legumes for their suitability for distribution in Ukraine (2016). The frequency (4) and degree (5) of transgressions were calculated according to the method of G. Voskresenskaya & V. Shpota (1967) using the following formulas:

$$Td = \frac{Ph-Pr}{Pr} \times 100, \quad (4)$$

where Td is the degree of transgression; Ph is the highest parameter for the hybrid; Pr is the highest value of the parameter in the best output form.

$$Tf = A \times 100/B, \quad (5)$$

where Tf is the frequency of transgression (%); A – excess of the best parental form in hybrid generations (averaged from the three best hybrid plants); B – the total number of plants analysed for this trait by combination.

Ecological plasticity (6) was assessed by the regression coefficient (b_i), and stability (7) by the variant of the trait (S^2_{di}). The calculations were performed according to the method of Eberhart & Russel (1966) using the formulas:

$$b_i = \sum_j Y_{ij} I_j / \sum_j I^2, \quad (6)$$

where b_i is the regression coefficient of the variety sample in environments with contrasting growing conditions; Y_{ij} – the yield of a particular variety under any growing conditions; Ij – index of year conditions.

$$S^2_{di} = \sum_i \delta^2_{ij} / (n-1), \quad (7)$$

where δ^2_{ij} is the difference between theoretical and actual yields:

The samples were ranked by environmental plasticity according to the following gradation: 1) b_i>1 – highly plastic, well adapted to unfavourable growing conditions genotype; 2) b_i=0 – medium plastic sample; 3) b_i<1 – low plastic genotype. Homeostatic (8) and breeding value coefficient (9) were determined according to V. Khangildin & N. Lytvynenko (1981) using the following formulas:

$$Hom = \frac{x^2}{\sigma(X_{opt} - X_{\min})}, \quad (8)$$

$$Sc = X(X_{lim}/X_{opt}), \quad (9)$$

where X, X_{opt}, X_{lim} – the average, highest and lowest value of a feature; σ – standard deviation.

The experimental data were analysed statistically using Microsoft Excel 2010. The smallest significant difference ($HIP_{0.5}$) and coefficient of variation (V, %) were calculated according to the method of V. Yeshchenko *et al.* (2014).

RESULTS AND DISCUSSION

Triticum compactum Host. is a hexaploid wheat species with a similar genomic composition to spelt, so there are no significant difficulties associated with genetic incompatibility during hybridisation. In the hybridisation of *Triticum spelta* L. × *Triticum compactum* Host. in the first generation, the hybrids occupied an intermediate position between the original species. F_1 hybrids were similar in spike morphology and general plant habit. They had a pubescent spike of medium length

and density. The analysis of the degree of dominance and the level of heterosis revealed that plant height, spike length and density, and grain weight per spike in F_1 hybrids *Triticum spelta* L. × *Triticum compactum* Host. are inherited by the type of intermediate inheritance (Table 1). A negative level of true heterosis was also recorded. Seeds of F_1 hybrids were sown in a breeding nursery at sparse sowing. Self-pollination of the first-generation hybrids provided a significant range of variability in the phenotype of F_2 samples, in populations of which, after the end of the growing season, the phenotypic manifestation of the characteristics of ear morphology, in particular, the shape of the ear, its pubescence and filminess, was evaluated.

Table 1. *The degree of dominance and level of heterosis in F_1 hybrids of Triticum spelta L. × Triticum compactum Host., 2017 year*

Indicator	♀	♂	F_1	LSD_{05}	Degree of dominance, (hp)	Nature of inheritance	True heterosis, %	Hypothetical heterosis, %
Plant height	118	105	114	4	0.4	PU	-3.4	102.2
Length of the ear	15.8	6.5	10.1	0.5	-0.2	PU	-36.1	90.6
Ear density	15.4	21.5	16.8	0.8	-0.5	PU	-21.9	91.1
Grain weight per ear	1.38	1.05	1.15	0.09	-0.4	PU	-16.7	94.7

Source: *compiled by the authors*

Hybridological analysis showed that in the second generation of crosses of *Triticum spelta* L. × *Triticum compactum* Host. the spike shape was inherited according to the monogenic scheme 1:2:1 (Table 2). That is, in the F_2 descendants there was a quantitative advantage of intermediate forms over speltae and compactoids.

Table 2. *Descendants F_2 breakdown by grain morphological traits*

Breakdown	Plant correlation			χ2 factors	χ2 theories
By ear form (1:2:1)					
Phenotype	speltoid	Intermediary	compact	–	
Expected amount	21	42	21	–	3.84
Factual amount	24	39	21	0.642	–
By ear pubescence (3:1)					
Phenotype	pubescent	non-pubescent		–	
Expected amount	63	21		–	3.84
Factual amount	65	19		0.126	–
Based on the ear filminess (3:1)					
Phenotype	membranous	naked		–	
Expected amount	63	21		–	3.84
Factual amount	65	19		0.126	–

Source: *compiled by the authors*

The original form of *Triticum compactum* Host. was characterised by a pubescent ear, while spelt wheat was not pubescent. In the F_2 progeny of 84 plants, 65 pubescent and 19 non-pubescent were obtained. The hybridological analysis showed that the actual results obtained are a consequence of monohybrid cleavage, which corresponds to the theoretical ratio 3:1. Thus, the trait "pubescence of the ear", which is phenotypically manifested in *the Triticum compactum* Host. was dominant to the alternative trait of spelta. The analysis of the splitting of the filminess trait in plants of the F_2 population of *Triticum spelta* L. × *Triticum compactum* Host. hybrids revealed that ear filminess is controlled monogenically, with filminess being the dominant trait concerning hulling. Similar results were obtained by R. Yakymchuk *et al.* (2020) when hybridising durum wheat with mutant forms of spelt. This indicates the dominant nature of spelt grain hulling genes concerning alternative traits of other hexaploid wheat species.

In the third-fourth hybrid generation, a wide formation process was observed. A significant range of

variability was observed in almost all morphological and physiological traits, in particular, length, shape, density, colour, pubescence and graininess of the ear, plant height, shape and colour of the leaf blade and grains, pubescence and stiffness of the spike scales and the nature of threshing, duration of the growing season and plant productivity. It was found that in 2019 (F_3) the frequency of positive transgressions for ear productivity ranged from 18.8-28.1% (Table 3). The highest proportion of positive transgressions was recorded for the number of spikelets (28.1%) and grains (23.4%) in the ear. In 2020 (F_4), the frequency of positive transgressions increased. This is consistent with the scientific literature, which states that the degree and frequency of positive transgressions increase in later generations. The frequency of positive transgressions was the highest for grain weight per ear (32.1%) and for the number of grains per ear (26.8%) (Table 3).

Table 3. *Degree and frequency of transgressions in F_{3-4} hybrids of Triticum spelta L. × Triticum compactum Host., 2019-2020*

Indicator	Td, %	Tf, %	Pr	Ph
		F_3		
Length of the ear	2.5	18.8	16.1	16.5
Number of grains per ear	2.0	23.4	51	52
Number of spikelet per ear	-10.7	28.1	28	25
Grain weight per ear	1.9	20.3	1.58	1.61
		F_4		
Length of the ear	1.6	25.6	18.2	18.5
Number of grains per ear	3.9	26.8	51	53
Number of spikelet per ear	-6.0	26.5	28.5	26.8
Grain weight per ear	3.7	32.1	1.62	1.68

Note: *Pr is the maximum value of the trait in the best parental form; Pg is the maximum value of the trait in hybrids; Td is the degree of transgression; Tf is the frequency of transgression*
Source: *compiled by the authors*

A significant disadvantage of many introduced lines is their high stemness, late maturity and tendency to lodging, which often causes their rejection in the early stages of the breeding process. It is known that only forms with a short and strong stem can ensure the practical realisation of the high genetic potential of productivity. Comparative analysis of the created samples and the original forms indicates a different nature of inheritance of plant height. Most of the forms were tall or medium tall and were close to spelt wheat in this respect (Fig. 1). Further breeding work with the created forms consisted of individual selections, which reduced the proportion of tall-stemmed forms. To preserve the valuable genetic material of the created high- and medium-stemmed forms, they were hybridised with low-stemmed forms of spelt wheat obtained in our previous studies. As a result, undersized (81-95 cm) samples were obtained.

Another disadvantage characteristic of *Triticum spelta* L. × *Triticum compactum* Host. hybrids are their late maturity. The species *Triticum spelta* L. is late ripening (vegetation period is about 300 days). Although *Triticum compactum* Host. ripens 5-6 days earlier, it is also late-ripening. As a result of hybridisation, the vast majority of the offspring were mid-season. Individual selection allowed to selection of a small number of mid-season (vegetation period 275-285 days) introgressive lines of spelt wheat, which matured simultaneously with mid-season durum wheat varieties.

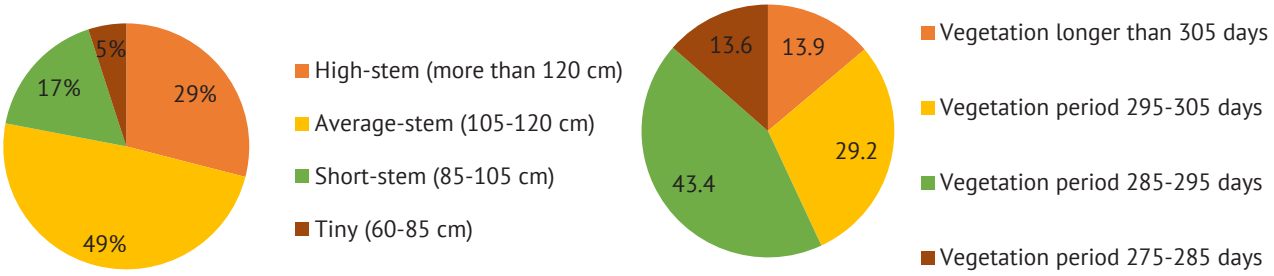

Figure 1. *Distribution (%) of created lines by plant height and length of the growing season, 2019-2020*

The parameters of adaptability, in particular, ecological plasticity and stability, of the created forms were determined in the course of the research to differentiate them by the level of adaptive potential. The analysis of yields for 2019-2021 shows that on average they ranged from 4.64-5.11 t/ha (Table 4).

Table 4. *Parameters of stability and adaptability of introgressive forms of spelt wheat in terms of yield*

Genotype	Yield, t/ha			Average	Stability parameters		Adaptiveness parameters		
	2019	2020	2021		b_i	S^2_{di}	Hom	V, %	Sc
Zoria Ukrainy (st)	5.45	4.27	5.64	5.12	1.05	0.042	175.7	4.0	3.9
192	5.15	4.21	5.28	4.88	0.83	0.031	175.7	4.0	**3.9**
195	5.25	4.32	5.36	4.98	0.81	0.035	164.3	4.1	3.7
201	5.08	4.12	5.24	4.81	0.86	**0.027**	**193.3**	4.4	**3.9**
205	4.95	4.01	5.24	4.73	0.91	0.037	170.7	4.0	3.8
206	5.38	4.41	5.54	**5.11**	0.86	0.061	**182.3**	4.1	**3.9**
208	5.05	3.98	5.21	4.75	**0.95**	**0.025**	143.5	5.6	3.2
210	4.89	3.92	5.12	4.64	0.90	0.031	170.7	4.0	3.8
LSD_{05}	*0.26*	*0.19*	*0.28*			–			

Source*: compiled by the authors*

The three studied samples were found to be not significantly inferior to the variety Zorya Ukrayiny in terms of yield. The highest yield was recorded in sample 206 – 5.11 t/ha. Calculations of the regression coefficient of the studied genotypes showed that all samples are low plastic, except for sample 208 (b_i=0.95), which belongs to medium plastic forms.

Calculations of S^2_{di} showed that the best stability parameters were characterised by samples 201 (S^2_{di}=0.027) and 208 (S^2_{di}=0.025). The relationship between homeostasis (Hom) and the coefficient of variation (V) characterises the stability of a genotype under changing environmental conditions. The most stable among the created samples were numbers 201 and 206, as indicated by a high level of homeostasis (182.3 and 193.3) and a low coefficient of variation (V=4.1-4.4%). The coefficient of breeding value (Sc) made it possible to identify highly productive forms characterised by stable realisation of productivity potential in a changing environment. In our research, the highest coefficient of breeding value was 206 (Sc=4.1), 195 (Sc=4.0) and 192 (Sc=3.9). Line 206, characterised by high yield (5.11 t/ha) and breeding value coefficient (3.9), was singled out.

Thus, the introgressive hybridisation of *Triticum spelta* L. × *Triticum compactum* Host. resulted in the creation of new source material and analysis of the patterns of trait inheritance and the formation process. A comparative analysis of productivity and adaptability parameters was carried out, which allowed the selection of valuable genotypes. Modern breeding and genetic studies have shown that morphological traits of hexaploid wheat species are determined by a small number of genes. The analysed morphological traits are controlled by the "large morphology" genes, as they significantly affect the plant phenotype. F. Hao & N. Goncharov (2019) note that the division into hexaploid wheat species is based on the shape and morphological structure of the ear, which is determined by the allelic state of these genes.

B. Xu *et al.* (2018), studying the shape of the spike in spelt, found that it is controlled by the *Q* gene, which is located on chromosome 5A. Recessive alleles of this gene determine the speltaic type of spike. M. Wen *et al.* (2022) noted that in wheat *Triticum compactum* Host. the spike shape is controlled by the *C* gene, which has the opposite effect of the *Q* gene. It causes a reduction in the length of the ear and stem, which leads to the formation of a stunted plant with a short, superdense ear and determines the main phenotypic differences of the *Triticum compactum* Host species. The spike density is also regulated by the length genes L_1, *and* L_2. In the presence of recessive alleles of all these genes (genotype cc$l_1l_1l_2l_2$), hexaploid wheat develops a short, dense club-shaped ear of the "squarhead" type.

M. Wen *et al.* (2022) noted that in the hybridisation of *Triticum compactum* Host. with durum wheat, the compact form of the ear dominates in the first generation. R. Yakymchuk *et al.* (2020) found a dominant inheritance of speltoid mutants with different types of wheat when hybridising speltoid traits with different wheat species. The results of the study indicate an intermediate inheritance of the spike shape in the hybridisation of *Triticum spelta* L. × *Triticum compactum* Host. The emergence of speltoid and compacted forms in studies is associated with different dosages of the *Q* gene in the offspring. Plants homozygous for the *qq* locus have a speltoid ear type. Increasing the dose of the *Q* gene produces offspring with a compactoid ear shape. Heterozygous genotypes (*Qq*) – have an intermediate phenotype. The presence of a significant number of plants with an intermediate spike shape can be explained by the independent recombination of genes controlling

the spike shape in the original species and the presence of modifier genes.

M. Haas *et al.* (2019), while studying genetic changes in wheat during domestication, noted that the determining factor of the free threshing phenotype is the gene for coarse spike scales (*Tg*), which is located on chromosome 2DS. Spelta contains the dominant *Tg* gene in the homozygous state, which, in combination with recessive alleles of the q gene, causes the formation of a speltoid ear type with coarse spike scales and difficult threshing. The species *Triticum compactum* Host. contains recessive alleles of the *Tg* gene, which causes free threshing of grain. This is confirmed by the lack of data in the scientific literature on filmy forms of wheat compactum with coarse spikelet scales. The conducted studies show that in the hybridisation of *Triticum spelta* L. × *Triticum compactum* Host. the progeny in the F_1 generation were characterised by a filmy ear with difficult threshing of grain. In the F_2 generation, a split was obtained in the ratio 3:1 ratio, where three parts are filmy forms and one part is hulled. Thus, filmy grain and complicated threshing of spelta are dominant traits to alternative traits of *Triticum compactum* Host.

The groups of researchers N. Grant *et al.* (2018) and U. Devi *et al.* (2019) independently found that the progeny obtained from interspecific hybridisation of wheat occupy an intermediate position between the original forms in terms of plant height. However, W. Sannemann *et al.* (2018) describe the fact of dominance and predominance of the tall parental form. They also recorded selection (additive interaction of genes) and hybrid (complementary interaction of genes) dwarfism in the offspring. There is data in the studies on the genetic control of plant height in *Triticum spelta* L. and *Triticum compactum* Host. Most scientists point to the dominant tallness of spelt. D. Packa (2018) and N. Grant *et al.* (2018) note that hybridisation of spelt with wheat forms with dominant or recessive dwarfism results in different types of gene interaction (complementary, epistatic, polymeric) and the formation of progeny with a wide range of plant height variability. In our studies, similar results were obtained with significant variation in plant height of F_{3-4} offspring (from 60 to 130 cm).

For successful breeding, positive transgressions obtained in hybrid offspring as a result of the formation of recombinations for individual valuable traits are of practical importance. N. Dubovik *et al.* (2018), studying the elements of wheat ear productivity, pointed out the wide range of transgressive variability, in particular, the frequency of positive transgressions for the number of grains per ear ranged from 16.0 to 62.0%, and for the weight of grains per year from 6.0 to 30.0%. M. Lozinsky & G. Ustinova (2020) note that the maximum frequency of positive transgressions in ear productivity is observed in later generations of hybrids, which in the first generation have a high degree of positive dominance in such important traits as grain weight per ear and number of grains per ear. Similar trends were observed in the studies conducted, where the frequency of positive transgressions for ear productivity increased in later generations.

The analysis of breeding material by stability and adaptability parameters provides researchers with information on the peculiarities of the normal response of genotypes to changing environmental conditions. The groups of researchers Y. Kuzmenko *et al.* (2023) and S. Popov *et al.* (2019) analysing wheat source material for adaptability concluded that these indicators can be most fully assessed by environmental plasticity or regression coefficient (b_i), environmental stability or stability variant (S^2_{di}) and homeostasis. Genotypes with low ecological plasticity and low S^2_{di} values are considered extensive and unprofitable. Genotypes with high environmental plasticity and low S^2_{di} are considered intensive. The results of the study indicate a high yield of sample 206 combined with high homeostatic and medium ecological plasticity and high stability of sample 208, which makes it possible to use them for breeding improvement of spelt by adaptive potential.

CONCLUSIONS

The hybridisation of *Triticum spelta* L. × *Triticum compactum* Host. resulted in the production of new source material with a broad genetic basis and the identification of valuable forms with a high level of manifestation of economically valuable traits. It was found that in F_1 hybrids *Triticum spelta* L. × *Triticum compactum* Host. morphological traits of plants (plant height, length and density of spikelet, weight of grain per spike) are inherited by the type of intermediate inheritance. The pubescence and filminess of the ear are dominantly monogenic. The shape of the ear is inherited monogenically by the type of incomplete dominance. In hybrids F_{3-4} *Triticum spelta* L. × *Triticum compactum* Host. the highest frequency of positive transgressions was recorded for the number of spikelets (28.1-28.5%) and grains (23.4-51.0%) in the ear and grain weight per ear (20.3-32.1%).

The calculation of adaptability indices allowed to differentiate the created forms by the norm of reaction to changing growing conditions. The line 201, characterised by high stability (S^2_{di}=0.027), homeostasis (Hom=193.3) and breeding value (Sc=3.9), and line 206, combining high yield (5.11 t/ha) with high homeostasis (Hom=182.3) and breeding value (Sc=3.9), were identified. A promising direction for further research is the involvement of the created forms in the selection and genetic improvement of spelt wheat to induce the formative process and isolate valuable genotypes with improved quantitative and qualitative productivity.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Створення та аналіз вихідного матеріалу отриманого за гібридизації *Triticum spelta* L. × *Triticum compactum* Host.

Ірина Павлівна Діордієва
Кандидат сільськогосподарських наук, доцент
Уманський національний університет садівництва
20300, вул. Інститутська 1, м. Умань, Україна
https://orcid.org/0000-0002-8534-5838

Валентин Сергійович Кочмарський
Доктор сільськогосподарських наук, директор
Державне підприємство «Дослідне господарство «Еліта»
Миронівського інституту пшениці ім. В. М. Ремесла НААН України
08853, вул. Центральна 68, с. Центральне, Україна
https://orcid.org/0009-0005-9786-4685

Людмила Олегівна Рябовол
Доктор сільськогосподарських наук, професор
Уманський національний університет садівництва
20300, вул. Інститутська 1, м. Умань, Україна
https://orcid.org/0000-0001-8988-4874

Ярослав Сергійович Рябовол
Доктор сільськогосподарських наук, доцент
Уманський національний університет садівництва
20300, вул. Інститутська 1, м. Умань, Україна
https://orcid.org/0000-0003-4325-5313

Світлана Петрівна Каричковська
Кандидат педагогічних наук, доцент
Уманський національний університет садівництва
20300, вул. Інститутська 1, м. Умань, Україна
https://orcid.org/0000-0002-0620-4392

Анотація. У проведених дослідженнях в результаті гібридизації *Triticum spelta* L. × *Triticum compactum* Host. отримано декілька нових форм, які відрізняються за морфобіологічними та господарсько-цінними ознаками. Метою дослідження є розширення генетичного різноманіття пшениці спельти та отримання нових інтрогресивних форм з високим рівнем прояву господарсько-цінних ознак. У роботі використовували такі методи: польовий, лабораторний, гідрологічний аналіз, статистичний аналіз. В результаті досліджень отримано новий вихідний матеріал та виділено цінні генотипи з унікальним поєднанням генетичного матеріалу вихідних видів. Встановлено проміжний тип успадкування морфологічних ознак рослин (висота рослин, довжина і щільність колоса, маса зерна з колоса) у гібридів F_1. У гібридів F_2 розщеплення 3:1, що свідчить про домінантний моногенний характер успадкування. Форма колоса успадковується моногенно за типом неповного домінування. Аналіз трансгресивної мінливості у F_{3-4} гібридів *Triticum spelta* L. × *Triticum compactum* Host. показав, що найбільша частка позитивних трансгресій зафіксована за кількістю колосків (28,1-28,5 %) і зерен (23,4-51,0 %) у колосі та масою зерна з колоса (20,3-32,1 %). Аналіз показників стабільності та адаптивності дозволив виділити лінію 201, що характеризується високою стабільністю (S^2_{di}=0,027), гомеостазом (Hom=193,3) та селекційною цінністю (Sc=3,9), та лінію 206, що поєднує високу врожайність (5,11 т/га) з високим гомеостазом (Hom=182,3) та селекційною цінністю (Sc=3,9). В результаті інтрогресивної гібридизації з *Triticum compactum* Host. виявлено нові генетичні джерела, які характеризуються наявністю дефіцитних для селекції ознак і мають велике практичне значення для подальшої селекції та генетичного поліпшення спельти, оскільки вони можуть збагатити існуючий генофонд культури

Ключові слова: пшениця спельта; успадкування; розщеплення; трансгресія; урожайність; адаптивність

SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 120-130

UDC 633.174:631.5
DOI: 10.48077/scihor9.2023.120

Sorghum (*Sorghum bicolour* (L.) Moench) growth and development features under the influence of growth regulator

Liudmyla Pravdyva*
PhD in Agricultural Sciences, Senior Research Fellow
Institute of Bioenergy Crops and Sugar Beet of NAAS
03110, 25 Klinichna Str., Kyiv, Ukraine
https://orcid.org/0000-0002-5510-3934
Oleg Prysiazhniuk
Doctor of Agricultural Sciences, Professor
Institute of Bioenergy Crops and Sugar Beet of NAAS
03110, 25 Klinichna Str., Kyiv, Ukraine
https://orcid.org/0000-0002-4639-424X
Valerii Khakhula
PhD in Agricultural Sciences, Associate Professor
Bila Tserkva National Agrarian University
09110, 8/1 Soborna Sq., Bila Tserkva, Ukraine
https://orcid.org/0000-0003-4100-262X
Lesia Kachan
PhD in Agricultural Sciences, Associate Professor
Bila Tserkva National Agrarian University
09110, 8/1 Soborna Sq., Bila Tserkva, Ukraine
https://orcid.org/0000-0001-5374-3252
Taras Panchenko
PhD in Agricultural Sciences, Associate Professor
Bila Tserkva National Agrarian University
09110, 8/1 Soborna Sq., Bila Tserkva, Ukraine
https://orcid.org/0000-0003-1114-5670

Article's History:
Received: 21.04.2023
Revised: 10.08.2023
Accepted: 27.09.2023

Abstract. There has been a growing interest in growing sorghum (*Sorghum bicolor* L. (Moenh) as a bioenergy crop, as it can be used to produce biofuels (ethyl alcohol) and solid fuels (pellets and briquettes from the above-ground mass). Sorghum grain is characterised by a high starch content of up to 80%. The research topic is relevant but poorly understood. The research aims to study the effect of growth regulators on the growth and development of sorghum in the conditions of the Right-Bank Forest-

Suggested Citation:
Pravdyva, L., Prysiazhniuk, O., Khakhula, V., Kachan, L., & Panchenko, T. (2023). Sorghum (*Sorghum bicolour* (L.) Moench) growth and development features under the influence of growth regulator. *Scientific Horizons*, 26(9), 120-130. doi: 10.48077/scihor9.2023.120.

*Corresponding author

Steppe of Ukraine. The following research methods were used: field, measuring and weighing, mathematical and statistical. The study presents the results of research for 2016-2019, where the influence of elements of cultivation technology on the peculiarities of the formation of biometric parameters was studied. plant growth regulator had an impact on field germination, vegetation period, biometric indicators of plant growth, and leaf area. The closeness of the correlations of the studied indicators was established, and a correlation-regression analysis of the data was carried out, which showed a strong correlation between the growing season and plant height, where the coefficient was R=0.9264, and the coefficient of determination R^2=0.9864. There was also a strong relationship between stem diameter and plant height, where the correlation coefficient was R=0.9767 and the coefficient of determination was R^2=0.954. The conducted studies confirm the feasibility of using a growth regulator that improves plant development and will contribute to increasing the yield and quality of grain and biomass of sorghum. The obtained results of the research give grounds to believe that growth regulators contribute to the production of environmentally friendly products and are components of environmentally friendly and energy-saving cultivation technology

Keywords: sorghum; growth regulator; seed quality parameters; vegetation period; biometric parameters; leaf area; correlation

INTRODUCTION

Establishing the feasibility of using a growth regulator by treating seeds and spraying sorghum crops to ensure high yields and quality of grain and biomass as raw material for biofuel production determines the research relevance. Following R. Saxena *et al.* (2018), R. Ortiz-Cruz *et al.* (2020) and S. Rajini *et al.* (2020) sorghum (*Sorghum bicolour* (L.) Moench) is a cereal crop belonging to the Poaceae family, originating in Africa, and domesticated 3-5 thousand years ago. According to D. Fuller & C. Stevens (2018), and B. Pandian *et al.* (2022), sorghum can withstand a variety of environmental conditions, including low soil fertility, high temperatures, and insufficient rainfall. It is cultivated primarily in semi-arid areas of Africa and Asia, where it is used for human consumption and is a staple food for local populations.

K. Aregawi *et al.* (2022), P. Espitia-Hernández *et al.* (2022) and Q. Yang *et al.* (2022) note that sorghum is the fifth most important grain crop in the world after wheat, rice, corn and barley. In some regions, it is replacing maize due to its high yields and resistance to drought and heat. As noted by B. Pandian *et al.* (2022), it is a short-day crop that uses moisture and precipitation efficiently, resumes growth after a long dry period, and generates high productivity, which means that sorghum can be grown in arid areas and the face of climate change.

Following C. Mundia *et al.* (2019) and T. Begna (2021a, b) sorghum is used as food, feed, fodder and fuel, so it has new market needs in the world. M. Nurmet *et al.* (2019) note that the world is facing the problem of depletion of conventional fossil fuel sources and a forecast of a doubling of energy demand within a decade, thus raising concerns about unreliable energy supply in the future. J. Dahlberg (2019), L. Pravdyva (2021) and L. Pravdyva *et al.* (2022) found that sorghum is an energy crop, as it can be used for the production of bioethanol (ethyl alcohol) and solid fuel: the aboveground mass is used to produce briquettes and pellets. Therefore, given the versatility of sorghum use, it is relevant and integral to study the elements of cultivation technology, in particular, an important element that affects the formation of many processes of growth, development and productivity of the crop is the use of growth regulators.

According to T. Makoveichuk *et al.* (2018), one of the ways to solve this problem is to use biologically active substances in modern crop cultivation technologies that can regulate plant growth processes and protect them from abiotic and biotic stresses. Plant growth and development regulators are synthetic and natural organic chemicals that are biologically active, even in small quantities, and cause changes in the physiological and biochemical processes of plants. According to A. Panfilova *et al.* (2019), biological products stimulate the growth and development of agricultural plants, increase resistance to stress and disease, and provide balanced nutrition. This effect is achieved by live bacteria converting insoluble compounds into available forms, providing nitrogen nutrition, and protecting plants from bacterial and fungal diseases. V. Hamaiunova *et al.* (2019) argue that the use of mineral fertilisers and growth regulators in cereal crop cultivation technologies helps to optimise plant nutrition throughout the growing season. Plant growth stimulants increase the intensity of metabolic and growth processes in plants, resulting in increased crop productivity and improved product quality.

The research aims to determine the effect of the growth regulator on the quality of sorghum seeds, the duration of the growing season, biometric parameters, and correlation in the conditions of the Right-Bank Forest-Steppe of Ukraine.

MATERIALS AND METHODS

The research was carried out in 2016-2019 at the Bila Tserkva Experimental Breeding Station of the Institute

of Bioenergy Crops and Sugar Beet of the National Academy of Agrarian Sciences of Ukraine – the Right-Bank Forest-Steppe zone of Ukraine. The research scheme envisages a two-factor experiment: seed treatment (factor A) and treatment of crops with a growth regulator (Factor B) (Table 1).

Table 1. *Study plan*

Factor A: Seed treatment	**Factor B: Treatment of crops with a growth regulator**
1. No treatment – control (water)	1. Untreated seeds + spraying of crops with a regulator
2. Seeds treated with a regulator	2. Treated seeds + and spraying of crops with a regulator

Source: *compiled by the author*

The area of the sowing plot is 50 m^2, the accounting plot is 25 m^2, and the replication of the experiment is four times. The plots were arranged by the method of systematic replication: in each replication, the experimental variants were placed in the plots sequentially. Sowing of seeds was carried out at a depth of 4-6 cm, row spacing of 45 cm, and density of 200 thousand seeds/ha. Following the methodology of M. Roik *et al.* (2020) and L. Pravdyva *et al.* (2021), crops were recorded and monitored. To establish the peculiarities of growth and development of sorghum plants, the following were determined: laboratory and field germination of seeds, duration of the growing season from emergence to full maturity; and biometric parameters.

The soil of the experimental site is typical deep low-humus chernozem of coarse-dusty medium loamy granulometric composition. Magnesium and calcium carbonates occur at a depth of 55-65 cm. The topsoil (0-30 cm) contains about 17% of silt particles and 46-54% of coarse dust. The terrain is flat, with a groundwater table of 8 m. The agrophysical and agrochemical properties of the arable (0-30 cm) soil layer are characterised by the following indicators: humus – 3.5%, total nitrogen – 0.31%; hydrolytic acidity – 2.41 mg-eq; easily hydrolysed nitrogen (N) – 134 mg, P_2O_5 – 276 mg, K_2O – 98 mg per 1000 g of soil. The degree of saturation with bases is 90%.

Weather conditions in the years of study during the growing season were characterised by minor deviations with an excess of long-term indicators (Figs. 1, 2). In 2016, the average air temperature during the growing season was 17.9°C, which is 2.48°C higher than the average long-term indicators. In 2017, the average air temperature was 17.2°C, which exceeded the long-term average by 1.85°C. In 2018 and 2019, the average air temperature for the growing season exceeded the long-term average by 2.91 and 1.83°C.

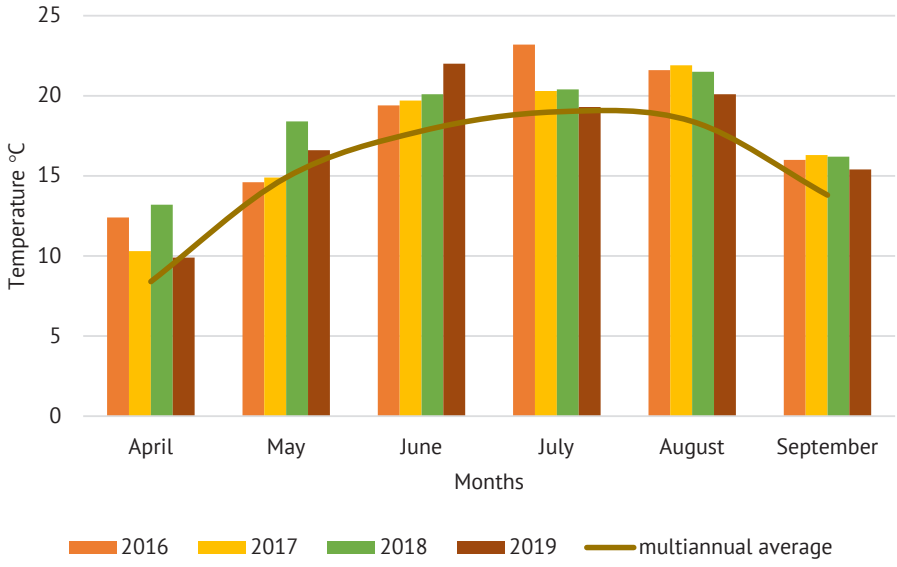

Figure 1. *Air temperature during the growing season in 2016-2019*

The amount of precipitation was uneven across months and below the long-term average. In 2016, during the growing season, the amount of precipitation was 102.6 mm less than the long-term average. In 2017, the amount of precipitation was 186.8 mm, which is 159.2 mm less than the long-term average. In 2018-2019, the amount of precipitation was 84.8 and 72.7 mm less than the long-term average.

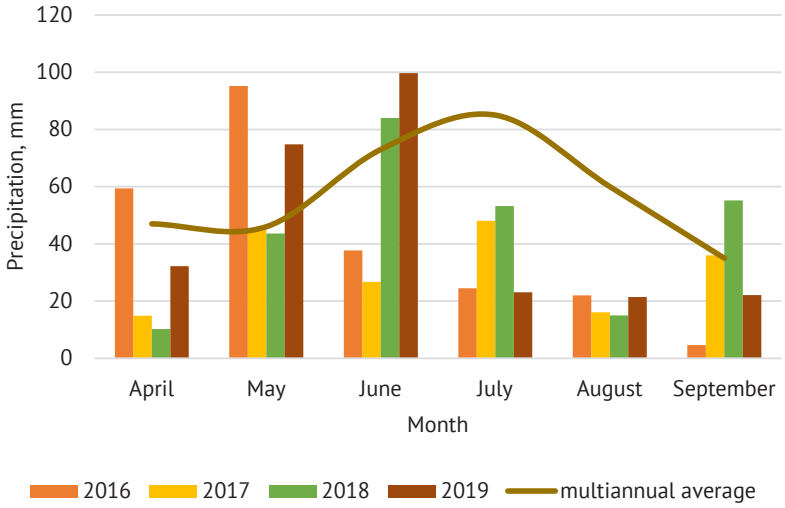

Figure 2. *Precipitations during the growing season in 2016-2019*

However, for the cultivation of sorghum, the soil and climatic conditions were typical of the conditions of the research and favourable for the cultivation of this crop. The experiment used the growth regulator Vermistim, a complex preparation made as an extract from vermicompost obtained through vermiculture as a product of worms' vital activity after they process organic waste. It consists of a wide range of organic and mineral substances, including humates, fulvic acids, and strains of beneficial soil organisms in dissolved and active form. The complex of organic substances also includes regulatory, micro, and macro elements, vitamins, and phytohormones.

Statistical analysis. To determine the statistical significance of the treatment effects (P=0.05 or less), after first undergoing an analysis of variance (ANOVA), all data were analysed with the software SAS (SAS Institute Inc., USA). Significant differences between individual means were determined using the least significant difference (LSD) test. Correlation and regression analysis was carried out using a PC in Excel based on the research results.

RESULTS AND DISCUSSION

Analysing the research results, it was found that sorghum responds positively to the treatment of seeds and crops with a growth regulator. Thus, the germination energy and laboratory germination of seeds were 93.4 and 97.8%, which is 2.3 and 3.2% less than the control (seed treatment with water). Field germination was slightly lower than the laboratory germination and amounted to 87.2% in the variant without treatment with the growth regulator, 91.5% in the variant where only seeds were treated with the preparation, 87.4% in the variant with untreated seeds but sprayed crops, and 90.8% with seed and crop treatment (Table 2). The use of a growth regulator provided a significant increase in field germination of sorghum seeds.

Table 2. *Quality indicators of common sorghum seeds depending on the method of seed preparation for sowing (average for 2016-2019)*

Seed quality metrics	Seed treatment		Treatment of crops with a growth regulator	
	No treatment – control (water)	Seeds treated with a regulator	Untreated seeds + spraying of crops with a regulator	Treated seeds + and spraying of crops with a regulator
Seed germination energy, %	91.1	93.4	–	–
Laboratory germination of seeds, %	94.6	97.8	–	–
Field germination of seeds, %	87.2	91.5	87.4	90.8
$LSD_{0.5}$ (seed germination energy) - 1.30				
$LSD_{0.5}$ (seed laboratory similarity) - 0.39				
$LSD_{0.5}$ (seed field similarity) - 0.84				

Source: *compiled by the author*

The duration of the growing season varied depending on the use of the plant growth regulator (Fig. 3). It was proved that seed treatment and spraying of crops reduced the vegetation period of plants to 92 days, while in the untreated variant, the period from germination to full maturity was 105 days.

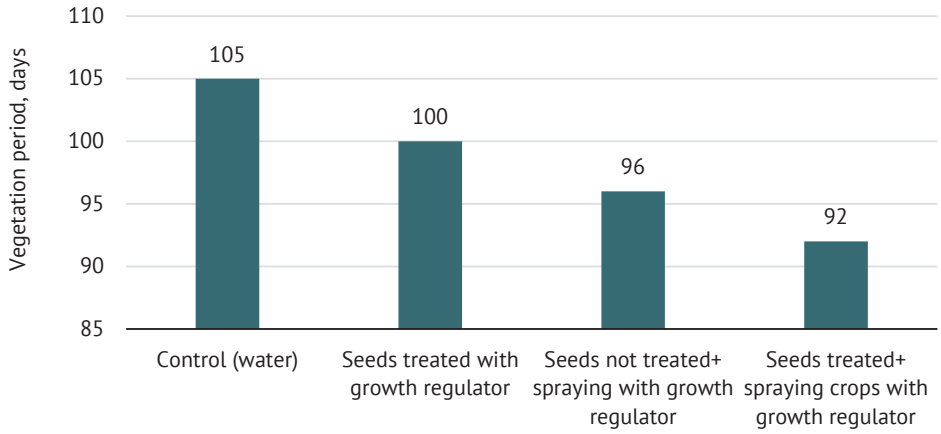

Figure 3. *Duration of the growing season of sorghum depending on the method of seed preparation for sowing (average for 2016-2019)*

The use of the growth regulator significantly improved the biometric parameters of the studied plants and the crop as a whole (Table 3). The lowest indicators were obtained in the variant where the growth regulator was not used (control), with a plant height of 118.6 cm, stem diameter of 1.4 cm, tillering and plant weight of 1.8 pcs/plant and 88.2 g. In the variant where only seeds and sowings were treated, the height of plants was on average 2.0-4.2 cm higher, the diameter was 1.5 cm, the number of tillers was up to 2.0 per plant, and the average plant weight was 2.1 and 3.6 g higher. The highest indicators of plant growth and development were observed in the variant where both seeds and crops were treated: plant height significantly increased – 130.2 cm, diameter – 1.7 cm, plants bushy – 2.5 pieces/plant and plant weight – 93.5 g.

Table 3. *Biometric indices of growth and development of sorghum plants depending on the method of seed preparation for sowing (average for 2016-2019)*

Seed sowing treatment method		Indicator for the harvest period			
		Plant height, cm	Stem diameter, cm	Bushiness of plants, pcs./plant	Plant mass, g
Seed treatment (Factor A)	No treatment – control	118.6	1.4	1.8	88.2
	Seeds treated with a plant growth stimulant	120.4	1.5	2.0	90.3
Crop treatment (Factor B)	Untreated seeds + spraying of crops with a plant growth stimulant	122.8	1.5	2.0	91.8
	Treated seeds + and spraying of crops with a plant growth stimulant	130.2	1.7	2.5	93.5
	$LSD_{0.5}$	1.39	0.20	0.28	1.30

Source: *compiled by the author*

The use of a growth regulator provided an increase in the area of the assimilative apparatus of plants (Table 4), and, accordingly, photosynthetic activity. The growth of leaf surface area in sorghum plants occurred from germination to flowering, where the maximum values of the assimilation surface area were obtained. Thus, in the variant without the use of a growth regulator, the leaf surface area was 20.4 thousand m^2/ha. Seed treatment and the use of a growth regulator on sorghum crops increased the leaf surface area by 2.6 and 5.4 thousand m^2/ha. In the variant where seeds and crops were treated, the assimilation surface area reached a maximum and amounted to 40.32 thousand m^2/ha.

Table 4. *Influence of growth regulator on the change of leaf surface area of sorghum, thousand m²/ha, (average for 2016-2019)*

Seed sowing treatment method		Leaf area, thousand m²/ha, per phase:			
		bushing	tube exit	blooming	maturity
Seed treatment (Factor A)	No treatment – control	6.83	20.4	33.5	3.19
	Seeds treated with a plant growth stimulant	7.92	27.2	36.1	4.12
Crop treatment (Factor B)	Untreated seeds + spraying of crops with a plant growth stimulant	8.15	28.6	38.9	4.68
	Treated seeds + and spraying of crops with a plant growth stimulant	8.56	29.6	40.32	4.97
	$LSD_{0.5}$	0.11	0.98	1.36	0.13

Source: *compiled by the author*

The correlation and regression analysis of the data between the growing season and plant height is represented by a second-order polynomial and the equation is *y = 0.0831x2 – 17.229x + 1011.7*. A strong correlation was found, with the coefficient being R=0.9264 and the coefficient of determination R^2=0.9864 (Fig. 4).

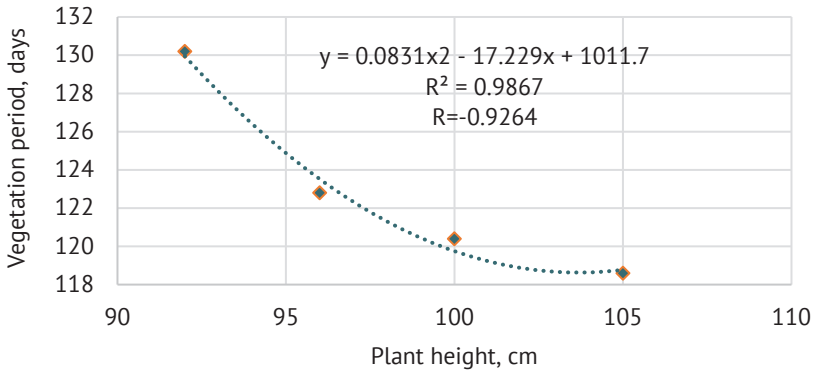

Figure 4. *Correlation and regression relationship between the growing season and plant height (average for 2016-2019)*

Source: *compiled by the author*

Correlation and regression analysis of the data showed a strong linear correlation between stem diameter and plant height, with the equation *y = 0.0241x – 1.4396* (Fig. 5). The correlation coefficient was R=0.9767 and the coefficient of determination was R^2=0.954.

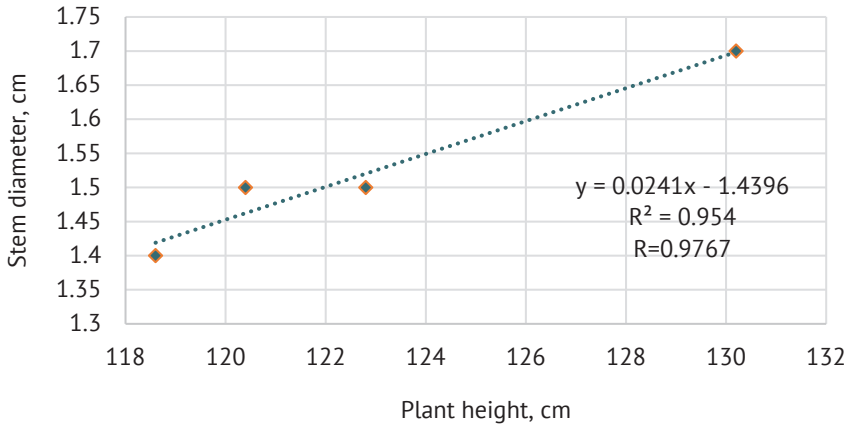

Figure 5. *Correlation and regression relationship between stem diameter and plant height (average for 2016-2019)*

Source: *compiled by the author*

When studying correlations, two main questions arise: the closeness and the shape of the relationship.

Special statistical methods called correlation are used to measure the strength and shape of the relationship. The data in Table 5 show that there are strong correlations associated with biometric traits of sorghum, depending on the use of plant growth regulators.

Table 5. *Correlation matrix (Pearson)*

Biometric parameters	Plant height, cm	Stem diameter, cm	Bushiness of plants, pcs./plant	Plant mass, g	Leaf area, thousand m^2/ha (tillering)	Leaf surface area, thousand m^2/ha (tube yield)	Leaf area, thousand m^2/ha (flowering)	Leaf area, thousand m^2/ha (full maturity)
Plant height, cm	1	0.972	0.980	0.928	0.827	0.738	0.890	0.842
Stem diameter, cm	0.972	1	0.972	0.923	0.869	0.788	0.868	0.848
Bushiness of plants, pcs./plant	0.980	0.972	1	0.904	0.837	0.748	0.847	0.818
Plant mass, g	0.928	0.923	0.904	1	0.966	0.927	0.991	0.982
Leaf area, thousand m^2/ha (tillering)	0.827	0.869	0.837	0.966	1	0.989	0.847	0.985
Leaf surface area, thousand m^2/ha (tube yield)	0.738	0.788	0.748	0.927	0.989	1	0.928	0.973
Leaf area, thousand m^2/ha (flowering)	0.890	0.868	0.847	0.991	0.847	0.928	1	0.988
Leaf area, thousand m^2/ha (full maturity)	0.842	0.848	0.818	0.982	0.985	0.973	0.988	1

Note: *Significant correlation at $p < 0.05$*
Source: *compiled by the author*

The correlation calculation shows that the most important indicators that will influence the formation of crop productivity are plant weight, leaf area, height, and tillering.

Figure 6 shows a sorghum trial plot. The plants are in the waxy ripeness phase (third decade of August). Area where seeds and crops were treated with a growth regulator.

Figure 6. *Sorghum in the experimental plots*

Source: *authors' photo*

It is worth noting that during the vegetation period, natural genetically determined changes occurred in sorghum plants under the influence of the growth regulator, which was reflected in the consistent development of plants and crops in general. Research by T. Asami & Y. Nakagawa (2018) has shown that plants treated with growth regulators suffer less from unfavourable environments and unstable weather conditions. Growth regulators promote the formation of nutrients that enhance the enzymatic activity of plant cells and the development of stimulatory connections by the plant itself. As a result, the permeability of the root cell membrane increases, and the absorption of mineral nutrients from the soil solution by plants improves. The use of growth

regulators accelerates the absorption of oxygen and the process of photosynthesis. Accordingly, the photosynthetic activity of agrocenoses and crop yields increase.

According to M. Szczepanek (2018), the high efficiency of growth regulators on cereals is associated with their ability to increase the accumulation of macro- and microelements, the concentration of photosynthetic pigments and, as a result, to activate photosynthesis and increase crop productivity. In addition, growth regulators help to control the duration of certain stages of plant growth and development and also contribute to the correction of crop conditions under the influence of adverse abiotic factors.

Observations of V. Lyubich *et al.* (2020) show that the use of a plant growth regulator affects the germination energy and laboratory germination of sorghum seeds, increasing it by 4-7% compared to the control variant. L. Storozhyk *et al.* (2020) show that the sowing qualities of sugar sorghum of the studied varieties and hybrid decrease even with short-term storage of grain. The laboratory germination rate after 1 year of storage of sugar sorghum is 80-81%, germination energy – 6-67% without the use of biological products. The use of the biological product Phytocide P increased the quality indicators to 83-87% and 73-75%, respectively. Laboratory germination of ⩾50% ensures storage of sugar sorghum grain for 4 years. The germination energy is 47-54%, depending on the variety. Treatment of grain with biological products can extend the storage period to 6 years with a germination energy of 50-54%. It is optimal to use the Medovyi variety for the production of sugar sorghum seeds, which have the highest sowing properties with the treatment of grain with a biological product. The laboratory germination rate is 65-87%, and the germination energy is 54-75%, depending on the storage period.

According to O. Prysiazhniuk *et al.* (2022), foliar application of a plant growth regulator at microstage 21 (BBCH) contributed to faster development of sorghum plants and increased grain yield of sorghum varieties (0.19 t/ha of Odesskyi 205 and 0.12 t/ha of Lan 59) compared to application at stage III (Kuperman). S. Dhaliwal *et al.* (2020) argue that the use of growth regulators at the optimal time helps to avoid plant stress caused by unfavourable growing conditions during critical growth stages.

According to the research of Dyomin *et al.* (2021), the use of the product was found to be detected at the initial stages of plant ontogeny and did not affect the rate of seed ripening in the panicle. Changes in the duration of the interphase periods (sowing-seedlings, seedlings-bush, bush-emergence into the tube) mainly depended on the method of application of the preparation. However, the periods of panicle ejection-flowering and flowering-seed ripening were not affected by the experimental variants. At the same time, the total duration of the growing season of perennial sorghum varied depending on the factor under study: in the context of the experimental variants, it ranged from 122 to 132 days. That is, the use of "Agrostimulin" in pre-sowing seed preparation reduces this period by 5 days, treatment during the growing season – by 6 days, and the combined use of these measures – by 10 days.

Thus, based on the conducted research, it should be noted that the use of plant growth regulators on seeds and crops of sorghum allows for a reduction in the rates of fertiliser and pesticide application. It also has a positive effect on the quality and biometric parameters of seeds, which was confirmed by correlation analysis. However, the issue of using growth regulators on sorghum crops requires further study, as there is virtually no information on such studies.

CONCLUSIONS

The results of the research are statistically significant, and the elements of the cultivation technology studied are likely to be used for growing other varieties in different soil and climatic conditions. The results of the research showed that the use of the growth regulator changed the quality indicators of sorghum seeds, namely seed treatment and spraying of crops increased germination energy and laboratory and field germination by 2.3, 3.2 and 4.3% compared to the control. The duration of the growing season without seed and crop treatment was 105 days, while with treatment it was reduced by 13 days.

It was proved that the use of the growth regulator significantly increases biometric parameters: plant height – up to 130.2 cm, stem diameter – up to 1.7 cm, increase in productive stems – up to 2.5 pieces per plant and plant weight – up to 93.5 g. The leaf surface area, depending on the period of development, in the variant where seeds and crops were treated, reached a maximum and ranged from 8.56 to 40.32 thousand m^2/ha: the smallest was in the period of full ripeness – 4.97 thousand m^2/ha in the same experiment.

A close correlation was established between the studied parameters of plant growth and development, which have a significant impact on the yield of sorghum as an energy crop. Given the significant effectiveness of growth regulator application in studies to improve grain and biomass productivity of sorghum and considering climate change, namely, increasing temperature and decreasing precipitation, the study of the effectiveness of growth regulators in sorghum crops is a relevant and promising area of research. Sorghum is considered as a bioenergy crop, so it is worth investigating the use of plant growth regulators in combination with other elements of cultivation technology, which will determine the correct planning of crops and processing in the future.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

None.

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Особливості росту та розвитку сорго (*Sorghum bicolor* (L.) Moench) за дії регулятора росту

Людмила Анатоліївна Правдива
Кандидат сільськогосподарських наук, старший науковий співробітник
Інститут біоенергетичних культур і цукрових буряків НААН України
03110, вул. Клінічна, 25, м. Київ, Україна
https://orcid.org/0000-0002-5510-3934

Олег Іванович Присяжнюк
Доктор сільськогосподарських наук, професор
Інститут біоенергетичних культур і цукрових буряків НААН України
03110, вул. Клінічна, 25, м. Київ, Україна
https://orcid.org/0000-0002-4639-424X

Валерій Семенович Хахула
Кандидат сільськогосподарських наук, доцент
Білоцерківський національний аграрний університет
09110, Соборна площа, 8/1, м. Біла Церква, Україна
https://orcid.org/0000-0003-4100-262X

Леся Михайлівна Качан
Кандидат сільськогосподарських наук, доцент
Білоцерківський національний аграрний університет
09110, Соборна площа, 8/1, м. Біла Церква, Україна
https://orcid.org/0000-0001-5374-3252

Тарас Валентинович Панченко
Кандидат сільськогосподарських наук, доцент
Білоцерківський національний аграрний університет
09110, Соборна площа, 8/1, м. Біла Церква, Україна
https://orcid.org/0000-0003-1114-5670

Анотація. Останнім часом зростає інтерес до вирощування сорго звичайного двокольорового як біоенергетичної культури, так як його можна використовувати для виробництва біопалива (етиловий спирт) та твердого палива (пелетів та брикетів із надземної маси). Зерно сорго характеризується високим вмістом крохмалю – до 80 %. Тема досліджень актуальна, але маловивчена. Мета досліджень полягала у вивченні впливу регулятора росту на ріст та розвиток сорго звичайного двокольорового в умовах Правобережного Лісостепу України. Використовувались такі методи досліджень: польовий, вимірювально-ваговий, математично-статистичний. У статті наведено результати досліджень за 2016-2019 роки, де вивчали вплив елементів технології вирощування на особливості формування біометричних показників. Досліджено, що застосування регулятора росту рослин впливало на польову схожість, вегетаційний період, біометричні показники росту рослин, площу листкової поверхні. Встановлено тісноту кореляційних зв'язків досліджуваних показників, та проведено кореляційно-

регресійний аналіз даних, який показав сильну кореляцію між вегетаційним періодом і висотою рослин, де коефіцієнт склав R=0,9264, а коефіцієнт детермінації R^2=0,9864. А також сильний зв'язок між діаметром стебла та висотою рослин, де коефіцієнт кореляції склав R=0,9767, а коефіцієнт детермінації відповідно становив R^2=0,954. Проведені дослідження підтверджують доцільність застосування регулятора росту, що покращує розвиток рослин і сприятиме підвищенню урожайності та якості зерна і біомаси сорго звичайного двокольорового. Отримані результати досліджень дають підстави вважати, що регулятори росту сприяють отриманню екологічно чистої продукції та виступають складовими екологічно чистої та енергозберігаючої технології вирощування

Ключові слова: сорго звичайне двокольорове; регулятор росту; якісні показники насіння; вегетаційний період; біометричні показники; площа листкової поверхні; кореляція

SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 131-142

UDC 633.11:631.95:575.21
DOI: 10.48077/scihor9.2023.131

Cytogenetic activity of a mutagenic factor with high damaging capacity in winter wheat

Vladislav Horshchar
PhD in Agricultural Sciences, Associate Professor
Dnipro State Agrarian and Economic University
49600, 25 S. Efremova Str., Dnipro, Ukraine
https://orcid.org/0000-0002-6604-0123
Mykola Nazarenko*
Doctor at Agricultural Sciences, Professor
Dnipro State Agrarian and Economic University
49600, 25 S. Efremova Str., Dnipro, Ukraine
https://orcid.org/0000-0002-6604-0123

Article's History:
Received: 11.05.2023
Revised: 29.08.2023
Accepted: 27.09.2023

Abstract. The analysis of cytological abnormalities is an important method for identifying the potential of a chemical as a mutagen for future heritable genetic changes, the level of genotype-mutagen interaction and site-specific activity for the nature and/or different concentrations of the mutagen. The research aims to determine the limits of the variability of genotypes of different origins, especially those with wide ecological and genetic variability, and to show the interaction between the variety and the mutagen. Winter wheat grains of several varieties (Balaton, Borovytsia, Zelenyi Hai, Zoloto Ukrainy, Kalancha, Niva Odeska, Polianka, Pochayna) were treated with dimethyl sulphate at concentrations of 0.0125%, 0.025%, 0.05%, and exposure was 24 hours. The cytogenetic activity was studied by the frequency and spectrum of chromosomal rearrangements in the corresponding phases of cell division, depending on the variety and concentration of the mutagen as the main factors affecting these parameters, as well as the main features of the spectrum, such as the overall rate of chromosomal rearrangements, the number of fragments and double fragments, bridges, micronuclei, and lagging chromosomes. The studied concentrations of the supermutagen were found to have a significant effect on all analysed parameters and can be classified as optimal and high concentration levels in terms of the effect on cytological activity and mitotic problems for the factor, despite previous studies. The variety factor has a much greater impact on the nature and frequency of certain types of aberrations than an increase in the mutagen concentration, it was characterised by a much greater site-specific effect than other chemical agents, and various variants in mutagenic effects were identified according to the subject's genotype. It was generalised that the features that reproduced the effect of the mutagen, according to the discriminant

Suggested Citation:
Horshchar, V., & Nazarenko, M. (2023). Cytogenetic activity of a mutagenic factor with high damaging capacity in winter wheat. *Scientific Horizons*, 26(9), 131-142. doi: 10.48077/scihor9.2023.131.

*Corresponding author

analysis, were the total frequency, the frequency of fragments and double fragments, and bridges. In practical use in the genetic improvement of plants and for obtaining valuable traits, the optimal concentrations are 0.0125%, and 0.025%, which is planned to be further confirmed on a wider range of genotypes and by mutation studies for the next (second or third) generations

Keywords: chromosomal aberrations; mutagen; winter wheat; dimethyl sulphate; cytological analysis

INTRODUCTION

The study of the chemical factors' activity identifies the success of the respective substance in inducing both general variability in plant material and specificity in the possibility of detecting certain types of changes in treated plants in subsequent generations. As long-term studies have shown, the level of activity depends on the future overall frequency of changes and is significantly adjusted by the subject of the action, while the specificity of the action directly depends on the reproduction of the specifics of the factor's effect by individual indicators.

Following previous studies (Küçük & Liman, 2018; Cann *et al.*, 2022), the use of mutagenic factors for genetic improvement of cultivated plants should be preceded by careful monitoring of their mutational activity and damage capacity. The study was carried out both at the level of the plant as a whole (mutagenic depression in terms of plant growth and development in the first generation) and at the level of the organism, to determine cytogenetic activity at the cellular level (analysis of chromosomal aberrations, their frequencies depending on the genotype and dose (concentration) of the mutagen, characteristics of the spectrum of cytogenetic variability and analysis of the ratios of certain types of rearrangements).

Monitoring of cytogenetic activity allows not only to establish the potential activity of a chemical as a mutagen, as indicated in previous studies (Ariraman *et al.*, 2018; Abaza *et al.*, 2020; Yali & Mitiku, 2022) but also to conclude the nature of the aberrations formed and their correlation with the possible genotype-mutagenic interaction and the suitability of individual factors for acting on certain genotypes, which has already been established many times. This is of particular importance for chemical mutagenesis, given the significant specific feature of chemicals (mechanism of action through structural correspondence to certain DNA sites) established by previous studies (Von Well *et al.*, 2018; Chaudhary *et al.*, 2019).

Several researchers (Yang *et al.*, 2019; Hussain *et al.*, 2021) have shown that varietal material is rather poorly studied in this area, and even for classical factors, there is still variability that can reach quite significant limits. S. Nurmansyah *et al.* (2018) and E. El-Azab *et al.* (2018) proved that only by using the DNA structure of the original forms, firstly, it is possible to increase mutational variability or reduce possible depressive effects when using forms that are very sensitive to this factor (especially on the verge of semi- and sublethality of certain indicators), and secondly, to predict the level of variability of the resulting material. H. Ram *et al.* (2019) found that an important feature of identifying a mutagenic effect at the cellular level is the ability to determine the lethality of a particular factor and its practically useful concentrations in terms of its genetic activity. When certain thresholds are reached, the use of higher concentrations is inappropriate and leads, instead of increasing, to a decrease in the induction of new forms and their variability, which is associated with a too-high toxic effect and an increase in the magnitude of changes in DNA (Hong *et al.*, 2022).

M. Spencer-Lopes *et al.* (2018) showed that sensitivity to mutagens is genetically determined, and while two main genetically determined resistance mechanisms are known for physical factors, in particular gamma rays, summarised in the relevant protocols, a significantly larger number of them are predicted for chemical agents. It should be noted that one of these mechanisms was discovered precisely when studying the cytogenetic activity of local forms of cereal crops, so cases where individual genotypes in studies (Yang *et al.*, 2019; Hase *et al.*, 2020) demonstrate a rather specific, uncharacteristic genotype response to a particular factor may indicate a new or modified mechanism of resistance and/or sensitivity to mutagenic effects. This is especially true given the predominantly recessive control of these types of resistance.

The research aims to identify the peculiarities of genotype mutagenic action given the ecological and genetic variability of subjects in terms of cytogenetic activity parameters under the influence of a factor with high damaging ability.

MATERIALS AND METHODS

Cytological analysis studies were conducted in the laboratory at the Department of Breeding and Seed Production of Dnipro State Agrarian and Economic University in 2021-2022. The experiment was conducted on the seeds of eight winter wheat varieties, which were selected in such a way as to maximise the biodiversity of the crop varieties used for agriculture (Balaton, Borovytsia, Zelenyi Hai, Zoloto Ukrainy, Kalancha, Nyva Odeska, Polianka, Pochaina). These varieties in the first generation were classified into two groups in the field studies under the action of the mutagen according to the effects of depression, so to simplify the research

design, we further studied not only the behaviour of each genotype separately but also linked the data obtained to the groups.

Samples of winter wheat grain were treated with an aqueous solution of DMS (dimethyl sulphate, hereinafter referred to as DMS) at concentrations of 0.0125, 0.025 and 0.05%. The control was soaked in distilled water. The number of treated grains should have ensured the maintenance of at least 25-30 samples with a developed primary root system in the future (at least 100 grains). The selected concentrations were experimentally proposed for protocols for the mutational improvement of winter wheat. The exposure period was 24 hours.

The cytological analysis of chromosomal abnormalities under the action of the mutagen was performed on temporary preparations of the primary root system meristem at the corresponding stages of mitosis in late metaphase and early anaphase. All types of aberrations were studied using Micromed XS-3330 light microscopy with a 600x magnification on pressed root tip preparations (2 mm with root length of 1.0-1.2 cm). In each variant, up to 1000 corresponding cells were used if available. At a high level of elimination, at least 500 were used.

After exposure to the appropriate concentration of DMS, the samples were cultured in Petri dishes on filter paper with distilled water in a thermostat at 20-22°C. The tips of the roots were then cut off and fixed in Clark's fixative, consisting of 3 parts 96% medical alcohol and 1-part ice-cold acetic acid, for at least 24 hours. The samples were stored in a solution of 70% ethyl alcohol at 2°C in a refrigerator. For each variant, up to 30 samples were prepared (but not necessarily all were used). The resulting pressed root tip preparations were stained with acetocarmine. For this method of cytogenetic analysis, such abnormalities as fragments and double fragments, chromatid and chromosomal bridges, micronuclei, and lagging chromosomes were examined. Cells with multiple abnormalities were counted separately.

The values in the tables are presented as means with standard deviation x±SD. The difference between the variants was determined by factor analysis (ANOVA module) and was considered significant at $P<0.05$. The normality of the distribution was investigated by the Shapiro-Wilks test, pairwise comparison was performed by the Tukey HSD test, and discriminant analysis was performed by the multiple statistics modules of Statistica 10.

RESULTS AND DISCUSSION

The study of the cytogenetic activity of a mutagenic factor includes such steps as calculating the total frequency of chromosomal aberrations, indicators of the spectrum of existing changes, and studying the nature and concentration of mutagens on the variability of these indicators. The total frequency of chromosomal aberrations generally depended on the change in concentration according to the factor analysis ($F=46.17$; $F_{0.05}=3.07$; $P=3.19*10^{-6}$) and, especially, on the genotype of the source material ($F=112.47$; $F_{0.05}=2.48$; $P=8.26*10^{-9}$), which significantly differed from the previously studied genetic activity of other, less harmful mutagens (Table 1). The total frequency, in general, varied in the control from 0.6 % in Polianka variety to 1.00% in Balaton variety, from 13.47 (Nyva Odeska variety) to 17.78% in Balaton variety under the influence of DMS concentration 0. 0125%, under the action of DMS 0.025% from 14.18% in Kalancha to 20.26% in Balaton, from 12.99% in Nyva Odeska to 23.91% in Balaton with increasing concentration to DMS 0.05%. In contrast to the previously studied mutagens, the specificity of the dynamics of frequency changes in different genotypes was noted. Thus, the frequency gradually increases in Balaton and Polyanka, the frequency remains at the same level and then increases in Zelenyi Hai, Zoloto Ukrainy, Borovytsia, and Kalancha (i.e. this is the most typical reaction), the frequency first increases and then decreases in Nyva Odeska, increases and then remains at the same level in Pochaina.

Table 1. *The total frequency of chromosomal rearrangements in mitotic cells in the corresponding phases (x±SD, n=500-1000)*

Variety	Concentration	Mitoses, pcs.	Chromosomal rearrangements	
			pcs.	%
Balaton	water	1002	9	0.90±0.11[a]
Balaton	DMS 0.0125%	1007	179	17.78±0.51[b]
Balaton	DMS 0.025%	923	187	20.26±0.48[c]
Balaton	DMS 0.05%	640	153	23.91±0.37[d]
Zelenyi Hai	water	1008	8	0.79±0.12[a]
Zelenyi Hai	DMS 0.0125%	1001	175	17.48±0.39[b]
Zelenyi Hai	DMS 0.025%	910	161	17.69±0.50[b]
Zelenyi Hai	DMS 0.05%	610	123	20.16±0.69[c]
Zoloto Ukrainy	water	1001	8	0.80±0.21[a]

Table 1, Continued

Variety	Concentration	Mitoses, pcs.	Chromosomal rearrangements	
			pcs.	%
Zoloto Ukrainy	DMS 0.0125%	1008	174	17.26±0.39[b]
Zoloto Ukrainy	DMS 0.025%	890	150	16.85±0.37[b]
Zoloto Ukrainy	DMS 0.05%	610	117	19.18±0.43[c]
Nyva Odeska	water	1009	9	0.89±0.19[a]
Nyva Odeska	DMS 0.0125%	1010	136	13.47±0.40[b]
Nyva Odeska	DMS 0.025%	889	130	14.62±0.28[c]
Nyva Odeska	DMS 0.05%	670	87	12.99±0.47[b]
Borovytsia	water	1001	7	0.70±0.20[a]
Borovytsia	DMS 0.0125%	1009	157	15.56±0.32[b]
Borovytsia	DMS 0.025%	930	148	15.91±0.33[b]
Borovytsia	DMS 0.05%	578	106	18.34±0.41[c]
Kalancha	water	1000	10	1.00±0.15[a]
Kalancha	DMS 0.0125%	1003	139	13.86±0.34[b]
Kalancha	DMS 0.025%	924	131	14.18±0.39[b]
Kalancha	DMS 0.05%	619	97	15.67±0.41[c]
Polianka	water	1007	6	0.60±0.26[a]
Polianka	DMS 0.0125%	1005	148	14.73±0.36[b]
Polianka	DMS 0.025%	917	145	15.81±0.39[c]
Polianka	DMS 0.05%	734	129	17.57±0.51[d]
Pochaina	water	1005	8	0.80±0.06[a]
Pochaina	DMS 0.0125%	1002	139	13.87±0.35[b]
Pochaina	DMS 0.025%	901	152	16.87±0.33[c]
Pochaina	DMS 0.05%	712	118	16.57±0.37[c]

Note: *indicates a significant difference at P<0.05 for the Tukey test with Bonferroni correction. Comparison within the same variety*

Source: *developed by the authors*

Tukey's test for pairwise comparison showed that Balaton (F=11.09; $F_{0.05}$=2.48; P=0.001), Nyva Odeska (F=8.34; $F_{0.05}$=2.48; P=0.005), Pochaina (F=4.53; $F_{0.05}$=2.48; P=0.02) were significantly different from other varieties. Thus, regarding the difference in response between genotypes, the following was found in the pairwise comparison: varieties Zelenyi Hai, Zoloto Ukrainy, Borovytsia, Kalancha, and Polianka form a more or less homogeneous group, while varieties Balaton, Nyva Odeska, Pochaina differ from them and each other.

Considering such an indicator as the number of cells studied, it can be established that only a concentration of 0.0125% of DMS allowed us to study the appropriate number of cells. Already at a concentration of 0.025%, there was a sufficiently high elimination of the genetic apparatus of cells to fail to collect such a number (the difference between genotypes is insignificant), and a concentration of 0.025% of DMS led to high cell death in the corresponding phases, especially for the varieties Balaton, Zelenyi Hai, Zoloto Ukrainy, Borovytsia, Kalancha. The varieties Nyva Odeska, Polianka, and Pochaina were more resistant (F=8.90; $F_{0.05}$=4.10; P=0.004), but they have a more characteristic plateau in the effect of the mutagen at high concentrations (except for Polyanka). That is, the overall decrease in frequency at high concentrations cannot be explained by cell death alone; the mechanism is more complex and includes a ban on the rearrangement of individual sites after reaching a certain limit in aberrations. The peak of genetic activity should be between the second and third concentrations.

Individual indicators of the chromosomal aberration spectrum are presented following the level of depression at the level of the organism as a whole in Tables 2 (higher depression) and 3 (more stable). The analysis was based on the following parameters: the presence of bridges, fragments, the ratio between fragments and bridges (since the first type of aberration is mainly induced by chemical mutagens, the second – by physical ones), the presence of less frequent types of aberrations such as micronuclei and lagging chromosomes, and the presence of cells with two or more aberrations were calculated separately.

Table 2. *Parameters of the spectrum of adjustments. First group (x±SD, n=500-1000)*

Variety	Concentration	Fragments (single + double)		Bridges (chromosomal + chromatid)		fragments/ bridges	Other (micronuclei, lagging chromosomes)		Double or more	
		n	%	n	%		n	%	n	%
Balaton	water	4.0±0.4[a]	44.4	4.0±1.7[a]	44.4	1.0	2.0±0.9[a]	22.2	0.0±0.0[a]	0.0
Balaton	DMS 0.0125%	96.0±1.6[b]	53.6	61.0±2.1[b]	34.1	1.6	22.0±1.7[b]	12.3	32.0±1.7[b]	17.9
Balaton	DMS 0.025%	101.0±1.9[b]	54.2	64.0±2.2[b]	34.2	1.6	22.0±1.5[b]	11.8	30.0±2.7[b]	16.0
Balaton	DMS 0.05%	75.0±2.4[c]	49.0	62.0±3.4[b]	40.5	1.2	16.0±1.9[c]	10.5	22.0±1.5[c]	14.4
Zelenyi Hai	water	4.0±1.0[a]	50.0	3.0±0.6[a]	37.5	1.3	1.0±1.1[a]	12.5	0.0±0.0[a]	0.0
Zelenyi Hai	DMS 0.0125%	109.0±1.2[b]	62.3	47.0±3.1[b]	26.9	2.3	19.0±2.5[b]	10.9	26.0±2.0[b]	14.9
Zelenyi Hai	DMS 0.025%	97.0±1.3[c]	60.2	46.0±2.7[b]	28.6	2.1	18.0±2.2[b]	11.2	23.0±2.5[b]	14.3
Zelenyi Hai	DMS 0.05%	75.0±2.2[d]	61.0	34.0±2.5[c]	27.6	2.2	14.0±2.1[b]	11.4	16.0±2.3[c]	13.0
Zoloto Ukrainy	water	5.0±1.5[a]	62.5	3.0±1.2[a]	37.5	1.7	0.0±0.0[a]	0.00	0.0±0.0[a]	0.0
Zoloto Ukrainy	DMS 0.0125%	93.0±3.0[b]	53.5	59.0±2.4[b]	33.9	1.6	22.0±1.8[b]	12.6	29.0±1.4[b]	16.7
Zoloto Ukrainy	DMS 0.025%	78.0±3.3[c]	52.0	52.0±3.1[b]	34.7	1.5	20.0±2.0[b]	13.3	29.0±2.3[b]	19.3
Zoloto Ukrainy	DMS 0.05%	61.0±3.1[d]	52.1	42.0±2.9[c]	35.9	1.5	14.0±2.1[c]	12.0	17.0±3.0[c]	14.6
Nyva Odeska	water	4.0±1.6[a]	44.4	4.0±1.2[a]	44.4	1.0	1.0±1.2[a]	11.1	1.0±1.5[a]	11.1
Nyva Odeska	DMS 0.0125%	79.0±2.3[b]	58.1	35.0±1.8[b]	25.7	2.3	22.0±2.0[b]	16.2	32.0±2.0[b]	23.5
Nyva Odeska	DMS 0.025%	74.0±3.2[b]	56.9	33.0±2.1[b]	25.4	2.2	23.0±2.1[b]	17.7	29.0±2.0[b]	22.3
Nyva Odeska	DMS 0.05%	55.0±4.1[c]	60.9	20.0±2.1[c]	23.0	2.7	14.0±2.2[c]	16.1	19.0±2.2[c]	21.8

Note: *indicates a significant difference at P<0.05 for the Tukey test with Bonferroni correction. Comparison within the same variety*

Source: *developed by the authors*

Analysing the data for individual types of rearrangements, it can be seen that according to the factor analysis, the frequency of fragments is highly dependent on the concentration (F=34.33; $F_{0.05}$=3.07; P=1.16*10^{-5}) and, to a lesser extent, on the genotype of the source material (F=29.90; $F_{0.05}$=2.48; P=4.17*10^{-4}). In general, the number of fragments varied at 0.0125% DMS from 79 in Nyva Odeska in the first group (Table 2) and 73 in Pochaina and Kalancha (second group, Table 3) to 109 in Zelenyi Hai and 85 in Polianka. Under the influence of 0.025% DMS, from 74 in Nyva Odeska in the first group, 70 in Kalancha to 97 in Zelenyi Hai (first group), 87 in Polianka (second group). Under the influence of DMS, 0.05% from 55 in Nyva Odeska to 75 in Balaton and Zelenyi Hai (first group), from 54 in Kalancha to 76 in Polianka (second group).

In the first group, the absolute amount is higher, and the relative weight in the spectrum is lower in the second group, except for the variety Polyanka, i.e. the effect of the mutagen is less pronounced. At the same time, in Balaton, Nyva Odeska, Borovytsia, Polianka, Pochaina (F=3.11; $F_{0.05}$=4.10; P=0.07) there is no statistically significant difference between the effect of the first and second concentration, while in Zelenyi Hai, Zoloto Ukrainy and Kalancha (F=11.25; $F_{0.05}$=4.44; P=0.004) the difference at each concentration is significant. The number of fragments between the control and the first concentration, the second and the third concentration always changes significantly within the grade. It should be noted that, in general, the proportion of this type of chromosomal aberration varies between 50 and 60% of the total number in the spectrum. The following groups can be distinguished by variability: in the Balaton variety (F=4.45; $F_{0.05}$=2.48; P=0.01), the proportion is generally decreasing, in all other varieties it is at the same level, without significant variations.

As for the frequency of bridges, there is a characteristic dependence on the concentration (F=5.09, $F_{0.05}$=3.07, P=0.02), and a relatively stronger dependence on the genotype of the source material (F=9.34, $F_{0.05}$=2.48, P=0.007). In general, the number of bridges varied at 0.0125% DMS from 35 in Nyva Odeska in the first group (Table 2) and 34 in Kalancha (second group, Table 3) to 61 in Balaton and 47 in Borovytsia. Under the influence of 0.025% DMS, from 33 in Nyva Odeska in the first group, 30 in Borovytsia to 64 in Balaton (first group), and 44 in Borovytsia (second group). Under the influence of DMS 0.05% from 20 in Nyva Odeska to 62 in Balaton (first group), from 23 in Kalancha to 37 in Pochaina (second group).

Table 3. *Parameters of the restructuring spectrum. The second group (x±SD, n=500-1000)*

Variety	Concentration	Fragments (single + double)		Bridges (chromosomal + chromatid)		fragments/ bridges	Other (micronuclei, lagging chromosomes)		Double or more	
		n	%	n	%		n	%	n	%
Borovytsia	water	3.0±1.0[a]	42.9	3.0±1.1[a]	42.9	1.0	1.0±1.6[a]	14.3	0.0±0.0[a]	0.0
Borovytsia	DMS 0.0125%	82.0±3.1[b]	52. 2	47.0±2.0[b]	30.0	1.7	28.0±1.9[b]	17.8	26.0±2.0[b]	16.6
Borovytsia	DMS 0.025%	78.0±1.9[b]	52.7	44.0±2.5[b]	30.0	1.8	26.0±2.4[b]	17.6	25.0±2.2[b]	16.9
Borovytsia	DMS 0.05%	57.0±3.0[c]	53.7	30.0±3.0[c]	28.3	1.9	19.0±2.0[c]	17.9	20.0±3.1[b]	18.9
Kalancha	water	4.0±0.7[a]	40.0	5.0±1.7[a]	50.0	0.8	1.0±0.6[a]	10.0	0.0±1.0[a]	0.0
Kalancha	DMS 0.0125%	73.0±2.2[b]	52.5	34.0±1.8[b]	24.5	2.2	32.0±1.4[b]	23.0	29.0±1.5[b]	20.9
Kalancha	DMS 0.025%	70.0±2.0[b]	53.4	32.0±2.2[b]	24.4	2.2	29.0±2.1[b]	22.0	28.0±2.0[b]	21.4
Kalancha	DMS 0.05%	54.0±1.1[c]	55.7	23.0±2.2[c]	23.7	2.4	20.0±2.2[c]	20.7	19.0±2.5[c]	19.6
Polianka	water	2.0±1.2[a]	33.3	2.0±2.0[a]	33.3	1.0	2.0±1.3[a]	33.3	0.0±0.0[a]	0.0
Polianka	DMS 0.0125%	85.0±2.1[b]	57.4	43.0±2.1[b]	29.0	2.0	20.0±1.4[b]	13.5	23.0±1.4[b]	15.4
Polianka	DMS 0.025%	87.0±2.4[b]	60.0	41.0±2.6[b]	28.3	2.1	17.0±2.0[b]	11.7	22.0±2.1[b]	15.1
Polianka	DMS 0.05%	76.0±2.9[c]	58.9	31.0±3.1[c]	24.0	2.5	22.0±3.0[b]	17.1	31.0±2.6[c]	24.0
Pochaina	water	3.0±1.4[a]	37.5	5.0±1.2[a]	62.5	0.6	0.0±0.0[a]	0.0	0.0±0.0[a]	0.0
Pochaina	DMS 0.0125%	73.0±2.1[b]	52.5	40.0±2.6[b]	28.8	1.8	26.0±2.4[b]	18.7	25.0±1.4[b]	18.0
Pochaina	DMS 0.025%	78.0±2.5[b]	51.3	42.0±3.3[b]	27.6	1.9	32.0±3.0[c]	21.1	28.0±1.5[bc]	18.4
Pochaina	DMS 0.05%	60.0±3.1[c]	50.9	37.0±3.4[b]	31.3	1.6	21.0±3.3[b]	17.8	24.0±1.9[b]	20.3

Note: *indicates a significant difference at P<0.05 for the Tukey test with Bonferroni correction. Comparison within the same variety*

Source: *developed by the authors*

The absolute amount is again higher in the first group, and the relative weight in the spectrum is approximately equal in both groups and depends more on the individual variety, but it is impossible to establish patterns. The variability in concentrations is much lower. Thus, in the Balaton and Pochaina varieties (F=2.46, $F_{0.05}$=4.40, P=0.09) there is no statistically significant difference between the effects of individual concentrations, while in other varieties there is no difference between the first and second concentration (F=1.34, $F_{0.05}$=2.55, P=0.08), only between the control and the first and third and all other concentrations. In general, the proportion of this type of chromosomal aberration varies between 20-40% of the total number in the spectrum. The following groups can be distinguished by their variability: in the Balaton variety, the proportion is generally increasing, in the Polianka variety it is decreasing, and in all other varieties it is at the same level, without significant variations. As for the integrative index of the ratio of bridges to fragments, in all cases under the action of the mutagen it exceeded 1, which is typical for the action of chemical mutagens. This is least pronounced in the Balaton variety, with a significant predominance of fragments in the varieties Zelenyi Hai, Nyva Odeska, Kalancha, and Polianka.

As for the presence of liquid aberrations, their number depends on the concentration (F=3.91; $F_{0.05}$=3.07; P=0.04) but does not depend on the genotype of the source material (F=2.40; $F_{0.05}$=2.48; P=0.06). In general, the number of micronuclei and lagging chromosomes varied at 0.0125% DMS from 19 in Zelenyi Hai in the first group (Table 2) and 20 in Polianka (second group, Table 3) to 22 in Balaton, Zoloto Ukrainy, Nyva Odeska and 32 in Kalancha. Under the effect of 0.025% DMS, from 18 in Zelenyi Hai in the first group, 17 in Polianka to 23 in Nyva Odeska (first group), and 32 in Pochayna (second group). Under the influence of 0.05% of the DMS, from 14 in three varieties to 16 in Balaton (first group), there is virtually no variation, from 19 in Borovytsia to 22 in Polyanka (second group). In general, the number is much lower than other types of aberrations.

In this case, the absolute amount is higher in the second group, and the relative weight in the spectrum is relatively higher in the second group (except for Polianka and considering that Nyva Odeska is approximately at the same level as other varieties in the second group) and is more dependent on the individual variety. The variability in concentrations is even lower than that of bridges. Thus, in the varieties Zelenyi Hai and Polianka (F=2.99; $F_{0.05}$=4.40; P=0.08) there is no statistically significant difference between the effect of individual concentrations, while in other varieties there is no difference between the first and second concentration (F=2.55; $F_{0.05}$=3.01; P=0.07), only between the control and the first and third concentrations, and all other varieties with a significant decrease in the

number under the highest concentration (except for Pochaina, which stood out with a higher number under the second concentration). In general, the proportion of this type of chromosomal aberration varies between 10 and 25% of the total number in the spectrum. The following groups can be distinguished by their variability: in the first group and varieties Borovytsia and Kalancha, it is approximately at the same level, in the variety Polianka it decreases at the second concentration and increases again to the same level at the third concentration, in the variety Pochaina it increases to increases to the second concentration and then decreases to the same level.

As for the presence of cells with complex aberrations (two or more rearrangements), their number depends on the concentration (F=5.99, $F_{0.05}$=3.07, P=0.03), but again does not depend on the genotype of the source material (F=2.12, $F_{0.05}$=2.48, P=0.07) according to the factor analysis. When counting the number of cells with complex chromosomal rearrangements, the effect of 0.0125% DMS varied from 26 in Zelenyi Hai in the first group (Table 2) and 23 in Polyanka (second group, Table 3) to 32 in Balaton, Nyva Odeska and 29 in Kalancha. Under the influence of DMS 0.025% from 23 in Zelenyi Hai in the first group, 22 in Polyanka to 30 in Balaton (first group), and 28 in Polianka, Pochaina (second group). Under the influence of 0.05% DMS, from 16 in Zelenyi Hai to 22 in Balaton (first group), from 19 in Kalancha to 31 in Polianka (second group). In general, the number of such cells is quite high in comparison with other mutagens, but the lethal effect of high concentrations is affected.

In this case, in the second group, the absolute number is higher at higher concentrations, which is associated with increased cell elimination in the first group, the relative weight in the spectrum is relatively higher in the second group (considering the lower one in the variety Polyanka from the second group and the higher one in the variety Nyva Odeska), this parameter shows a high genotype-mutagenic interaction in the form of a variety reaction as a subject of mutagenic action. The variability in concentrations is quite high but lower than in the previous parameters. Thus, only in the Pochaina variety, the effect of the second concentration is partially different, while in other varieties there is no difference between the first and second concentration (F=2.01; $F_{0.05}$=3.40; P=0.09), there is a statistically significant difference only between the control and the first and second concentration, between the first and second concentrations and the third, highest concentration. In general, the proportion of this type of chromosomal aberration varies between 10-25% of the total number in the spectrum. Such varieties as Polianka (the number of cells with multiple aberrations increases at the third concentration) (F=9.46; $F_{0.05}$=2.48; P=0.001) and Pochaina (the indicator does not reflect any increase in concentration) (F=4.00; $F_{0.05}$=2.48; P=0.03) stood out for their variability in pairwise comparison, which indicates the absence of threshold concentrations of significance for viability. All other varieties are characterised by approximately the same value at the first and second concentrations and a sharp decline at the third.

The analysis of the factor loadings of individual signs of cytogenetic variability (Table 4) showed that for the genotype, such parameters as the total frequency, fragments, bridges, rare types of aberrations, and the presence of cells with multiple rearrangements were significantly variable, although some were on the verge of being unreliable. That is, concentration as a factor was more significant, since in the case of genotype, two indicators of the presence of other types of rearrangements and complex rearrangements were not significant, indicating the fact of the reliability of deviations in the reaction of only two varieties of Polianka and Pochaina (in one case, somewhat Zelenyi Hai).

Table 4. *Factor load by cytogenetic parameters*

Parameter	Concentration	Genotype
General frequency	0.817*	-0.934*
Fragments	0.816*	0.844*
Bridges	0.790*	-0.749*
Other types of aberrations	-0.678*	0.436
Complex	0.717*	0.501
Overall explained	2.983	2.114
Unexplained	0.990	1.095

Note: ** – important parameters*

Source: *developed by the authors*

The discriminant analysis (Table 5, Fig. 1) confirmed the data obtained, somewhat specifying the traits that reproduced the effect of the mutagen. The total frequency, number of fragments, and bridges depended on the genotype. It should be noted that for chemical mutagens, especially weaker ones, the number of bridges is not always included in the model. All parameters changed when the concentration of DMS changed. In the classification

space of the canonical functions, the control was clearly distinguished with a large distance. The grouping also showed a statistically significant difference in the case of higher concentrations. Concentrations of 0.0125 and 0.025% of DMS did not differ in the factor space and formed one continuous mixed group.

Table 5. *Discriminant function values for cytogenetic activity parameters*

Parameter	Genotype			Concentration		
	Wilks' - Lambda	F_{remove} (7.85)	p-level	Wilks' - Lambda	F_{remove} (3.89)	p-level
General frequency	0.022	10.87	0.01	0.017	8.07	0.01
Fragments	0.020	11.34	0.01	0.018	9.76	0.01
Bridges	0.015	8.77	0.03	0.015	4.56	0.03
Other	0.004	3.67	0.07	0.014	4.66	0.04
Two or more	0.003	3.16	0.08	0.014	4.19	0.05

Source: *developed by the authors*

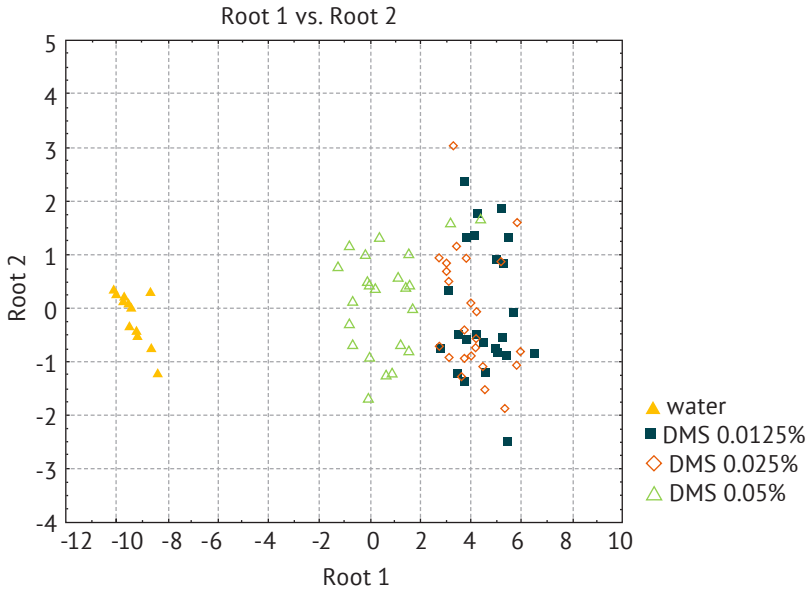

Figure 1. *Classification by concentrations in the factor space of canonical functions*

The strength of the classification of individual parameters was the same for both genotypes and concentrations, which is significantly different from previous studies, where concentrations had a significantly (50%) higher strength when divided into groups (Table 6). First of all, this is due to the low potency for the first two concentrations, while for the control and 0.05% DMS, it is quite high. Among the genotypes, we can hope for a reliable classification for Balaton, Nyva Odeska, Polianka, and Pochaina, especially for the first and third. While the other genotypes form a separate group, with no significant difference between them. That is, the studied varieties can be divided into three separate groups according to their efficiency: Balaton and Polianka, with high genotype-mutagenic interaction (although it can be different in consequences, always with high identification ability), Nyva Odeska and Pochaina – mediocre genotype-mutagenic interaction (can be different) and the other 4 varieties with low genotype-mutagenic interaction.

Table 6. *Classification ability of objects belonging to separate concentrations and genotypes*

	water	100
Objects in the model, concentration, %	DMS 0.0125%	32.0
	DMS 0.025%	32.0
	DMS 0.05%	83.5
	Total	62.0

Table 6, Continued

	water	**100**
Objects in the model, genotype, %	Balaton	92.5
	Borovytsia	37.5
	Zelenyi Hai	52.5
	Zoloto Ukrainy	37.5
	Kalancha	37.5
	Nyva Odeska	75.0
	Polianka	92.5
	Pochaina	75.0
	Total	62.5

Source: *developed by the authors*

According to such an integrative sign of mutagen concentration lethality as the number of cells with multiple aberrations (with the addition of the total frequency), for most varieties, the critical concentration can be between the DMS 0.025% and DMS 0.05% with a plateau in the range of DMS 0.0125% and DMS 0.025% or to the right of the higher one. Based on the nature of the expression of individual parameters, it can be concluded that the first hypothesis is more justified. For the Polianka and Balaton varieties, the critical concentration lies further away from the 0.05% DMS, while the Pochaina variety is characterised by a long plateau under the influence of all concentrations.

This study is part of a series of studies analysing the effects of a genotype-mutagenic set of chemical factors on a corresponding set of genotypes. Previous analyses of the data obtained showed (Nazarenko, 2017; Beiko & Nazarenko, 2022; Horshchar & Nazarenko, 2022) a decrease in site specificity with increasing mutagen activity, but when using DMS, the trend reversed, and site specificity increased significantly. This is evident from the pairwise comparison of genotypes but was not reflected in the number of traits with a significant effect of genotype, which may indicate the contrast of only individual varieties.

The plateau found in the studies, depending on the increase in the dose of the mutagen and the variety, has already been noted by researchers (Oney-Birol & Balkan, 2019), with a general decrease in mitotic activity and a gradual increase in the frequency of rearrangements in other material. This effect is typical in the range of high values of the agents of action. In addition, it demonstrated the ability to reduce the genotoxicity index, which in total allowed the researchers to conclude that the mutagenic effect is highly dependent on the characteristics of a particular variety as its subject.

Protocols of cytogenetic studies of mutagenic factors show (Spencer-Lopes *et al.*, 2018) that such values, although they belong to high and ultra-high doses and concentrations, have quite wide limits of variability in the case of continuous mutagens, but are 1.5-2 times lower for chemical supermutagens. The difference in recommendations is the use of such doses in the case of physical factors, while for chemical factors these are pre-critical concentrations (Bezie *et al.*, 2020), given the maximum values of usefulness in future generations (in the case of the studied material, DMS 0.0125 and 0.025%, except for the varieties Polyanka and Pochayna, for which the use of DMS 0.05% is more effective for obtaining complex changes)

Previous studies (El-Azab *et al.,* 2018; Pane *et al.,* 2018) have often found a lower significance of the subject of mutagenic influence, mainly at the level of the organism as a whole, The experiment confirms the significance of this conclusion and proves the preservation of the identified trends in a partial form at the cellular level, taking into account some changes that are related to the structural features of the genome of certain varieties and that may not affect the level of fixation of depressive effects in terms of phenotypic indicators in the first generation. This may indicate the possible presence of separate genetically determined mechanisms of resistance to the mutagenic factor and their increasing importance with the increase in the strength of the mutagen, even though previous studies (Nader *et al.,* 2022) also associated a decrease in the site specificity of some chemicals, especially new classes of nanoparticles, which, despite the overall negative effect on mitotic activity and induction of a significant number of rearrangements, may not have shown any dependence on the subject of action.

Despite the peculiarities of the action of each mutagen in the group as a whole, the greater significance of such classical indicators (Nurmansyah *et al.*, 2018) as the total frequency, number and ratio of fragments and bridges does not decrease. They are the main indicators of mutagenic activity at the cytogenetic level, as established by cycles of previous studies (Bhat & Wani, 2017), especially in terms of identifying the properties of a chemical agent, while other indicators are more complementary (Nurmansyah *et al*, 2018), even when applying agents with high correspondence to DNA structures and using insertional mutagenesis (Ram *et al.*, 2019), which has a higher level of dependence on the subject

of action. Of great importance for establishing the significance of individual concentrations in terms of peak activity is the rate of complex aberrations, which has been observed in both less harmful agents (Nazarenko & Izhboldin, 2017; Bhat & Wani, 2017) and aspects of continuous action (Pramanik *et al.*, 2018), although in the latter case, it is proposed to give priority to the use of sublethal and lethal doses. In the first case, the recommendation to use more moderate options remains, which can be based on both mutagenic depression and monitoring of cytogenetic activity.

CONCLUSIONS

According to the pairwise comparison of cytogenetic activity for individual varieties, the role of genotype in determining the reaction of the subject of mutagenic action to the increase in the damaging properties of the mutagen (considering both its nature and the increase in the concentration of the factor) was found to be increasing. As the concentration of the agent of genotype-mutagenic interaction increases, the difference between individual varieties in the variability of cytogenetic parameters of the spectrum increases, which is reproduced by the power of classification analysis of objects by a set of indicators and by the significance of three parameters (total frequency, number of bridges and fragments) according to factor and discriminant analysis. The sensitivity of the varieties manifested at the organism level in terms of mutagenic depression is partially preserved, although it may differ for individual varieties (Niva Odeska, Polyanka). A rather significant variety of reactions to the action of the studied mutagenic factor was shown. The data obtained on predicting the effectiveness of individual concentrations (DMS 0.0125 and 0.025%) in terms of inducing certain types of mutations require both the expansion of the source material to confirm the obtained patterns and further research at the level of consideration of visual and biochemically identified micro- and macro- changes that are heritable during field trials in the next generations, which is included in the plans (involvement of additional varieties of Western European ecotypes, research of the second or third generation).

ACKNOWLEDGEMENTS

The authors are grateful to the Ministry of Education and Science of Ukraine for supporting the research within the theme “Use of induced biodiversity of cereal crops”.

CONFLICT OF INTEREST

None.

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Цитогенетична активність мутагенного чинника з високою ушкоджувальною здатністю у пшениці озимої

Владислав Іванович Горщар
Кандидат сільськогосподарських наук, доцент
Дніпровський державний аграрно-економічний університет
49600, вул. С. Єфремова, 25, м. Дніпро, Україна
https://orcid.org/0000-0001-9175-9749

Микола Миколайович Назаренко
Доктор сільськогосподарських наук, професор
Дніпровський державний аграрно-економічний університет
49600, вул. С. Єфремова, 25, м. Дніпро, Україна
https://orcid.org/0000-0002-6604-0123

Анотація. Аналіз цитологічних аномалій є важливим методом ідентифікації можливостей хімічної речовини як мутагену для майбутніх спадкових генетичних змін, рівня генотип-мутагенної взаємодії та сайт-специфічної активності для природи та/або різних концентрацій мутагену. Метою експерименту було визначити межі мінливості генотипів різного походження, насамперед із широкою еколого-генетичною мінливістю, показати взаємодію між сортом та мутагеном. Зерно пшениці озимої кількох сортів (Балатон, Боровиця, Зелений Гай, Золото України, Каланча, Нива Одеська, Полянка, Почайна) обробляли диметилсульфатом у концентраціях 0,0125 %, 0,025 %, 0,05 %, експозиція становила 24 години. Було досліджено цитогенетичну активність досліджували за частотою та спектром хромосомних перебудов у відповідних фазах клітинного поділу в залежності від сорту та концентрації мутагену як основних факторів, що впливають на ці показники, також основні ознаки спектру, такі як загальна швидкість хромосомних перебудов, кількість фрагментів і подвійних фрагментів, містків, мікроядер і відстаючих хромосом. Було встановлено, що досліджувані концентрації супермутагену суттєво вплинули на всі аналізовані параметри, їх можна віднести до оптимальних і високих рівнів концентрацій за впливом на цитологічну активність і проблемами у мітозі, для чинника, незважаючи на попередні дослідження, фактор сорт значно більше впливає на природу та частоту деяких типів аберацій, ніж збільшення концентрації мутагену, він характеризувався значно більшою сайт-специфічною дією, ніж інші хімічні агенти, різні варіанти по змінам мутагенної дії були ідентифіковані відповідно до генотипу суб'єкту. Було узагальнено, що ознаками, які відтворювали вплив мутагену, згідно дискримінантного аналізу, є загальна частота, частота фрагментів і подвійних фрагментів, містки. При практичному використані в генетичному поліпшені рослин та для отримання цінних ознак оптимальним є використання концентрацій 0,0125 %, 0,025 %, що планується додатково підтвердити на більш широкому спектрі генотипів та дослідженнями мутацій для наступних (другого-третього) поколінь

Ключові слова: хромосомні аберації; мутаген; пшениця озима; диметилсульфат; цитологічний аналіз

SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 143-152

UDC 630:614.8
DOI: 10.48077/scihor9.2023.143

Assessment of wildfires forecast performance in Albania: Case study

Orjeta Jaupaj*
Doctor of Sciences, Researcher
Polytechnic University of Tirana
1024, 60 Don Bosko Str., Tirana, Albania
https://orcid.org/0000-0002-0175-5005
Alban Doko
PhD, Researcher
Polytechnic University of Tirana
1024, 60 Don Bosko Str., Tirana, Albania
https://orcid.org/0009-0006-4079-0125
Ardit Dervishi
Researcher
Polytechnic University of Tirana
1024, 60 Don Bosko Str., Tirana, Albania
https://orcid.org/0009-0002-4679-6019
Florinda Kadria
Master of Sciences, Technician
Polytechnic University of Tirana
1024, 60 Don Bosko Str., Tirana, Albania
https://orcid.org/0009-0005-5597-0324
Klodian Zaimi
PhD, Researcher
Polytechnic University of Tirana
1024, 60 Don Bosko Str., Tirana, Albania
https://orcid.org/0000-0003-0495-586X

Article's History:
Received: 16.05.2023
Revised: 26.08.2023
Accepted: 27.09.2023

Abstract. The harmful impacts of climate change caused by wildfires are substantially harming the people of mainland Europe, as well as damaging species biodiversity and the ecosystem. It can be minimised by improving the effectiveness of fire risk forecasting and mitigation strategies. The aim of this paper was to investigate the accuracy of forest fire forecasts in Albania produced by the FWI (Fire Weather Index) system. During the summer of 2022, observations and data were collected on expected and actual fires in the prefectures of Albania, which were previously divided into four

Suggested Citation:
Jaupaj, O., Doko, A., Dervishi, A., Kadria, F., & Zaimi, K. (2023). Assessment of wildfires forecast performance in Albania: Case study. *Scientific Horizons*, 26(9), 143-152. doi: 10.48077/scihor9.2023.143.

*Corresponding author

categories according to the level of fire risk: high, moderate, low, and zero. It was determined that, in the summer of 2022, Albania happened a grand total of 620 wildfires. The data were analysed using two indicators: the probability of fire occurrence for a particular prefecture and the number of fires per prefecture. The analysis revealed varying degrees of accuracy in fire predictions across different prefectures, with higher precision observed in high-risk regions but diminishing as the risk level decreased. The most dependable indicator of forecast accuracy, reaching 75%, was observed in high-risk areas during the month of August. Predicting fire localization within moderate-risk zones consistently achieved results above 50% but fell short of the 60% threshold. Overall, the results confirm the effectiveness of using data to predict the probability of fires for prefectures with a high and increased level of the relevant threat. This will make it possible to more effectively deploy and mobilise the resources needed to overcome them and substantially reduce the losses associated with them

Keywords: fire prevention; predicting fires in forested areas; natural hazards; risk assessment; Albanian forests

INTRODUCTION

Wildfires, often fueled by dry conditions, high temperatures, and flammable vegetation, can unleash destructive and uncontrollable forces that devastate landscapes and endanger lives. The frequent occurrence of natural forest fires in Europe and around the world leads to many adverse consequences for the environment and the population of the country's most often affected by this problem. Studying the ability to predict the possibility of localization, as well as the period and scale of fire occurrence is relevant, because it allows you to assess the need for evacuation, as well as to mobilize the necessary resources in time to overcome the fire and minimize possible consequences.

Albanian scientists E. Muça *et al.* (2022) determined that gas emissions from the combustion of high-carbon biomass not only increase the greenhouse effect and, in the long term, can substantially affect the composition of the atmosphere, but also reduce the number of plants that contribute to the conversion of carbon dioxide and the transfer of carbon into a form accessible to living organisms, playing an important role in its transformation. O. Jaupaj *et al.* (2023) conducted a survey of forecast warnings and fire events in the summer seasons for 2017-2021 in Albania. Scientists have confirmed that gas emissions affect the condition of soils and soil microbiota, which also takes an active part in the transformation of elements. The increase in the greenhouse effect and global warming trends only increase the probability of fires. Massive fires also pose a threat to the lives, health, and property of people living nearby and have a negative impact on their well-being which also increases the relevance of the research.

L.M. Johnston *et al.* (2020), in their work on defining the prerequisites for fire prediction, identify the following main components of natural fires: probability, which includes fire behaviour and the possibility of its occurrence in a particular place, impacts and consequences, and contact. Based on these key points, as well as on the array of data collected on fires that have already occurred, it appears possible to see the preconditions for a natural fire in the future.

Currently, there are two global European databases that collect the works of most researchers and the content of the archives of numerous research centres in different countries – the Global Fire Monitoring Centre (UNDRR, 2017) and the European Forest Fire Information System (EFFIS) (European Commission, 2023).

S. Sudhakar *et al.* (2020) were among the first to reach the next stage – the creation of a specific index for characterising fires. They used data from unmanned aerial vehicles (UAVs) to calculate the Fire Detection Index (FDI) and the Forest Fire Detection Index (FFDI). However, the most commonly used is the Canadian Fire Weather Index (FWI) by C.E. van Wagner and T.L. Pickett (Archibald *et al.*, 2018), developed within the framework of the Canadian Forest Fire Detection and Rating System (CFFDRS). For European conditions, this index is adapted by EFFIS, and for Albania, since 2016, is being used by the Institute of Geosciences (IGEO) (n.d.). The actual work on the study of fires in the country is carried out by a subdivision of this institute – the Centre for Forecasting and Monitoring of Natural Hazards (CFMNH) (Jaupaj & Zaimi, 2021). Despite the diversity of methods explored in the listed works for fire prediction, it is evident that a unified and comprehensive methodology is crucial for effectively forecasting not only the onset and progression of fires but also their potential consequences.

Thus, the purpose of this paper was to establish the degree of reliability of the forecasts obtained using the FWI system for prefectures of Albania with different levels of fire risk according to the CFMNH classification.

MATERIALS AND METHODS

To determine the factual reliability of the fire weather index (FWI), were assessed the correspondence between the real frequency of fires in the territory of certain prefectures of Albania and the risk levels obtained from the FWI. Observations of the fact and location of fires were recorded every day throughout the summer of 2022 by the National Civil Protection Agency (NCPA), whose data was used during the study. The data were further processed in three main periods: July and August, as

months of increased risk, and the entire summer. The results of the observations were compared with FWI data obtained by getting EFFIS and distributed in 4 categories of Risk. The information was posted on the center's website in the open access format "today for tomorrow", i.e., one day in advance (European Commission, n.d.).

Depending on the probability of fires occurring and their real occurrence, prefectures were assigned to high, moderate, low, and no risk zones, and the assessment was conducted within these zones by analysing two main factors: the coincidence of the expected and real number of fires within the risk level and the ratio of the number of fires per coincidence.

Thus, the first factor was expressed through the probability of fires in the prefecture (PHP). This indicator was calculated using the following formula (Formula 1):

$$\text{PHP}_{\text{Level}} = \sum_{i=0}^{n} \frac{n^0\text{Hits}}{n^0\text{Alerts}} \text{ x } 100\% , \quad (1)$$

where, PHP – is the probability of fires for the prefecture; n^0 of HITs – is the number of prefectures where fires occurred in the risk group under consideration; n^0 of Alerts – is the number of fire forecasts for a given risk level.

The second equally important factor under study was the average number of fires per prefecture (AFH). This indicator was defined as the ratio of the number of fire observations to the number of prefectures and was calculated separately for prefectures of each risk level. To simplify the assessment, the data were categorised into P_{C1} and P_{C2} (category 1 and category 2 performance) for PHP and AFH respectively and interpreted qualitatively in the format "very good", "good", and "bad", according to the template (Table 1).

Table 1. *Thresholds of PHP and AFH used for categorising the wildfire forecast Performance-Component 1 & 2*

PHP						
	P_{C1}	PHP, %	P_{C1}	PHP, %	P_{C1}	PHP, %
High risk	VERY GOOD	70-100	GOOD	50-70	BAD	>50
Moderate risk		50-70		40-50		>40
				70-80		<80
Low risk		20-40		10-20		>10 <50
				40-50		
Very low risk		0-10		10-20		<20
AFH						
	P_{C2}	AFH	P_{C2}	AFH	P_{C2}	AFH
High risk	VERY GOOD	<1.5	GOOD	1.01-1.5	BAD	1
Moderate risk		1.2-1.5		1.5-1.8 1-1.2		<1.8
Low risk		1.02-1.2		1.2-1.5 1-1.2		<1.5
Very Low risk		1-1.02		1.02-1.16		<1.16

Source: *compiled by the authors based on O.E. Jaupaj and K. Zaimi (2021)*

The final fire forecasting performance (P) was determined based on the obtained indicators and evaluated on a scale from 1 to 5, according to the scheme (Table 2). All indicators were evaluated for the entire summer, as well as separately for July and August.

Table 2. *Categorising the wildfire forecast performance-final evaluation*

Final evaluation	P_{C1}	P_{C2}	P_{C1}	P_{C2}	P_{C1}	P_{C2}	P_{C1}	P_{C2}	P_{C1}	P_{C2}
	VG	VG	G	VG	G	G	B	G	B	B
			VG	G			G	B		
	1		2		3		4		5	

Source: *compiled by the authors*

RESULTS

To begin with, it's essential to discern the distinctions between the IGEO and EFFIS classifications, as well as the units used to measure fire intensity. This differentiation is visually presented in Table 3.

***Table 3**. IGEO vs. EFFIS fire danger classes*

Fire danger classes	FWI ranges	
	EFFIS	IGEO/CFMNH
No risk		<5.2
Very low	<5.2	-
Low	5.2-11.2	5.2-11.2
Moderate	11.2-21.3	11.2-38.0
High	21.3-38.0	>=38.0
Very high	38.0-50.0	-
Extreme	>=50.0	-

***Source:** compiled by the authors based on O.E. Jaupaj and K. Zaimi (2021)*

EFFIS identifies 6 levels of risk, while IGEO has only four, the ranges of which coincide with the corresponding levels in the EFFIS classification. So, in general, the difference is that the European system distinguishes two more levels of "very high" and "extreme" danger, which are not distinguished by the Institute of Sciences of Albania. The assessment of the possibility of predicting the risk of fire occurrence in Albania using FWI methods included the collection of two types of data: real fire occurrences and fire forecasts in the respective prefectures. In July, 305 fires broke out across the country. They were most often recorded in moderate-risk prefectures – in 61.3% of cases. High-risk prefectures accounted for 37.7% of the total number of fires, and less than 1% of fires occurred in low- and zero-risk prefectures (Table 4).

***Table 4.** Data on forecast alerts, fire occurrence and HITS by risk levels, 2022*

	July	August	Summer
FA_{High}	74	55	129
FO_{High}	115	90	205
Hits	50	41	91
$FA_{Moderate}$	206	139	345
$FO_{Moderate}$	187	166	353
Hits	106	82	188
FA_{Low}	14	68	82
FO_{Low}	2	40	42
Hits	2	23	25
FA_{No}	18	86	104
FO_{No}	1	19	20
Hits	1	15	16
FA_{Total}	312	348	660
FO_{Total}	305	315	620

***Source:** compiled by the authors*

The forecasts for this period showed a slightly different picture: 312 forecast warnings predicted that 23.7% of the probable fire occurrences would occur in high-risk areas, and 4.5% and 5.5% in low- and no-risk prefectures. The forecast for the number of fires in moderate risk prefectures was close to reality – 66%. Almost the same total number of fires occurred in August – 315 recorded incidents. Between different risk levels, the ratio of fires was as follows: 28.6% in high-risk prefectures, 52.7% in moderate-risk areas, 12.7% in low-risk areas, and 6% of the total number of fires occurred in prefectures with no risk. Therewith, 348 alerts about possible fires were recorded. Forecasts for high-risk, moderate-risk, and low-risk prefectures were close to reality – 25.8%, 47.7%, and 11.5%, respectively. As in the previous month, the number of real fires in areas of no risk was considerably lower than the estimated 15% (Table 4).

A total of 620 fires were recorded in Albania during the summer of 2022. Of these, the prefectures in the moderate risk zone accounted for the largest share

(56.9%), while in the high-risk zone the figure was 31.1%, and the lowest number of fires was observed in the prefectures in the low and no risk zones – 6.7% and 3.3%, respectively. The corresponding data obtained as a result of the daily collection of forecasts regarding the risk of fire occurrence in the territories of individual risk zones correlate well with the real map of fires in the prefectures of the moderate risk zone – 52.3% of the predicted probability compared to 56.9% – the proportion of recorded fires in this zone from all that occurred in all zones during the summer. Fires in the high-risk zone really occurred more often than expected, accounting for 19.6% of the total. It was also assumed that fires would occur more frequently in low and zero-risk areas, accounting for 12.4% and 15.7% of the total. The predicted (FA) and real (FO) quantitative distribution of fires in prefectures of different hazard levels is presented in the graph (Fig. 1).

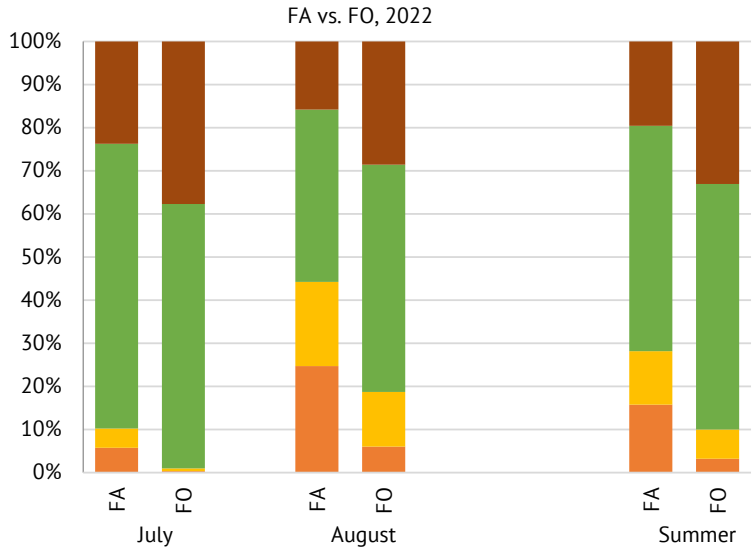

***Figure 1**. The distribution of Forecast Alerts (FA) and Fire Occurrence (FO) by risk levels*
***Source:** compiled by the authors*

It shows that the percentage of fires that occurred in moderate-risk prefectures was predicted quite accurately both in the context of individual months and for the entire season. The largest difference between the predicted and real situation in all cases concerned the prefectures with no-risk. Therewith, predictions of fire occurrence in particular prefectures were more accurate for prefectures in high-risk areas, worse for moderate-risk prefectures, and very poor for low- and no-risk prefectures (Fig. 2).

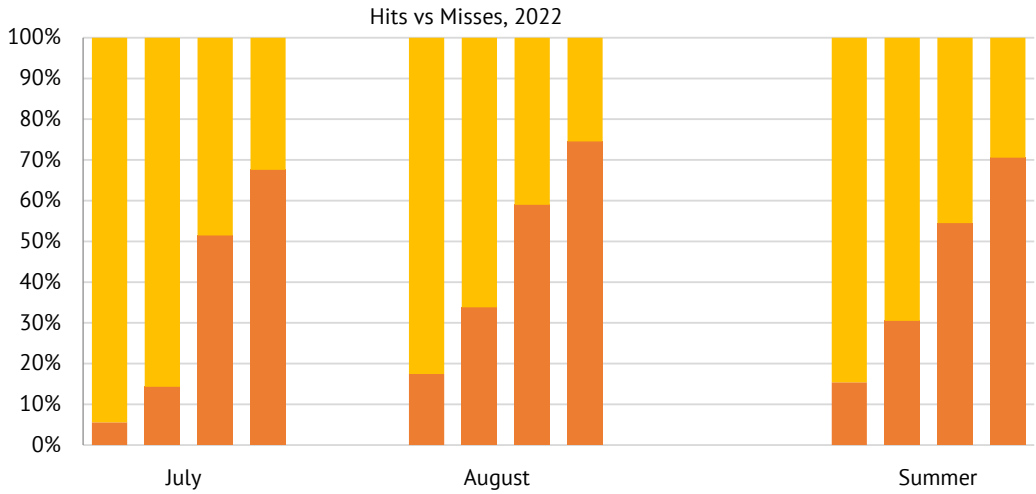

***Figure 2**. The distribution of Hits and Misses by risk levels*
***Source:** compiled by the authors*

The hit rates of the latter did not exceed 34%, 30%, or even 20%. The best indicator of forecast accuracy and fire occurrence in specific locations was 75% for high-risk areas in August. This high result could also be explained by the relatively small number of risk areas of this type. Predicting fire localisation within moderate hazard zones gave stable results, which, although exceeding 50%, did not reach 60%. However, apart from the ratio of correct to incorrect predictions, it is also worth considering that during the observation period, especially in higher risk areas, more fires almost always occurred than predicted, and therefore the ratio between the predicted and real number of fires differs for these prefectures (Table 5). Thus, for high-risk prefectures, about 45% of fires were predicted for each of the time periods under study, and for moderate risk prefectures, from 49% to 53%. In the case of low- and zero-risk areas, the percentage of predicted fires ranged from 57% to 100% and 79% to 100%, respectively.

The average frequency of prefectural fires (AFH) was highest for high-risk prefectures, with approximately two or more per prefecture, with no statistically significant difference between the periods under study. In moderate-risk prefectures, 1-2 fires occurred during the monitoring period, with a slightly higher frequency in August. In July, approximately the same number of fires – one or none – were observed in low and no probability prefectures, while in August, as in the entire summer period, these figures were slightly higher for no probability prefectures and higher for low probability areas (Fig. 3).

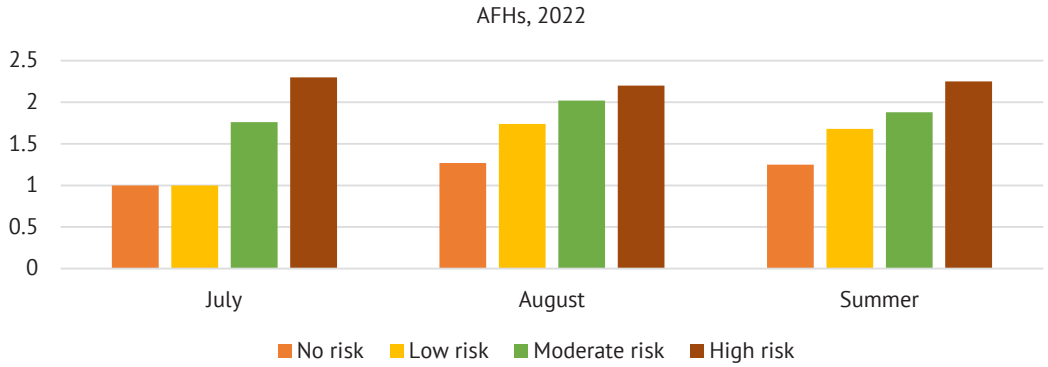

Figure 3. *The distribution of Average Fires per Hits by risk levels*

The prefectural fire probability assessment for July was most effective for moderate risk prefectures, and, like the number of fires per prefecture, reached the "very good" level. In addition, both indicators for the absence of risk were rated "very good". For high-risk prefectures, the result was worse – the first indicator reached only 68%, which corresponds to the "good" mark for this level of danger. Therewith, the second indicator was rated as "very good". The low-risk prefectures performed even worse: both indicators reached the "good" level, as they were too low (28% and 1.0) to reach the "very good" level. The final performance of the forecasts made in July was assessed as: "2" or "good", although the PHP values were above the "very good" threshold for two risk levels – moderate and no risk – and the AFH values reached this threshold for two risk levels – high and no risk. In general, all levels had different scores in both components, which allowed them to take certain places in the ranking as follows: $P_{high\ risk}$"2", $P_{moderate\ risk}$→"2", $P_{low\ risk}$→"3", $P_{no\ risk}$→"1", according to the data obtained (Table 4).

Table 5. *Wildfire Forecast Performance on a monthly duration basis and season duration basis PATH 2 raw data, PHP, AFH, 2022*

	July						August					Summer				
	PHP, %	AFH	P_{C1}		P_{C2}	P_F	PHP, %	AFH	P_{PHP}	P_{AFH}	P	PHP, %	AFH	P_{PHP}	P_{AFH}	P
High risk	68	2.30	G	Low PHP	VG	2	75	2.2	VG	VG	1	71	2.25	VG	VG	1
Moderate risk	51	1.8	VG		G	2	5%	2.0	VG	√++	2	51	1.9	VG	√++	2
Low risk	14	1.0	G	Low PHP	√-	3	34	1.7	VG	√++	2	28	1.7	VG	√+	2
No risk	6	1.0	VG		VG	1	17	1.3	G	High PHP	√++	3	14	1.3	G	High PHP	√+	3

Source: *compiled by the author of this study*

The effectiveness of risk assessment in August was somewhat lower overall, due to the unsatisfactory results of the assessment of the number of fires per prefecture. In turn, the prefectural fire probability score was slightly better, with three prefectures in the high, moderate, and low hazard levels achieving a "very good"

rating, up from two in July. Therewith, the forecasting efficiency for the zero-risk group dropped to "good", with a result of 17%, which is too high for this level of danger. AFH values stayed at the "very good" level for high-risk prefectures, but were too high for moderate, low, and zero risk (2.0, 1.7, and 1.3, respectively) and scored "BAD" on the rating scale, which is significantly worse than the previous month.

Fulfilment of forecasts for August, due to improved PHP results, eventually reached the mark of "2", which corresponds to a "good" rating. Forecasts for high-risk prefectures have become more effective, with the "good" rating changing to "very good", and the forecasts for low-risk prefectures improving from "bad" to "good". The performance of moderate-risk prefectures stayed generally unchanged, while the performance of zero-risk prefectures deteriorated from "good" to "bad". The rating of forecasts for different risk levels in August was as follows: $P_{high\ risk}$ "1", $P_{moderate\ risk}$→"2", $P_{low\ risk}$→"2", $P_{zero\ risk}$→"3".

Overall, during the summer of 2022, the forecasts for the high-risk prefectures were rated as "very good" for both indicators. For moderate-risk prefectures, the first component was also at the "very good" level, while the second exceeded the values allowed for a positive assessment and stopped at the "bad" level. The situation was similar for the low-risk prefectures, with PHP receiving a "very good" rating and AFH receiving a "bad" rating. The lowest level of PHP for the season was characterised by zero risk level, along with a rating "bad" for the second indicator. For the entire summer season, the predictions were fulfilled for prefectures with different levels of wildfire risk as follows: $P_{high\ risk}$ "1", $P_{moderate\ risk}$→ "2", $P_{low\ risk}$→ "2", $P_{zero\ risk}$→ "3", with the final score being "2" – "good". These figures are closer to those obtained in August, but the actual results for the season on average were slightly better, albeit within the respective levels and without any shifts to higher or lower efficiency ratings. There is also a slight correlation between the total number of predictions made in a month and the number of hits, but the number of misses is also increasing.

DISCUSSION

The probability of wildfires is a pressing issue in Europe and globally, especially during periods of heightened danger, during which historically more incidents have occurred in the respective regions. In this context, the problem of fire prediction is quite acute and consists of two main components: monitoring their actual occurrence and recording the facts of risk, and the development and use of algorithms for predicting the probability of fires in a particular area. The next step is to prepare both direct firefighting measures and the relevant infrastructure, and to work with the public. EFFIS currently has 40 member countries, which includes almost all European countries, regardless of their affiliation with other international organisations (European Commission, n.d.). However, only 22 countries have been part of the so-called "common core" since 2004, which is engaged in continuous monitoring of the fire situation and collecting relevant data for comparison in the community (European Fire Database, n.d.).

This study evaluated the effectiveness of fire forecasting using the FWI system for prefectures of four risk levels on a scale from "bad" to "very good", with a final score of 1 to 5. Thus, the prefectures in the no-risk zone in July and the high-risk zone in August and for the whole summer received "very good". However, the forecasting results in the no-risk zone were not very good, due to the AFH component. In previous years of analogous surveys, the results were quite comparable. In 2020, the observations for July and August resulted in "very good" for high-risk prefectures in July and August (no data for June), and a similar result for zero-risk areas in June and July (Jaupaj *et al.*, 2023). In 2017, the "very good" observation result was obtained for prefectures in high-risk and no-risk areas in June, and for high-risk areas in July and August (Jaupaj & Zaimi, 2021). All other territories received marks of "good" and "bad" in a roughly even distribution across months and years.

Presumably, forecasting in these areas turned out to be the easiest for several reasons. In the high-risk zone, the frequency of fires was very high in all months, as was the number of fires per prefecture of the corresponding risk level, and therefore, apart from the pronounced risk factors that allowed for more comprehensive assumptions, the statistical probability of a hit was also higher, but this could not affect the final result in the zone of statistical significance. In turn, for prefectures with no-risk of fire, 4-11 times more predictions were made than real fires. This has increased the hit rate, but this approach, especially in the context of prefectures with more hazardous situations, could potentially put added strain on the firefighting system in general or on certain days.

According to M.M. Müller *et al.* (2013), in Austria, local fires are often smaller than 30 ha, and therefore their detection is not satisfactory with the accuracy of EFFIS. Similar to Albania, the country uses adapted Canadian indices and conducts fire forecasting based on its own national forecasting centre, which stores data for the last 25 years. A study by R. Zotta *et al.* (2023) estimated the efficiency of using FWI in Austria at 68%, but when integrating other information levels, such as socio-economic parameters, vegetation parameters, etc., the forecasting accuracy increased to 87%. These results are comparable to the "good" assessment obtained in this study, but do not include assessments of individual risk groups for territories. A. Depicker *et al.* (2020) describe the approach of the National Fire Forecasting Centre of Belgium, which has been intensively involved in updating and improving cartography since 2013. Even though spontaneous fires are not a top priority in this country, fire forecasting is based on extensive data on the structure and distribution of wood, soil, land use

history, etc., and the territory is divided according to the expected level of risk. These results are consistent with research I. Vallejo-Villalta *et al.* (2019).

The study by J. Bedia *et al.* (2018) on the prospects for the adaptation and use of the FWI index in the Mediterranean countries showed mixed results. The first part of the study was an analogous approach to the one used herein, namely comparing predictions and real observations of fire occurrence. This resulted in "good" and "above average" predictive scores for some countries, such as Turkey, Greece, and Bulgaria, and partially for France, and Central Spain. The use of two correction methods slightly increased the probability of prediction but did not critically change the overall picture. Negative trends were observed for the southern coasts of the Adriatic and Ionian Seas. In general, the data presented by the authors correlate with those obtained in this study and strongly suggest that it is necessary to consider more variables to predict the probability of fire occurrence using the index under consideration in areas that differ climatically and in terms of vegetation and soil types from the corresponding indicators in Canada. On the other hand, the study in question assessed the effectiveness of forecasting in the longer term – on the ground, while one-day forecasts were more likely to fulfil their potential (Bedia *et al*., 2018).

Another analogous study is related to the Mediterranean region, specifically the Iberian Peninsula and Greece. There, the use of the index was more successful, and T. Giannaros *et al.* (2021) identified the consideration of wind strength and direction and precipitation as factors of problematic aspects of indexing under this system and called temperature and humidity characteristics as compensatory aspects. These difficulties are related to the proximity of these territories to the World Ocean, which is a considerable factor in the variability of weather conditions and, accordingly, impairs the ability to predict the phenomena that are largely dependent on them (Giannaros *et al.,* 2021). The study by A. Novo *et al.* (2020) was aimed at obtaining a high-quality mapping image with zoning corresponding to the level of fire risk in Galicia, Spain. During the study, the authors came to the unequivocal conclusion that the use of automated and semi-automated methods for processing and forecasting various risk factors, and the FWI index in particular, is much more effective than forecasting with a greater share of human involvement.

Similar to Austria and Albania, Greece also introduced the FWI index. According to V. Varela *et al.* (2018) and K. Papagiannaki *et al.* (2020), it was implemented in approximately the same period – 2013, after five years of monitoring and data collection within the country, by the national observation centre. And for the effective functioning of forecasting using this index, work has been done to consider various combinations of additional factors. In general, one can observe mixed, but rather positive experiences with the FWI index in different parts of Europe, where studies have been conducted to determine its effectiveness. A general conclusion from the experience of these countries is that the use of this factor outside of Canada and areas with appropriate climate, soil and vegetation should be complemented by other indicators that are key to the region of implementation.

CONCLUSIONS

The study evaluated the factual reliability of the Fire Weather Index (FWI) and examined the correlation between the actual frequency of fires in specific prefectures of Albania and the risk levels predicted by the FWI. It was observed that fire predictions in specific prefectures exhibited higher accuracy in high-risk regions, but declined for those with moderate risk. Predictions for low and no-risk prefectures performed notably poorly, with hit rates not surpassing 34%, 30%, or even 20%. The most reliable indicator of forecast accuracy, reaching 75%, was observed for high-risk areas in August. Conversely, forecasting fire localization within moderate-risk zones yielded consistent results, consistently surpassing 50% but falling short of 60%.

It was also determined, for high-risk prefectures, approximately 45% of fires were forecasted during each of the examined time periods, while for moderate-risk prefectures, the prediction rates ranged from 49% to 53%. In contrast, for low-risk and zero-risk areas, the range of predicted fires spanned from 57% to 100% and 79% to 100%, respectively. In general, the results of using indices to predict the occurrence of fires in prefectural areas belonging to the high, moderate, low, and no risk zones are mixed, but mostly positive. It worth to say, that the forecasting accuracy in prefectures is still unstable and shows rather erratic results.

Thus, in countries such as Albania, which are at risk of many types of natural disasters and their high frequency, such studies are mandatory. The wide variety and frequency of hazards, if not properly understood and controlled, can lead to an increase in fire risk over time. The prospect of further research in this area is determined by the consistently high risks of forest fires in the Mediterranean forestry sector and the associated need to investigate the possibilities of minimising these risks and implementing warning systems for the presence of such a danger. There is also a need for further research to analyse burnt forest areas in individual prefectures at all levels of fire hazard forecasting. Therewith, emphasis should be on particular losses that have been recorded according to the high-risk forecast.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

None.

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Оцінка ефективності прогнозування лісових пожеж в Албанії: тематичне дослідження

Оржета Яупай
Доктор наук, дослідник
Політехнічний університет Тирани
1024, вул. Дон Боско, 60, м. Тирана, Албанія
https://orcid.org/0000-0002-0175-5005

Албан Доко
Кандидат наук, науковий співробітник
Політехнічний університет Тирани
1024, вул. Дона Боско, 60, м. Тирана, Албанія
https://orcid.org/0009-0006-4079-0125

Ардіт Дервіші
Науковий співробітник
Політехнічний університет Тирани
1024, вул. Дона Боско, 60, м. Тирана, Албанія
https://orcid.org/0009-0002-4679-6019

Флорінда Кадрія
Магістр наук, технік
Політехнічний університет Тирани
1024, вул. Дона Боско, 60, м. Тирана, Албанія
https://orcid.org/0009-0005-5597-0324

Клодіан Заїмі
Доктор філософії, науковий співробітник
Політехнічний університет Тирани
1024, вул. Дона Боско, 60, м. Тирана, Албанія
https://orcid.org/0000-0003-0495-586X

Анотація. Шкідливі наслідки зміни клімату, спричинені лісовими пожежами, завдають значної шкоди населенню континентальної Європи, а також завдають шкоди біорізноманіттю видів та екосистемі. Його можна мінімізувати, підвищивши ефективність прогнозування пожежних ризиків та стратегій пом›якшення їх наслідків. Метою цієї статті було дослідити точність прогнозів лісових пожеж в Албанії, зроблених за допомогою системи FWI (Індекс пожежної погоди). Протягом літа 2022 року були зібрані спостереження та дані про очікувані та фактичні пожежі в префектурах Албанії, які раніше були розділені на чотири категорії за рівнем пожежного ризику: високий, помірний, низький та нульовий. Встановлено, що влітку 2022 року в Албанії загалом сталося 620 лісових пожеж. Дані аналізувалися за двома показниками: ймовірність виникнення пожежі для конкретної префектури та кількість пожеж на префектуру. Аналіз виявив різний ступінь точності прогнозів пожеж у різних префектурах, причому вища точність спостерігалася в регіонах з високим ризиком, але зменшувалася зі зниженням рівня ризику. Найбільш достовірний показник точності прогнозу, сягаючи 75 %, спостерігався в зонах підвищеного ризику протягом серпня місяця. Прогнозування локалізації пожежі в зонах помірного ризику постійно досягало результатів вище 50 %, але не досягло порогу в 60 %. У цілому результати підтверджують ефективність використання даних для прогнозування ймовірності виникнення пожеж для префектур з високим та підвищеним рівнем відповідної загрози. Це дозволить більш ефективно розгортати та мобілізувати ресурси, необхідні для їх подолання, та суттєво зменшити втрати, пов'язані з ними

Ключові слова: запобігання пожежам; прогнозування пожеж у лісових масивах; природні загрози; оцінка ризиків; ліси Албанії

SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
***Scientific Horizons*, 26(9), 153-166**

UDC 338.1: 338.43: 339.13
DOI: 10.48077/scihor9.2023.153

The agricultural machinery market for crop production and prospects for its development in the postwar period

Yaroslav Navrotskyi*
PhD in Economic Sciences
National Scientific Centre "Institute of Agrarian Economics"
03127, 10 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0003-4677-5306
Oleksandr Zakharchuk
Doctor of Economic Sciences, Professor, Corresponding Member of the National Academy of Sciences
National Scientific Centre "Institute of Agrarian Economics"
03127, 10 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0002-1734-1130
Oksana Vyshnevetska
PhD in Economic Sciences, Senior Research Fellow
National Scientific Centre "Institute of Agrarian Economics"
03127, 10 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0003-0978-2063
Beata Glinkowska-Krauze
PhD, Professor
University of Lodz
90-136, 68 Narutowicza, Lodz, Poland
https://orcid.org/0000-0002-6915-3297
Oleksandr Kuchmieiev
Doctor of Economics, Associate Professor
Open International University of Human Development "Ukraine"
03115, 23 Lvivska Str., Kyiv, Ukraine
https://orcid.org/0000-0003-1803-6231

Article's History:
Received: 28.04.2023
Revised: 30.08.2023
Accepted: 27.09.2023

Abstract. Due to the hostilities, the largest losses in the agricultural sector of Ukraine's economy were caused by the loss of agricultural machinery – 20.9% of it was destroyed or damaged. Therefore, ensuring the effective functioning of the market for agricultural machinery is a key factor in restoring the material and technical base of agricultural enterprises. The research aims to investigate the current state of the agricultural machinery market and the impact of military operations on it and to identify effective

Suggested Citation:
Navrotskyi, Ya., Zakharchuk, O., Vyshnevetska, O., Glinkowska-Krauze, B., & Kuchmieiev, O. (2023). The agricultural machinery market for crop production and prospects for its development in the postwar period. *Scientific Horizons*, 26(9), 153-166. doi: 10.48077/scihor9.2023.153.

*Corresponding author

ways of its development. The following methods were used in the study: dialectical method of scientific knowledge, monographic, statistical, and economic, economic and mathematical, expert evaluation, and graphical. The general trend towards a decrease in the share of households with certain types of machinery in 2015-2022 is determined. Disproportions between large and small forms of management in the provision of machinery, as well as restrictions on access to financial resources for small agricultural enterprises, have been identified. The study identified a general decline in the size of the Ukrainian machinery market due to the loss of production capacity in the east and south of the country. Imports of agricultural machinery – tractors by 57.0%, combine harvesters by 30.0%, seeders by 33.8%, disc harrows by 45.1%, and cultivators by 38.9% – also decreased significantly in quantitative terms. The analysis of state support for agricultural machinery and agricultural enterprises is carried out. A forecast of the development of the agricultural machinery market for crop production under positive and negative scenarios, including risks and challenges that may arise during and after military operations, is made. Recommendations for the development of the market of agricultural machinery for crop production have been developed, including implementation of the state's protectionist policy; preservation of human resources; evacuation of agricultural machinery enterprises from the war zone; simplification of business to stimulate investment activity; state support for small farms, etc. The implementation of these recommendations will help restore the effective functioning of the agricultural machinery market and the logistics of agricultural production in Ukraine

Keywords: agricultural machinery market; imports; exports; state support; logistics

INTRODUCTION

The market of agricultural machinery is a key element in the formation of an efficient material and technical base of agriculture. Unlike the markets of the vast majority of countries in the world, which are focused on small producers, the national market is oriented towards large and medium-sized enterprises. Machinery for small producers is of secondary importance in the market. Therefore, few studies reveal the specifics of the functioning of such a market. In addition, war has a significant negative impact on the functioning of the agricultural machinery market.

The current state of the market is characterised by fluctuations determined by the economic and political situation in a particular country and the world. Agricultural machinery and vehicles are distinguished by their specificity related to the working environment and conditions of use: difficult working conditions; seasonality of work; reliability; work on fields with uneven terrain; service maintenance, etc. (Durczak *et al.*, 2020).

J. Van Loon *et al.* (2020) identified the problems of small farms' access to mechanisation. The most common of these is the mismatch between the savings from machine power and the size of the farm – most of the world's food production is carried out by small farms, many of which have land in different places, which negates the efficiency of using more powerful machinery; the high cost of machinery makes it unaffordable for many, and financial support from the state and bank lending is limited; shortage of skilled workers and limited opportunities for their training. The authors see a way out in providing small farms with user-friendly machinery adapted to work in small fields and suitable for local business conditions. Technologies should be introduced that minimise negative social and environmental impacts.

Studying the evolution of agricultural mechanisation, D. Lewis *et al.* (2022) identified the most influential factors: the expansion of input markets; the development of urban-rural linkages; and the climate crisis. The authors drew attention to the need to formulate policies for the development of mechanisation of small farms and small and medium-sized enterprises by taking into account local and national characteristics, obtaining proposals from various sources, and creating a database of these enterprises, in particular through public-private partnership experiments.

The problems of mechanisation development for small agricultural enterprises are also considered by Y. Wang & G. Huang (2022). They see their solution in using the services of specialised enterprises. To solve the problem of allocating shared agricultural machinery according to time windows, the researchers developed a two-stage system for dispatching the use of agricultural machinery with time windows.

The level of mechanisation among small farms was studied by T. Qiu & B. Luo (2021). They found a relationship between the size of the farm and the level of use of mechanisation services in agricultural production. R. Markov (2019) notes the need to intensify the processes of technical support of crop production enterprises in Ukraine, especially small farms, due to their limited access to machinery. He substantiates the need to improve an integrated approach to providing state support for the development of crop producers and domestic agricultural machinery enterprises. V. Mukha (2022), studying the Ukrainian agricultural machinery market during the coronavirus pandemic, noted its significant development in 2021. With the beginning of Russian aggression in 2022, there was an absolute drop in sales. In 2022, Ukrainian-made machinery, except for tractors, was in particular demand, with Chinese manufacturers retaining leadership in this segment.

The research aims to identify effective ways to develop the agricultural machinery market in the context of military operations and after their end. The research objectives are as follows: to determine the theoretical foundations of the agricultural machinery market and its relationship with market actors; to analyse the dynamics of the provision of enterprises with agricultural machinery, its production and import; the impact of the war on market development; to summarise the findings and provide practical recommendations for improving the functioning of the agricultural machinery market.

MATERIALS AND METHODS

The analysis of the state of the agricultural machinery market and prospects for its development in the post-war period in Ukraine on a scientific basis is reflected in the relevant methodological approach. The scientific basis of the methodological approach to the analysis of technical and technological support of agricultural producers and export-import operations is theoretical support, practical principles of analysis and evaluation of this support, description of research methods, conditions of implementation, list of stages and expected results.

The methodological basis of the study is the dialectical method of cognition and a systematic approach to studying the development of the agricultural machinery market in Ukraine. At the same time, the following methods were used in the course of the study: monographic (in highlighting the views of scientists on the issues and problems studied in the work, in studying the current state of agricultural machinery in Ukraine); economic and statistical (in analysing foreign trade in agricultural machinery); tabular and graphical (in visualising the results of the study in the form of figures and tables); empirical (in studying the state of Ukrainian agricultural machinery and the sectoral machinery market in the wartime); comparative analysis (to identify the main problems of the development of the domestic agricultural machinery market in Ukraine); analytical forecasting method (to determine the tasks and recommendations for the organisational and economic mechanism of technical and technological support of agricultural producers in the post-war period); abstract and logical (generalisation and formulation of conclusions).

The statistical method is used to study the impact of factors of foreign economic activity and to forecast the situation on the agricultural machinery market in the post-war period. The characteristics of import operations change over time, and indicators of time series are used to assess the intensity of their development (Table 4, Figs. 2-4). The chain analysis method was used to obtain absolute and relative growth. The assessment is based on a comparison of imports and production volumes of machinery in the dynamics over different periods. The analysis was carried out both in general and by individual product groups in physical and value terms.

The study analyses the dynamics of import operations, in addition to studying the characteristics of import flows, and is supplemented by a study of the availability of agricultural machinery in enterprises and households, production volumes of the main types of agricultural machinery in Ukraine, the purchase of machinery under the partial compensation programme, and forecasts the development of the agricultural machinery market under optimistic and pessimistic scenarios. The system of indicators is built based on an indicative assessment using descriptive statistics and statistical analysis methods.

The information base of the study was based on the works of Ukrainian and foreign scholars, reference and information publications, and the regulatory framework of state economic regulation of the development of the agrarian sector of the economy (Official web portal of the Parliament of Ukraine, n.d.), statistical and analytical data of the Ministry of Agrarian Policy and Food of Ukraine (n.d.), the State Statistics Service of Ukraine (n.d.), the State Customs Service of Ukraine (n.d.), scientific electronic sources of information on the global computer network Internet, and the authors' observations.

RESULTS AND DISCUSSIONS

Crop production is the basis of human nutrition, the feedstock for livestock, raw materials for industry, and thus the basis of the country's food security. A key condition for its efficient production is to provide agricultural enterprises with modern and high-performance agricultural machinery, which is impossible without a functioning agricultural machinery market. In the pre-war period, agricultural enterprises and households had the opportunity to constantly upgrade machinery, which, although not fully and in insufficient quantities, met production needs, while maintaining the general trend towards a reduction in the number of machinery in both agricultural enterprises and households. The issue of the availability of modern and high-performance machinery is particularly acute for small and medium-sized farms (Table 1).

The number of available machinery in the surveyed enterprises remained almost unchanged over the period, except for a significant drop in the availability of combine harvesters (29.3%) and harrows (17.5%) in 2019 compared to 2015. This decline is explained by the periodic mismatch between purchases and write-offs and fluctuations in demand, and applies to all types of machinery, as evidenced by a significant decrease in combine harvesters imports in 2018, when the availability of this type of machinery decreased (2,197 units), after significant imports in 2017 (3,250 units). The situation is similar for other types of machinery. The values of the indicator of machinery availability in households are even more unstable than in enterprises, which is related to their solvency and directly depends on agricultural prices.

Since statistical observations on the availability of equipment have not been conducted since 2020, 2019 figures are taken as the baseline. During the year of full-scale war, the largest losses in tractors were 9.1% (18203 units were destroyed and 10068 units were partially damaged) and the smallest losses in ploughs were 2.1% (6363 units were destroyed and 241 units were partially damaged). The total losses of agriculture from the hostilities for the year amounted to 8717.7 million USD. USD, with losses of agricultural machinery destroyed and damaged amounting to 4655.7 million USD or 53.4%. USD or 53.4%.

Table 1. *Dynamics of agricultural machinery availability in enterprises and households in 2015-2019* and losses during full-scale military operations, thousand units*

Vehicle type	2015	2016	2017	2018	2019	as of 24.03.2023	War losses, %***
Tractors**	127.9	132.7	129.3	128.7	130.5	×	×
Tractors***	181.9	207.1	217.8	248.6	180.1		
Tractors, total	309.8	339.8	347.1	377.3	310.6	282.3	-9.1
Combine harvesters**	37.5	37.9	36.6	26.3	26.5	×	×
Combine harvesters***	18.8	14.8	15	16.6	14.6		
Combine harvesters, total	56.3	52.7	51.6	42.9	41.1	38.7	-5.8
Planters**	65.5	67.2	66.3	65.1	66.5	×	×
Planters***	93.8	98.8	127.5	130.9	122.5		
Planters, total	159.3	166	193.8	196	189	178.1	-5.8
Ploughs**	47.3	49.3	49.1	49.9	51.4	×	×
Ploughs***	313.2	284.3	287.6	301.9	258.8		
Ploughs, total	360.5	333.6	336.7	351.8	310.2	303.6	-2.1
Cultivators**	69.5	71.7	70.1	70.5	71.6	×	×
Cultivators***	129.4	112.1	119	139.7	121		
Cultivators, total	198.9	183.8	189.1	210.2	192.6	180.2	-6.4
Harrows**	194.0	192.0	181.4	161.1	160.0	×	×
Harrows***	427.4	378.6	400.2	363.7	342.6		
Harrows, total	621.4	570.6	581.6	524.8	502.6	473.2	-5.8

Note: **- since 2020, statistical observations on the availability of agricultural machinery in enterprises and households have not been conducted*
***- in agricultural enterprises*
****- In households*
*****- from 24.02.2022 to 24.02.2023*
Source: *compiled by the author based on State Statistics Service (2015-2019) and Agricultural War Damages (2023) data*

No statistical observations have been made since 2018. The calculated figure is based on the growth in the number and share of tractors with engine power over 90 kW in the total volume of tractor imports. Although the number of tractors in agricultural enterprises decreased from 1991 to 2017, from 497.3 thousand units to 129.3 thousand units, the average tractor engine power has been steadily increasing – from 63.2 kW in 1991 to 97.3 kW in 2017 (State Statistics Service of Ukraine, n.d.), and is estimated to reach 99 kW in 2021, which has increased their productivity. The growth of the average tractor power is influenced by the development of agricultural holdings that use powerful machinery to cultivate large areas. Thus, in 2021, 4129 units of tractors with an engine power of more than 90 kW (13.7% of all imported units) were imported into Ukraine, while in 2017, only 2857 units (10.7%) were imported. Even in 2022, despite the outbreak of a full-scale war, their share in total tractor imports was 12.2% or 2040 units (State Statistics Service of Ukraine, n.d.).

An analysis of the availability of certain types of machinery in households indicates a downward trend in the share of these farms, although the share of households that owned agricultural machinery increased from 17.5% in 2015 to 20.0% in 2019 (Table 2).

Table 2. *Share of households with agricultural machinery in use in 2015-2019* and in 2022, %*

Vehicle type	2015	2016	2017	2018	2019	2022**
Total	17.5	17.4	19.0	19.9	20.0	20.1
Tractors	31.2	24.6	23.7	27.2	19.2	19.4
Harvesters	2.3	1.8	1.7	1.7	1.6	1.7
Planters	13.9	12.4	14.5	14.3	13.1	13.2
Ploughs	37.3	34.6	31.3	32.0	27.3	27.5
Cultivators	15.8	14.1	13.4	15.0	13.2	13.3
Harrows	34.1	30.9	29.3	27.5	24.0	24.2

Note: **Since 2020, statistical observations on "Share of households with agricultural machinery in use" have not been conducted*
*** – calculated*
Source: *compiled by the author based on State Statistics Service (2015-2019)*

The largest decrease was in the share of households that owned harrows – from 34.1% to 24.0%. The share of combine harvesters gradually decreased from 2.3% to 1.6% due to the constant increase in the cost of servicing this complex machinery (fuel, spare parts, machinery repair services, etc.). For the same reason, the share of tractor owners decreased significantly from 31.2% to 19.2%. Changes in the share of seeders and cultivators' owners were insignificant. Fluctuations in this indicator are influenced by prices for agricultural products and energy resources, yields, crop cultivation technologies, cessation of agricultural activities and leasing of land to other farms, inflation, etc. During the period of full-scale military operations, considering 13% of the occupied territories, internal migration and physical loss of agricultural machinery, the share of households that had it in use decreased only in the temporarily occupied territories. In the territories of Ukraine free from occupation, the share of these farms increased slightly due to the evacuation of some of the equipment and its owners.

In 1991, independent Ukraine gained a powerful agricultural machine-building complex, which provided not only the Soviet Union with modern and highly productive agricultural machinery but also exported a significant part of it to the countries of the socialist camp, as well as to Western Europe, Latin America, Africa, etc. For example, in 1990, Ukrainian factories produced 106,000 agricultural tractors alone, while the production of seeders amounted to 57,100 units (State Statistics Service of Ukraine, n.d.). Gradually, due to a decline in demand in the CIS countries – the main buyers of machinery, the use of outdated technologies in production, and a decrease in investment in the sector, Ukraine has largely lost its production and, consequently, export potential (Table 3).

The data reflects a general downward trend in agricultural machinery production and significant volatility due to several factors. After a long period of economic growth, the global financial crisis of 2008 led to a significant decline in machinery production. The economic growth in 2011-2012 was followed by a decline due to the internal economic crisis, loss of territories and the outbreak of hostilities in eastern Ukraine in 2014. The economic development of the country was negatively impacted by the COVID-19 pandemic in 2019-2020. In 2021, a slight recovery began, which was interrupted by Russian aggression.

Table 3. *Dynamics of production of the main types of agricultural machinery for crop production in 2008-2021*

Vehicle type	YEARS									±2021 to 2008, cases
	2008	2009	2011	2013	2014	2018	2019	2020	2021	
Wheeled tractors with power over 59 kW, thousand units	6.3	1.4	5.4	2.9	4.1	2.4	1.4	0.9	1.2	-5.3
Combine harvesters, pcs.	309.0	56.0	399.0	68.0	c	47.0	c	c	c	–
Ploughs, thsnd pcs.	7.1	5.3	6.1	6.2	2.0	3.0	2.5	2.7	2.3	-3.1
Cultivators, thsnd pcs.	10.4	4.6	7.4	4.4	3.7	2.9	3.9	2.5	3.0	-3.4
Disc harrows, thsd. units	5.5	1.1	7.4	2.0	2.0	2.2	2.1	2.3	3.5	-1.6

Table 3, Continued

Vehicle type	YEARS									±2021 to 2008, cases
	2008	2009	2011	2013	2014	2018	2019	2020	2021	
Planters, thsnd pcs.	9.9	2.5	7.4	5.6	3.0	3.8	3.2	3.3	5.5	-1.8

Note: *c - Data are confidential following the Law of Ukraine "On State Statistics".*
Source: *compiled by the author based on State Statistics Service (2008-2021)*

During military operations, the impact of such factors as inflation, rising energy prices and the cost of logistics services is particularly acute. But without government support and foreign investment, the industry's recovery and innovative development is impossible. As a result of hostilities, seaport blockades, and inflationary processes in the country's economy, the level of agricultural machinery available to agricultural enterprises has significantly decreased. In addition, as of the beginning of 2023, 109.6 thousand units of agricultural machinery (14.3%) out of 764.3 thousand units were destroyed and 50.7 thousand units (6.6%) were damaged, and the total losses amounted to USD 4.7 billion – USD 4.3 and USD 0.4 billion respectively (Report on direct damage..., 2023)

The Kharkiv Tractor Plant, one of the country's largest manufacturers of a wide range of tractors of various capacities and tractors from 20 to 180 kW (Kharkiv Tractor Plant, 2023), was significantly damaged. The amount of damage is estimated at USD 44 billion (Report on direct damage..., 2022). The John Greaves plant in Berdiansk, which produced harvesters for various crops and tillage equipment, is now "producing anti-tank hedgehogs and stoves for the occupying army" (Who got MP Ponomaryov's plants in Berdiansk, 2023). The Orikhivsilmash plant in Orikhiv, which produced tillage equipment, trailers, spreaders, etc. before the war, was heavily damaged (Ukrainian Armed Forces..., 2022). A significant reduction in the production of domestic machinery will have a negative impact, first and foremost, on the logistics of small and medium-sized farms.

Therefore, in the context of military operations, the government needs to take measures aimed at restoring the material and technical base of agricultural enterprises. The Cabinet of Ministers of Ukraine (Resolution of the Cabinet of Ministers of Ukraine No. 324, 2022) amended the list of critical imports. In particular, several items under the UKTZED relating to agricultural machinery were classified as critical imports: sprayers and atomisers of plant protection products; machines for preparing or cultivating soil; (soil cultivation machinery, seeders and planters, distributors of mineral and organic fertilisers); tractors, in particular for agriculture and forestry. Before the outbreak of hostilities in 2022, the country's agricultural sector largely renewed its machine and tractor fleet with foreign machinery, which largely dominated the market. Often, agricultural enterprises prefer more efficient machinery from foreign manufacturers.

The leading countries producing tractors and other agricultural machinery – the United States, China and the European Union (Germany, France and the Netherlands) – were confidently promoting their products in Ukraine. Their share was 40-60% (State Customs Service of Ukraine). Manufacturers from such countries as the UK, Canada, Poland, and Belarus also held a prominent place in the market. The negative balance between exports and imports contributes to a significant outflow of foreign currency from the country, loss of competitive advantages of domestic machine builders' products in foreign markets, migration of skilled labour abroad, and undermining of the economy as a whole due to critical import dependence.

An analysis of the dynamics of machinery imports confirms the significant dependence of Ukrainian agricultural production on foreign machinery. The peculiarity of the machinery market is that a significant segment of it is occupied by Chinese products, which have attractive prices and are gradually improving in quality. Tillers are in particular demand among households and small farms, as well as mini tractors (Table 4).

Table 4. *Dynamics of imports of monoblocks and mini-tractors in 2017-2022*

Vehicle type	measurement unit	Years					2022 to 2021, ± %
		2018	2019	2020	2021	2022	
Motorblocks	thsnd pcs.	47.5	31.2	40.1	34.8	28.3	-18.7
	mln. USD	10.7	7.6	9.2	10.4	7.4	-28.8
Mini tractors with engine power up to 37 kW	thsnd pcs.	22.5	16.3	18.8	18.7	11.6	-37.9
	mln. USD	50.1	40.8	46.4	53.5	35.4	-33.8

Source: *compiled by the author based on State Statistics Service (2017-2022)*

Before the outbreak of hostilities, imports of monoblocks and mini-tractors were fairly stable. The hostilities led to an economic crisis and the loss of some territories contributed to a significant reduction in imports of not only mini-tractors and walk-behind tractors but also other machinery. Imports of tractors with engine power over 37 kW decreased by 57.1% in quantity and 44.1% in value (Fig. 1).

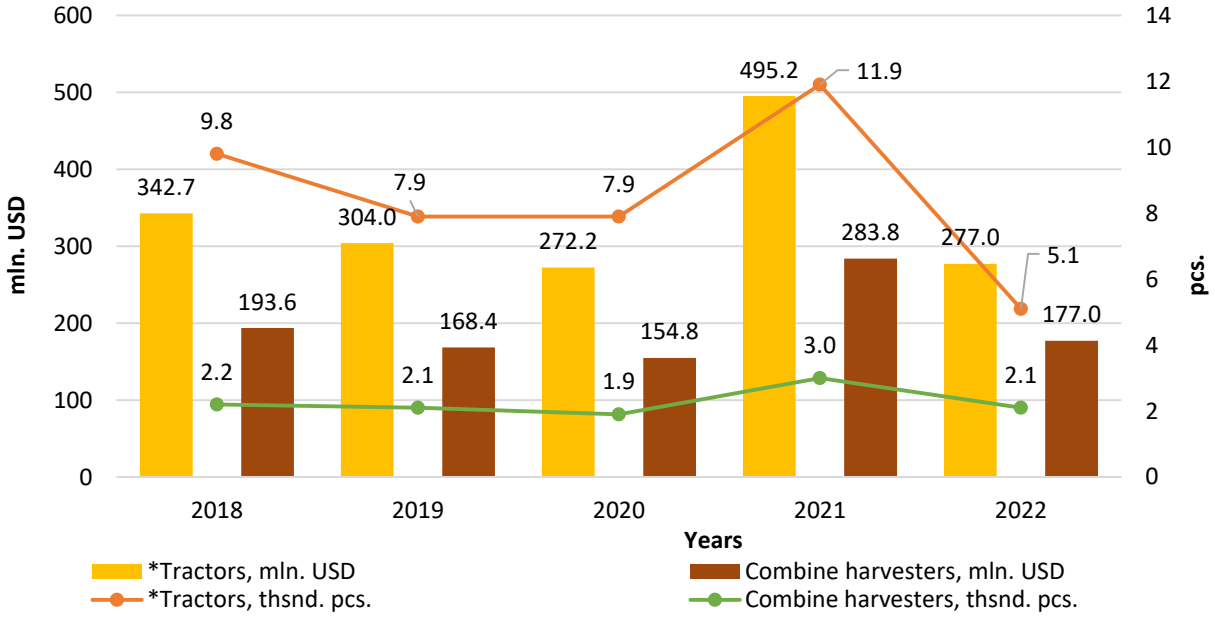

***Figure 1**. Dynamics of imports of tractors and combined harvesters in 2018-2022*
***Note:** * – Tractors with an engine power of more than 37 kW*
***Source:** compiled by the author based on State Statistics Service (2018-2022)*

This disproportion is explained by the rising cost of machinery due to increased production and logistics costs. The reduction in imports of combine harvesters in 2022 compared to 2021 is significant in terms of quantity and value (30.0% and 37.6%, respectively), but its volumes are at the level of 2019 and slightly higher than in 2020 due to unmet demand for this type of equipment and significant losses due to hostilities.

In 2018, 10.8 thousand units of ploughs (87.8%) for motorblocks and mini-tractors were imported from China with a customs value of only USD 288.1 thousand (0.82%), which explains the imbalance between the large number and relatively low value of imported machines. In 2021, for example, only 0.55 thousand units of ploughs (27.2%) were imported from China with a customs value of 29.9 thousand USD (0.1%), so the total customs value of imports is relatively high – 26.0 million USD. In 2022, 2.1 thousand ploughs were imported from China with a customs value of only USD 14 thousand (Fig. 2).

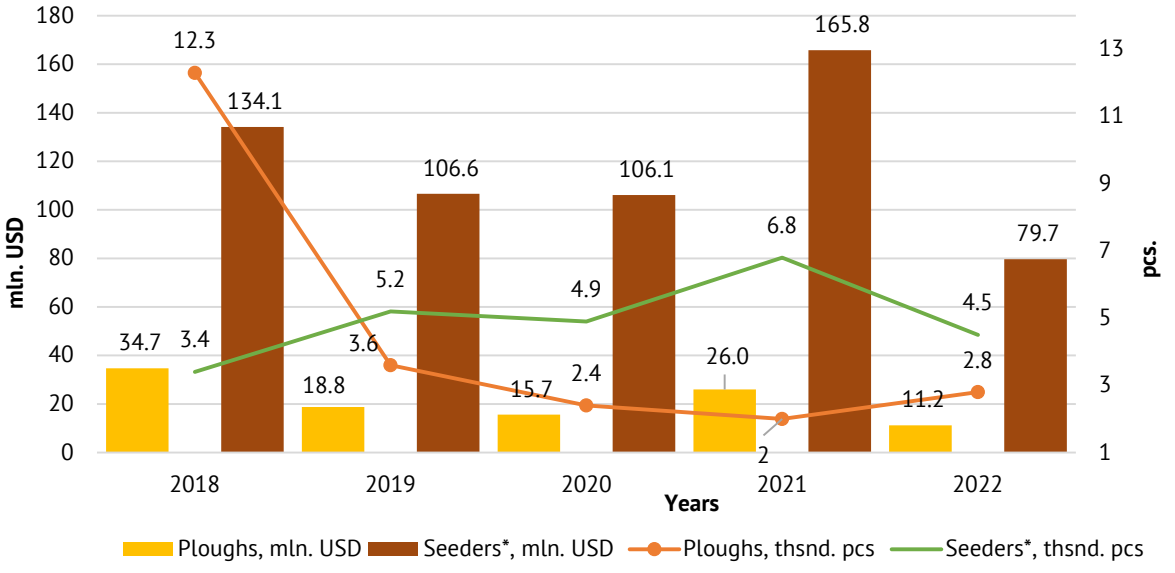

***Figure 2**. Dynamics of imports of ploughs and seeders in 2018-2022*
***Note:** * – Seeders, planters, and seedling machines*
***Source:** compiled by the author based on State Statistics Service (2018-2022)*

Seeders were imported from 3.4 thousand units in 2018 to 6.8 thousand units in 2021 with a customs value of 79.7 million USD during the study period. USD in 2022 to 165.8 million USD in 2021. In 2022, 69.8% of seeders were imported from China, and their customs value was only 3.5% of the value of all imported seeders, which indicates the demand for them among small farms and households. Disc harrows are imported to Ukraine in small quantities (Fig. 3), which is explained by their sufficient domestic production. In 2020, 3,929 units of harrows (86.2%) for motorblocks were imported from India with a low average customs value of only 4.6 USD (State Statistics Service of Ukraine). Despite significant domestic production of cultivators, the vast majority of miniature machinery is imported from China – in 2022, imports accounted for 96.9% in quantity and only 15.5% in value.

The expansion of Chinese machinery for use in small areas hurts the development of domestic agricultural machinery. While the limited production capacity of self-propelled machinery can be partially justified by the complexity of technological processes and lack of personnel and necessary equipment, Ukraine has all the capabilities to produce trailed implements and units for motorblocks and mini-tractors – production facilities, personnel, and raw materials. The production of mini-machinery during the post-war reconstruction period would create new jobs, increase tax revenues and save foreign currency, which is so necessary for a country in a state of war and post-war economic recovery.

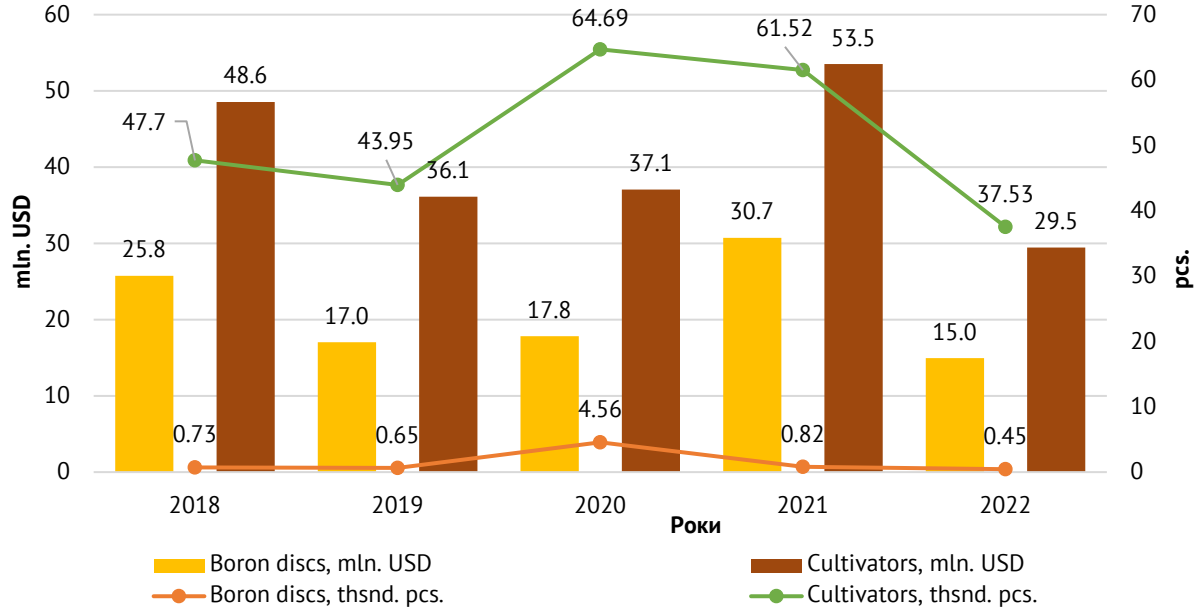

***Figure 3**. Dynamics of imports of disc harrows and cultivators in 2018-2022*
***Source:** compiled by the author based on State Statistics Service (2018-2022)*

It is worth noting that Chinese products are gradually occupying not only the segment of mini-machinery in the market but are also confidently covering the segments of more powerful machinery. For example, in 2017, only 40 units of Chinese-made tractors with an engine power of more than 90 kW were imported to Ukraine, while in 2018 the volume of imports was 216 units, and in 2019 – 241 units. In 2020, 547 units of tractors with an engine power of 75-130 kW were imported from China, and in 2021, imports reached a record high of 1,546 units. In the same year, tractors with a capacity of more than 130 kW were imported for the first time in the amount of 9 units. Due to the outbreak of hostilities and a decline in demand for Chinese high-power tractors, fewer Chinese tractors were imported in 2022 than in the previous year: 706 units with an engine power of 75-130 kW and 9 units with an engine power of more than 130 kW (State Statistics Service of Ukraine). In recent years, Chinese machinery started to confidently compete not only with domestic and CIS machinery but also with the best global brands due to its affordable price, improved quality, and performance.

At various times, Ukraine has adopted legislative acts and programmes aimed at stimulating the technical and technological modernisation of agricultural production, in particular by stimulating demand for domestic agricultural machinery. The most effective programme was the Programme of Partial Compensation for the Cost of Domestic Agricultural Machinery and Equipment, which was renewed in 2017 (Resolution of the Cabinet of Ministers of Ukraine No. 130). Although the programme did not start well in 2017, with only UAH 134.1 million of the planned UAH 550 million being used, it proved to be quite viable. A total of 1,220 companies participated in the programme and purchased 2,906 pieces of equipment for a total

of UAH 670.3 million (excluding VAT) with a 20% compensation. The farmers purchased 88 tractors, 1 grain carrier, 870 units of tillage and sowing equipment, and 1947 units of agricultural equipment (Fig. 4).

In 2018, the programme's funding increased, and the interest of participants grew: UAH 912.9 million (96%) of the UAH 955.0 million budgeted was spent, the number of participants reached 7,043, and the compensation rate increased to 25%. We purchased 17182 units of agricultural machinery and equipment worth UAH 3651.8 million. In 2021, with a budget of UAH 991.35 million, 5789 enterprises purchased almost 30 thousand units of machinery. Since the data on certain types of equipment are not published, the experts of the Institute of Agrarian Economics have calculated the approximate number of sales.

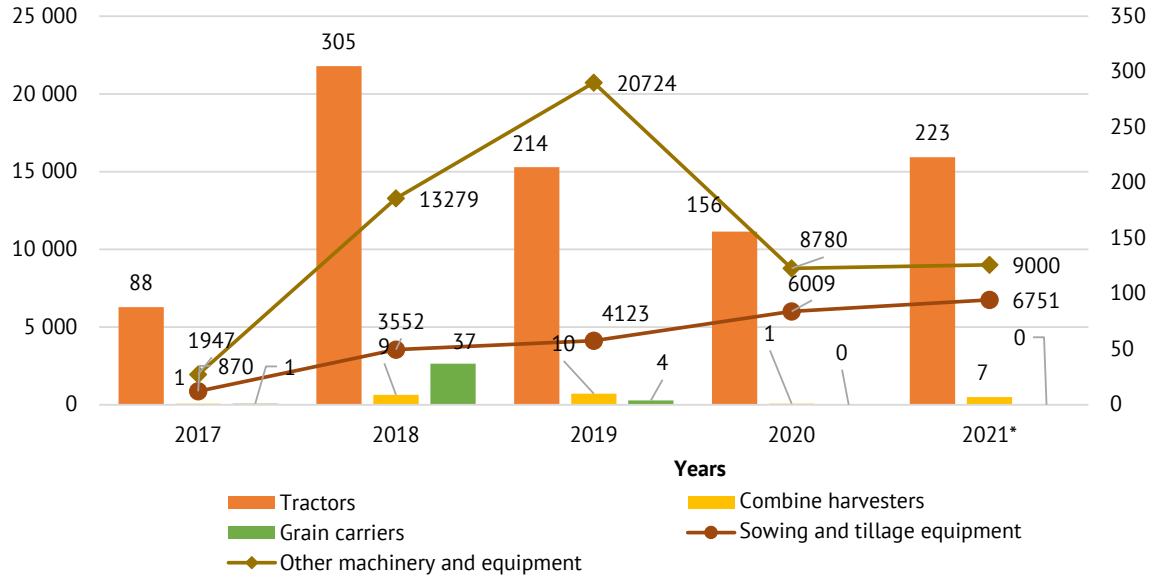

Figure 4. *Purchase of agricultural machinery under the partial cost compensation programme, units*
Source: *compiled by the author based on data (The Ministry of Agrarian Policy and Food of Ukraine, n.d.).*
Note: **2021 – estimated*

Due to the military operations in 2022 and 2023, the Partial Compensation Programme for Agricultural Machinery was suspended. Given the destruction of some agricultural machinery companies and the destruction of agricultural machinery, the resumption of the programme in 2024 could be a powerful compensation for the losses incurred. To support small and medium-sized agricultural producers during the war, a soft loan programme was introduced (Resolution of the Cabinet of Ministers of Ukraine No. 274, 2022). Only small and medium-sized agricultural producers can participate in this programme. Their turnover should not exceed EUR 20 million per year (equivalent to enterprises that cultivate up to 10,000 hectares). The maximum loan amount is UAH 50 million, the loan term is 6 months, and the purpose is to carry out agricultural activities for the period of martial law (at 0 per cent per annum, and in case of termination of martial law – until the end of the loan term). The maximum amount of the state guarantee for portfolio loans is up to 80%.

The proposed measures will ensure the timely and full implementation of a set of necessary technological operations in critical conditions of hostilities, which will help ensure a high level of food security in Ukraine. As of December 2022, the inflation rate for non-energy industrial products in the euro area was 6.4%, and as of 1 May 2023, although it decreased to 6.2%, it remains quite high, which will lead to an increase in prices for agricultural machinery from European manufacturers, the main suppliers to Ukraine. In addition, high logistics costs remain a significant factor behind the rise in machinery prices. After all, the inflation rate in the energy sector was 25.5% in December 2022 and decreased to 2.5% as of 1 May (Official website of the European Union, n.d.). This will increase demand for new and foreign-used equipment and decrease demand for new foreign equipment. Low domestic prices for agricultural products, primarily oilseeds and grains, will also reduce demand for expensive imported machinery.

The aforementioned factors will lead to a decrease in imports of machinery in quantitative terms in 2023 compared to 2022: tillage machinery – by 25-30%; harvesting machinery – by 15-20%; and agricultural tractors – by 20-25%. Considering the analysis of the current state of the agricultural machinery market, its further development can be seen in two scenarios – positive and negative.

The positive scenario envisages: rebuilding and modernising the domestic agricultural machinery industry through foreign investment; government support for agricultural machinery, which will increase demand

for Ukrainian-made machinery; entry of domestic machinery into new foreign markets in Eastern Europe and Africa, where it will become fully competitive; prioritising the production of mini-machinery and gradually driving cheap foreign machinery out of the market; favourable investment climate, cheap energy resources, and skilled personnel will facilitate the production of equipment of the world's best brands and the creation of joint ventures based on domestic facilities; creation of new jobs at the restored enterprises.

Under the negative scenario, the loss of the existing, most high-tech agricultural machinery enterprises and the lack of budgetary funds for their restoration should be expected; production of only simple, low-tech machinery and equipment for soil preparation or cultivation; minimisation of purchases of new machinery, restoration of existing machinery and purchase of used machinery by the vast majority of agricultural enterprises; unfavourable investment climate and difficult business conditions; expansion of foreign equipment, mostly of low quality; loss of human resources in the machine-building industry due to war losses and migration abroad.

Therefore, to overcome the negative effects of the war on the development of the agricultural machinery market and the logistics of agricultural production, it is necessary to create a favourable investment climate to attract investment in the development and restoration of domestic machinery production, and to organise joint ventures to produce innovative, high-performance and environmentally friendly machinery; simplification of the business and licensing system, reduction of bureaucratic procedures and elimination of corruption in the process of setting up enterprises; implementation of protectionist policies by the state to limit imports of low-quality machinery at dumping prices by introducing quotas and raising duty rates; resumption of state programmes aimed at financing the restoration and development of domestic agricultural machinery; and state incentives to increase the production of miniature machinery; involvement of households and small farms in state targeted programmes for the development of the agricultural sector; creation of the most favoured nation regime for critical imports of machinery through a mechanism of reducing tax pressure; evacuation of agricultural machinery enterprises and their employees from the war zone, and training of new specialists.

Only the adoption and clear implementation of a comprehensive programme to restore the agricultural machinery industry and support agricultural producers will contribute to the sustainable development of the agricultural sector in the post-war period. S. Késmárki-Gally *et al.* (2020) determined that adverse weather conditions limit the time spent on agricultural work and encourage farmers to consider purchasing more powerful machines. This is also caused by a significant and persistent labour shortage in agricultural production. At the same time, farmers are faced with a choice: to use modern, innovative machinery or to purchase simpler and cheaper equipment. For Ukraine, during and after the hostilities, the issue of combining these factors is acute, given the significant loss of equipment and the crisis economic situation. Therefore, the use of used, cheap but innovative equipment is particularly relevant today and will remain a priority for several post-war years.

G. Lobos *et al.* (2021) confirmed that the price of tractors decreases with age, increases with decreasing tractor power, and significantly depends on the manufacturer's brand. In Ukraine, used high-capacity tractors made in Europe and the US are preferred, while mini tractors are preferred from Japan. In 2022, imports of used tractors with an engine power of more than 130 kW from the US and Germany accounted for 84.5%, and from Japan – 97.8% (engine power up to 18 kW) (State Statistics Service of Ukraine). M. Jaleta *et al.* (2019) examined the mechanisation of agricultural production and the reduction of soil impact in the process of tillage in the context of sustainable intensification in developing countries. According to the authors, soil conservation, the speed of technological operations and energy savings in the production process are factors that contribute to the spread of minimum tillage. In Ukraine, the introduction of these technologies is particularly relevant during and after the war period. First and foremost, it saves energy resources in the face of their significant shortage and high cost, as well as reduces depreciation of technical equipment and the cost of their repair. This approach will have a positive impact on the efficiency of agricultural enterprises in the context of high inflation and low prices for agricultural products.

According to the authors, the statement proposed by S. Marchenko & A. Voropaev (2019) that "today, the equipment of Ukrainian manufacturers meets modern requirements for technical condition, build quality, safety, and price. And foreign consumers are ready to buy Ukrainian machinery..." is debatable. After all, exports of domestic machinery are quite low compared to imports of foreign machinery. Ukraine's partner countries were CIS countries with a relatively low level of agricultural machinery development. Until 2019, Russia was the main consumer of domestic products. For example, in 2017-2018, Russia ranked first among the importing countries of Ukrainian tractors in the amount of USD 13.1 (95.3%) and 5.0 (77.6%) million and seeders in the amount of USD 16.2 (82.1%) and 10.5 (73.6%) million, respectively. But first, it restricted the import of Ukrainian machinery into its territory, and from 24 February 2022, all exports to Russia were suspended. With the loss of Russian markets, there was a sharp drop in exports of certain types of domestic machinery (tractors, ploughs). In 2019-2021, exports of tractors amounted to only USD 1.9 million, USD 1.2 million, and USD 642.5

thousand, respectively. Import partners of domestic tractors were Romania, the Republic of Moldova, Bulgaria, Kazakhstan, etc. (State Customs Service of Ukraine). In 2019-2021, Ukrainian-made seeders were exported to Belarus, Kazakhstan, Moldova, Bulgaria, etc. It should be noted that seeders are one of the few types of machinery that meet high standards and whose exports have been consistently high.

According to O. Boltianskyi & N. Boltianska (2020), there are significant problems in the production of Ukrainian agricultural machinery due to the production of identical machinery in many enterprises. Each enterprise produces it using imperfect technology and low-quality materials, which makes the reliability and durability of the machine's working parts three times lower than that of foreign analogues. In addition, the decline in sales of Ukrainian-made machinery has led to a loss of human resources in the agricultural machinery industry, resulting in a decline in product quality and, consequently, a decrease in competitiveness.

R. Vidosa *et al.* (2022) studied the patterns of use of the ISO 11783 (ISOBUS) standard in the production of agricultural machinery. The ISO 11783 standard, which is widely used globally, will become mandatory for successful entry into foreign markets. The expansion of agricultural machinery by multinationals to Latin American countries may deprive local companies of the ability to maintain their market share. A similar situation may arise in the Ukrainian agricultural machinery market. After all, the Ukrainian machine-building industry operates according to old standards that currently meet the requirements of only the domestic market and the markets of a limited number of countries, including the CIS. Therefore, the issue of the transition of agricultural machinery production to new standards will arise after the end of hostilities in Ukraine. Otherwise, the expansion of foreign machinery will completely displace Ukrainian products due to their inability to compete with it.

In our opinion, it is reasonable to identify the factors that influence the decline in production of agricultural machinery enterprises during the crisis period: "The main negative factors that determine the decline in industry performance during the intensive unfolding of the economic crisis are a drop in the solvency of enterprises, and an increase in production costs. The synergistic effect of the simultaneous action of these destructive factors has led to the crisis rate of industrial decline" (Abuselidze *et al.*, 2022). It is quite appropriate that H. Tong *et al.* (2020) note the impact of state support on the quality of agricultural machinery and production costs, but it is not unambiguous – the growth of profits is not equivalent to quality improvement. Therefore, a condition for financing state support programmes for domestic machine building should be the condition of investing in the innovative development of the enterprise and improving product quality.

CONCLUSIONS

Exports of agricultural machinery from Ukraine are significantly lower than imports. Until 24 February 2022, Russia was the main importer of Ukrainian agricultural machinery. Since the outbreak of the war, this market has been lost, resulting in a sharp drop in exports of certain types of machinery. The key importing partners of domestic tractors were Romania, the Republic of Moldova, Bulgaria, Kazakhstan, and seeders – Kazakhstan, Moldova, and Bulgaria.

During the hostilities, the impact of inflation, rising energy prices and the cost of logistics services on the supply and demand for machinery was particularly acute. A significant number of agricultural machinery companies have been damaged, destroyed or are under occupation, which has also reduced the presence of Ukrainian machinery on the market. As a result of the hostilities, blocking of seaports, inflation and increased logistics costs, the availability of agricultural machinery to agricultural producers has decreased. As of 24 February 2023, 160.3 (20.9%) thousand units of equipment out of 764.3 thousand units were destroyed or damaged, and the total losses in value amounted to USD 4.7 billion.

The decline in machinery production in Ukraine will primarily hurt the availability of machinery for small and medium-sized agricultural producers. Therefore, the government's activities should be aimed primarily at restoring the logistics of agricultural enterprises, and the government's relaunch of the Programme for Partial Compensation of the Cost of Agricultural Machinery and Equipment should be a key factor in this recovery. In 2021, the budget of the Programme was UAH 991.35 million, which allowed 5789 agricultural producers to purchase almost 30 thousand units of equipment. However, the hostilities prevented the Programme from being funded in 2022 and 2023.

Foreign machinery plays a leading role in updating the material and technical base of agricultural enterprises. The main exporters of agricultural machinery to Ukraine were the United States, the European Union (Germany, France, and the Netherlands), Belarus and China. Their share was 40-60%. China became the main importer of mini-machinery. In 2023, due to the negative impact of hostilities, imports of machinery may decrease in quantitative terms compared to 2022: tillage machinery – by 25-30%; harvesting machinery – by 15-20%; and agricultural tractors – by 20-25%. The positive and negative scenarios for the development of the Ukrainian agricultural machinery market considered in the study indicate the possibility of a full recovery of the Ukrainian agricultural machinery industry, saturation of the market with domestic competitive machinery and access to new foreign markets for its sales, or the loss of Ukrainian machinery positions in the market and complete dependence on imported machinery.

Therefore, to overcome the negative effects of the war and intensify the development of the agricultural

machinery market, as well as to meet the full demand for agricultural machinery, it is necessary to improve the investment climate, organise joint production with the world's leading agricultural machinery manufacturers, and implement a protectionist state policy aimed at protecting domestic production, restoration of state support for the restoration and development of domestic agricultural machinery, support for the development of technical support for small businesses in rural areas. The prospect of further research will be to study the state of the Ukrainian agricultural machinery industry as a key link in the machinery market, to reveal the reasons for the decline in its efficiency and determine the volume of losses as a result of military operations, and to formulate effective proposals for its restoration and improvement.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTERESTS

None.

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Ринок сільськогосподарської техніки для рослинництва та перспективи його розвитку у повоєнний період

Ярослав Федорович Навроцький
Кандидат економічних наук
Національний науковий центр «Інститут аграрної економіки»
03127, вул. Героїв Оборони, 10, м Київ, Україна
https://orcid.org/0000-0003-4677-5306

Олександр Васильович Захарчук
Доктор економічних наук, професор, член-кореспондент НААН
Національний науковий центр «Інститут аграрної економіки»
03127, вул. Героїв Оборони, 10, м Київ, Україна
https://orcid.org/0000-0002-1734-1130

Оксана Василівна Вишневецька
Кандидат економічних наук, провідний науковий співробітник
Національний науковий центр «Інститут аграрної економіки»
03127, вул. Героїв Оборони, 10, м Київ, Україна
https://orcid.org/0000-0003-0978-2063

Беата Глінковська-Краузе
Доктор філософії, професор
Лодзький університет
90-136, 68 Нарутовіча, м. Лодзь, Польща
https://orcid.org/0000-0002-6915-3297

Олександр Олександрович Кучмєєв
Доктор економічних наук, доцент
Відкритий міжнародний університет розвитку людини «Україна»
03115, вул. Львівська, 23, м. Київ, Україна
https://orcid.org/0000-0003-1803-6231

Анотація. Через воєнні дії найбільшими втратами аграрного сектору економіки України стали втрати сільськогосподарської техніки – знищено і пошкоджено 20,9 %. Тому, забезпечення ефективного функціонування ринку технічних засобів для села є ключовим чинником відновлення матеріально-технічної бази сільськогосподарських підприємств. Метою роботи було дослідити сучасний стан ринку сільськогосподарської техніки та вплив на нього воєнних дій, визначити ефективні шляхи його розвитку. У процесі дослідження були використані наступні методи: діалектичний метод наукового пізнання, монографічний, статистико-економічний, економіко-математичний, експертних оцінок, графічний. Визначено загальну тенденцію до скорочення протягом 2015-2022 років частки господарств населення, які мають окремі види техніки. Виявлено диспропорції між великими та малими формами господарювання у забезпеченні технікою, а також обмеження доступу до фінансових ресурсів малих сільськогосподарських підприємств. У процесі дослідження визначено загальне скорочення обсягів ринку української техніки через втрату виробничих потужностей на сході та півдні країни. Також значно скоротився імпорт сільськогосподарської техніки – тракторів на 57,0 %, комбайнів зернозбиральних на 30,0 %, сівалок на 33,8 %, борін дискових на 45,1 %, культиваторів на 38,9 % у кількісному виразі. Здійснено аналіз державної підтримки сільськогосподарського машинобудування та аграрних підприємств. Сформовано прогноз розвитку ринку сільськогосподарської техніки для рослинництва за позитивним та негативним сценаріями, що включають ризики та виклики, які можуть виникнути під час воєнних дій та після їх завершення. Розроблено рекомендації щодо розвитку ринку сільськогосподарської техніки для рослинництва, що передбачають: здійснення протекціоністської політики держави; збереження кадрового потенціалу; евакуацію підприємств сільськогосподарського машинобудування із зони бойових дій, спрощення ведення бізнесу для стимулювання інвестиційної діяльності, державну підтримку малих фермерських господарств тощо. Виконання цих рекомендацій сприятиме відновленню ефективного функціонування ринку сільськогосподарської техніки та матеріально-технічного забезпечення аграрного виробництва в Україні

Ключові слова: ринок сільськогосподарської техніки; імпорт; експорт; державна підтримка; матеріально-технічне забезпечення

SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 26(9), 167-177

UDC 631.2
DOI: 10.48077/scihor9.2023.167

State-of-the-art technologies for remote sensing of crops water status and nutrients in agriculture: A review

Svetoslav Atanasov*
PhD Student, Magister in Computer System and Technologies
Trakia University
6015, Students campus, Stara Zagora, Bulgaria
https://orcid.org/0000-0002-2658-1611

Article's History:
Received: 16.05.2023
Revised: 19.08.2023
Accepted: 27.09.2023

Abstract. The research relevance is predetermined by the need to introduce effective methods and tools for monitoring water resources and tracking soil nutrient levels to improve agricultural production and sustainable use of natural resources. The research aims to provide a comprehensive overview of the latest technologies and techniques used in RS for accurately assessing water status and nutrient levels in crops, aiming to enhance agricultural productivity and sustainability. The latest advancements in remote sensing techniques that enable precise monitoring and assessment of water levels and nutrient conditions in crops, crucial for optimizing agricultural practices, were studied. The literature research was conducted by adapting the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Methods. The current study provides an overview of RS technology, with a special focus on establishing crops' water and nutrient status in agriculture. A thorough review of research focused on the applications and technologies of RS in agriculture, using a broad-to-narrow approach, was also conducted. The scientific studies analysed provide the following: RS crops on a large scale level, RS crops on a field level, RS crops on a greenhouse level, RS on a plant level and RS on a leaf level. Current cutting-edge technologies are also presented. The findings of this study could be beneficial to those involved in sustainable agriculture, such as researchers, academics, and aspiring students

Keywords: review; precision agriculture; precision irrigation; smart farming; remote sensing; crop monitoring; plant water response; non-invasive; non-destructive

Suggested Citation:
Atanasov, S. (2023). State-of-the-art technologies for remote sensing of crops water status and nutrients in agriculture: A review. *Scientific Horizons*, 26(9), 167-177. doi: 10.48077/scihor9.2023.167.

*Corresponding author

INTRODUCTION

The timely and reliable information on crops' water and nutrient status is of critical importance in the conditions of modern precision agriculture and smart farms. By 2050, the world population is expected to increase to almost 10 billion people, and to feed them, existing food production must be increased by 59-98% according to estimates by the Food and Agriculture Organization (FAO, 2023). It is also estimated that agriculture will use over 70% of the world's freshwater reserves, with about half of it being lost or wasted. Up to 95% of the world's food production depends on soil. As a result of unsustainable agricultural practices, overexploitation of natural resources, and growing populations, one-third of the soils have already degraded, and experts estimate that soil erosion could lead to a 10% loss of crop yields by 2050. Soils are also full of life and contain approximately 25% of the world's biodiversity. Globally, remote sensing (RS) has been supporting decision-making in the field of agriculture for many years.

Several previously published literature reviews partially examine the application of the RS in the crops' water status and nutrients determined in agriculture. M. Weiss *et al.* (2020) conducted a meta-review of agronomical variables and plant traits that can be estimated from remote sensing. They described different methodological approaches to retrieve them, discussed how these variables are employed by different stakeholders for specific applications and concluded with an overview of caveats and future challenges. A. Konings *et al.* (2019) presented a review of microwave remote sensing observations sensitive to plant water content. They introduced the principles behind microwave remote sensing observations to illustrate how they are sensitive to plant water content and discussed how various sensor types can be leveraged for specific applications depending on the spatiotemporal resolution needed. M. Bacco *et al.* (2019) provided a survey of the most recent research activities in the area of digitalization of agriculture, in the form of both research projects and scientific literature, to show the already achieved results, the current investigations, and the still open challenges, both technical and non-technical. G. Lassalle (2021) conducted a meta-review about the advances achieved in monitoring natural and anthropogenic plant stressors by hyperspectral remote sensing over the last 50 years. He presented advances in hyperspectral monitoring of plant stress with a total of 466 peer-reviewed articles.

N. Katsoulas *et al.* (2016) conducted a review of crop reflectance monitoring as a tool for water stress detection in greenhouses. They presented challenges of detecting water status in greenhouses by remote sensing, discussed sensors available for reflectance sensing and applications, effects of environmental, canopy structure and other parameters and proposed possible solutions to mitigate the effects of those parameters. U. Ahmad *et al.* (2021) conducted a review on crop reflectance monitoring as a tool for water stress detection in greenhouses. They reviewed past research and recent advances regarding the sensors and approaches used for crop reflectance measurements and the indices used for crop water and nutrient status detection. A. Damm *et al.* (2018) conducted a review of remote sensing of plant-water relations. They reviewed suitable remote sensing approaches to assess plant-water relations ranging from pure observational to combined observational-modelling approaches. They used a combined energy balance and radiative transfer model to assess the explanatory power of pure observational approaches focusing on plant parameters to estimate plant-water relations, followed by an outline for a more effective use of remote sensing by their integration into soil-plant-atmosphere continuum (SPAC) models. It is possible to conclude that there are similar studies, but they are not categorized as in this publication – from the general to the particular, from the global scale to the leaf level.

The research aims to present a thorough examination of contemporary technologies and methodologies employed in remote sensing to precisely evaluate the water conditions and nutrient concentrations in crops. The ultimate goal is to improve agricultural efficiency and promote sustainable practices.

REMOTE SENSING CROPS ON LARGE SCALE LEVEL

Improved satellite data resolution from platforms like Landsat 8 and Sentinel-2 has opened up new possibilities for monitoring crop conditions and mapping yields on a larger scale in agriculture. J. Clevers *et al.* (2017) utilized Sentinel-2 satellite images to estimate LAI, LCC, and CCC of potato crops using vegetation indices (VIs). Their study found that Sentinel-2 bands with a 10 m spatial resolution were sufficient, eliminating the need for red-edge bands with a 20 m resolution. J. Miao *et al.* (2022) employed machine learning models to estimate leaf nutrient levels in mangrove forests using Sentinel-2 images. They compared XGBoost, RF, and LightGBM models and mapped nutrient levels for multiple seasons, highlighting the importance of monitoring seasonal changes. A. Hama *et al.* (2022) introduced a low-cost LiDAR approach to estimate leaf water content (LWC) by measuring reflectance in the 905 nm band, which was found to be closely related to leaf structure. T. Dong *et al.* (2020) utilized Landsat 8 and Sentinel-2 data to estimate crop biomass in Manitoba, Canada, by parametrizing a crop growth model using remotely sensed LAI. Lastly, I. Sanches *et al.* (2014) used spectral feature analysis to detect plant stress in visible/near-infrared wavelengths, identifying a new index, PSDI, effective for detecting stress in hydrocarbon-impacted plants based on field and airborne data. Table 1 presents the above data in a summarized form:

Table 1. *Examples of RS crops on large scale level*

Source	Sensor	Crop	Estimation	Results
(Clevers *et al.,* 2017)	Sentinel-2 satellite images	Potato	LAI; LCC; CCC	At 10 m spatial resolution: WDVI estimating LAI with R^2=0.80, RMSEP=0.36; CVI estimating LCC: R^2=0.656; RMSEP=0.066 g·m^{-2}); CI_{green} linear estimator of CCC: R^2=0.818; RMSEP=0.29 g·m^{-2}; At 20 m spatial resolution: TCARI/OSAVI linear estimator of LCC: R^2=0.696, RMSEP=0.062 g·m^{-2}
(Miao *et al.,* 2022)	Sentinel-2 image	Mangrove	Seasonal leaf nutrients: carbon (C), nitrogen (N) and phosphorus (P)	XGBoost demonstrates high accuracy in estimating leaf C, N, and P levels with R^2 values of 0.655, 0.799, and 0.829 in spring, summer, and winter for leaf C, and R^2 values of 0.668, 0.743, and 0.704 for leaf N, and R^2 values of 0.539, 0.622, and 0.596 for leaf P
(Hama *et al.,* 2022)	LiDAR and hyperspectral data	leaves of sweet potatoes	LWC by LiDAR reflectance	Using LiDAR reflectance, the estimation of LWC (Liquid Water Content) achieves a high level of accuracy, with an R^2 value of 0.950, an RMSE (Root Mean Square Error) of 6.78%, and a MAPE (Mean Absolute Percentage Error) of 18.6%
(Dong *et al.,* 2020)	Landsat 8 and Sentinel-2 data	Anola, soybean, wheat, corn, oats and beans	Crop biomass using remotely sensed LAI	The accurate estimation of above-ground dry biomass for these six crops is achieved by assimilating LAI data obtained from satellite sources. The estimation yielded favourable results, with an R^2 value of 0.81, an RMSE of 135.4 g/m^2, a normalized RMSE (nRMSE) of 37.9%, and a relative per cent difference (RPD) of 2.26
(Sanches *et al.,* 2014)	ProSpecTIR-VS airborne imaging spectrometer for far-range field hyperspectral RS data	Forages brachiaria grass and perennial soybean	Plant stress in hydrocarbon-contaminated soil, true leaf data	Among the leaf data, the PSDI and chlorophyll feature area demonstrated the highest proportion (67-70%) of plants exhibiting signs of stress

Source: *developed by the author*

REMOTE SENSING CROPS ON THE FIELD LEVEL

T. Silva *et al.* (2022) conducted on-site measurements of chlorophyll levels in grapevines to assess their nitrogen status. The study established a relationship between chlorophyll meter readings and actual leaf nitrogen contents. C. Araújo-Paredes *et al.* (2022) evaluated the accuracy of using an aerial sensor to estimate plant water status (PWS) through the Crop Water Stress Index (CWSI). They compared CWSI values derived from aerial thermographic and portable thermal cameras with measurements of stem water potential and demonstrated the feasibility of estimating CWSI and generating spatial maps using aerial thermography. H. Zheng *et al.* (2018) assessed the performance of three sensors on an unmanned aerial system (UAS) for estimating nitrogen status in rice. They employed various Vis to estimate leaf and plant nitrogen accumulation and evaluated their integration with field data. P. Rosso *et al.* (2022) compared ground-based LAI data with remote sensing information using VIs, machine learning, and radiative transfer models in barley experiments. They determined that machine learning algorithms and VIs performed well, indicating the potential of using satellites and remotely piloted aircraft in precision agriculture. Du *et al.* (2022) used a multi-rotor UAV equipped with image sensors to gather maize canopy data and developed models for LAI estimation using different algorithms. N. Katsoulas *et al.* (2016) utilized ML algorithms to estimate NDVI in Arabica coffee cultivars using a passive RGB sensor in a UAV. They derived an NDVI equation based on RGB bands and found satisfactory agreement compared to in situ data, providing a simple method for evaluating vegetative vigour. Table 2 presents the above data in a summarized form:

Table 2. *Examples of RS crops on field level*

Source	Sensor	Crop	Estimation	Results
(Silva *et al.,* 2022)	Chlorophyll hand-held meter	Wine grape "Chardonnay"	Chlorophyll	The generated models produced estimations for chlorophyll a, b, and total with calibration errors of 0.98, 0.58, and 1.47μg ml^{-1} cm^{-2}, respectively. For prediction, the errors are 1.03, 0.67, and 1.49 μg ml^{-1} cm^{-2}. In terms of leaf nitrogen content, the calibration error is 1.49 g kg^{-1}, while the prediction error is 3.39 g kg^{-1}

Table 2, Continued

Source	Sensor	Crop	Estimation	Results
(Araújo-Paredes *et al.*, 2022)	Thermal Imagery	Grape vine	CWSI	Among the two models analysed, it is observed that for CWSITair, CWSIS demonstrated better evaluation of crop water stress compared to stem water potential, with an R^2 value of 0.55. Additionally, CWSIS exhibited a strong correlation with the portable sensor, with an R^2 value of 0.58, indicating its potential for practical application in field measurements
(Zheng *et al.*, 2018)	RGB, colour-infrared and multispectral cameras	Rice	Nitrogen status	The findings indicated that the red edge (VIs) derived from multi-spectral (MS) images achieved the highest level of accuracy in estimating Leaf Nitrogen Accumulation (LNA) with R^2 values ranging from 0.79 to 0.81 and RMSE values of 1.43 to 1.45 g m^{-2}. Similarly, for Plant Nitrogen Accumulation (PNA), the red edge VIs demonstrated strong performance with R^2 values ranging from 0.81 to 0.84 and RMSE values of 2.27 to 2.38 g m^{-2}
(Rosso *et al.*, 2022)	Sentinel-2, Landsat and Sen2-Agri	Barley	LAI	The coefficients of determination obtained from all approaches ranged from approximately 0.7 to 0.9. When only four Sentinel-2 bands are utilized instead of the full set of 12, the top-performing ML algorithms achieve even higher levels of accuracy
(Du *et al.*, 2022)	CMOS sensors (RGB bands of spectral and spatial information of image pixels	Maize	LAI	There is a strong correlation between RGB-based VIs and LAI. The (RF model outperformed other models across the entire observation period as well as during specific growth stages. It exhibited the highest coefficients of determination (R^2) ranging from 0.71 to 0.88 and the lowest RMSE values ranging from 0.12 to 0.25 on the test datasets. Following the RF model, the BPNN (Backpropagation Neural Network) model and LR models also showed good performance
(de Oliveira *et al.*, 2022)	Passive RGB sensor on UAS	20 Arabica coffee cultivars	Foliage level	A high ratio between the foliage level and the NDVI can be observed (r=0.97, p-value < 0.01)

Source: *developed by the author*

REMOTE SENSING CROPS ON THE GREENHOUSE LEVEL

Q. Li *et al.* (2022) investigated the relationship between soil water content (SWC), drought stages of wheat, canopy temperature, and spectral response characteristics. They found that red-valley position (RVP) and red-edge position (REP) parameters showed significant shifts during certain drought stages of wheat, while vegetation water indexes effectively distinguished different levels of water stress across various growth stages. A. Bianchi *et al.* (2017) developed a water balance model based on the FAO-56 method to schedule irrigation for greenhouse spinach crops. They found that nitrogen treatment had minimal impact on crop development and irrigation requirements. C. Ru *et al.* (2020) examined the effectiveness of using the CWSI based on leaf temperature to assess the water status of grapevines. They determined the optimal time and conditions for observing CWSI values and concluded that CWSI was more effective than Tc-Ta (canopy temperature minus air temperature) in monitoring plant water stress, with stomatal conductance (gs) showing the strongest correlation with CWSI. Table 3 presents the above data in a summarized form:

Table 3. *Examples of RS crops on the greenhouse level*

Source	Sensor	Crop	Estimation	Results
(Li *et al.*, 2022)	Ground hyper-spectral RS	Wheat	Water stress during tillering, jointing and milk maturity.	The presence of water stress at various growth stages resulted in noticeable variations in spectral characteristics. Notably, there are significant differences in SWC and canopy temperature across different stages of wheat drought, with the jointing stage experiencing the most pronounced changes in canopy temperature
(Bianchi *et al.*, 2017)	RGB images	Spinach (Spinacia oleracea)	Water requirements	The FAO-56-GH model demonstrated favourable performance during both the validation and calibration periods, as indicated by the evaluation metrics. In the validation period, the model exhibited a coefficient of determination (R^2) of 0.80, an RMSE of 0.41 mm day^{-1}, and an NSE of 0.78. Similarly, during the calibration period, the model achieved an R^2 of 0.84, an RMSE of 0.21 mm day^{-1}, and an NSE of 0.83

Table 3, Continued

Source	Sensor	Crop	Estimation	Results
(Ru *et al.*, 2020)	Infrared thermometer	Grapevines	Leaf temperature, Water status – water stress	The correlation between the leaf-air temperature difference (T_c-T_a) and indicators of PWS (φ_s, g_s, E) is statistically significant (P<0.05). Among these indicators, the relationship between g_s, E, and T_c-T_a is particularly strong, with R^2 values ranging from 0.530 to 0.604 and from 0.545 to 0.623, respectively. Additionally, there is a reliable linear correlation between the CWSI value and soil moisture at depths of 0-40 cm, with statistical significance (P<0.05)

Source: *developed by the author*

REMOTE SENSING ON PLANT LEVEL

N. Chandel *et al.* (2022) developed a non-invasive method using computer vision and thermal-RGB imagery to detect water stress in winter wheat crops. Deep learning models and function-approximation models are utilized to classify crops as stressed or non-stressed based on thermal-RGB images and various input parameters. P. López-García *et al.* (2022) conducted experiments to assess the impact of different water qualities and irrigation start times on crop growth. LR techniques and ANN models are trained using multispectral and RGB data to simulate water stress. J. Fernández-Novales *et al.* (2021) focused on assessing the water status of grapevines using leaf water potential and canopy temperature measurements. Spatial-temporal maps are generated to analyse variations in water status. S. Zhuang *et al.* (2017) developed a model for early identification of water stress in maize using image analysis. A two-stage detection model based on gradient-boosting decision trees is used to distinguish between adequate water supply and water stress conditions in maize fields. Table 4 presents the above data in a summarized form.

Table 4. *Examples of RS crops on the plant level*

Source	Sensor	Crop	Estimation	Results
(Chandel *et al.*, 2022)	RGB and thermal imagery plus computer vision	Winter wheat	Crop water stress	Different irrigation treatments significantly affect measurements of canopy temperature (Tc), relative water content (RWC), SMC, and RH concerning crop and soil responses. The 100% ETc treatment resulted in the lowest Tc (22.5±2°C), highest RWC (90%), and highest SMC (25.7±2.2%), while the 25% ETc treatment had the highest Tc (28±3°C), lowest RWC (74%), and lowest SMC (20.5±3.1%). ResNet50 performed the best among feature extraction models, achieving a discriminant accuracy of 96.9% with RGB inputs and 98.4% with thermal imagery inputs. Thermal imagery generally provided higher classification accuracy compared to RGB imagery. Among function approximation models, the DL-LSTM model achieved the highest discriminant accuracy of 96.7% and displayed lower errors in stress/non-stress classification
(López-García *et al.*, 2022)	Multispectral and RGB UAV Imagery	Vineyard	Water Status	Utilizing ML techniques, such as ANN models, is an efficient approach to analysing data obtained through UAV remote sensing for the estimation of S_Ψ. These models outperform LR models and using RGB bands and GCC as inputs leads to satisfactory outcomes. High-resolution RGB cameras are a cost-effective alternative to multispectral and thermal sensors, with promising results. To avoid soil effects and obtain precise GCC values, accurate vegetation segmentation is essential
(Fernández-Novales *et al.*, 2021)	Ground drone; Thermal infrared radiometry and multispectral sensor	Grapevine	PWS	The researchers employed Partial Least Squares (PLS) regression to develop calibration and prediction models. The findings revealed that the most effective prediction models for grapevine water status achieved a cross-validated determination coefficient (r^2_{cv}) of 0.57 in the morning and 0.42 at midday. The RMSE of cross-validation ($RMSE_{cv}$) was determined to be 0.191 MPa in the morning and 0.139 MPa at midday
(Zhuang *et al.*, 2017)	RGB outdoor camera	Maize	Early water stress detection	The accuracy of identifying three different water treatments is found to be 80.95%, while the accuracy of detecting water stress is 90.39%

Source: *developed by the author*

REMOTE SENSING ON LEAF LEVEL

In 2015, Dhillon introduced the "Leaf Monitor," a continuous leaf monitoring system specifically designed for measuring the PWS of almond and walnut trees. The Leaf Monitor system utilized a thermal infra-red sensor to measure leaf temperature and integrated additional sensors to monitor various environmental factors such as air temperature, relative humidity, photosynthetically active radiation (PAR), and wind speed. To optimize its performance, the system included a leaf holder, a solar radiation diffuser dome, and a wind barrier. These systems were interconnected in a wireless mesh network, allowing data collection and transmission every 16 minutes through the internet.

In a subsequent study published in 2019 by the same research team led by Dhillon, they developed a methodology to predict PWS in almond and walnut trees using the continuous leaf monitoring system. The researchers collected data on leaf temperature and environmental conditions using the Leaf Monitor system. These data were then utilized to calculate a Modified CWSI, which served as an indicator of the plant's water status. Before an irrigation event, MCWSI values were estimated and used to determine the appropriate irrigation amount for low-frequency variable rate irrigation (VRI). The irrigation amounts were based on the correlation between MCWSI and Drought Stress Water Potential (DSWP). By implementing variable rate irrigation, the researchers achieved a significant average reduction of 39% in water consumption compared to the fixed 100% evapotranspiration (ET) replacement irrigation method for all the trees.

Analysing strawberry leaf colour can effectively help evaluate soil status and protect against excess environmental nutrients and financial losses in strawberry crops (Madhavi *et al.,* 2022). The objective of this study was to create ML models, specifically multiple linear regression (MLR) and gradient boost regression (GBR), that can simulate variations in strawberry leaf colour. These colour changes are based on soil physicochemical properties and plant age, using mean values of the RGB channels. Accurate measurements of soil physicochemical components were obtained from the largest and most diverse coloured leaves of the strawberry plants using a multifunctional soil sensor in the rooting zones. Additionally, 400 strawberry leaflets were sampled at different stages of vegetative and reproductive growth, and individual leaves were captured using a digital imaging system. The RGB mean values of the coloured leaf images were extracted using image segmentation algorithms incorporated into image processing techniques. Subsequently, MLR and GBR models were developed to predict the RGB mean values of the leaves based on the soil physicochemical measurements and plant age. The GBR model demonstrated superior performance compared to the MLR model, achieving high-performance metrics.

Several other studies have explored the use of advanced techniques for assessing PWS and health in various crops. F. Hahn *et al.* (2021) focused on mango trees and employed dendrometers and capacitors with Teflon clamps to measure LWC and examine the influence of water on mango production. H. Skoneczny *et al.* (2020) investigated the potential of non-invasive proximal hyperspectral remote sensing (RS) to distinguish between healthy, infected, and dry leaves of apple trees using spectral bands and indices. F. Rojo *et al.* (2016) proposed a new method for calculating CWSI and Modified CWSI using continuous leaf monitor data in almond orchards and vineyards to implement site-specific irrigation management. J. Rodriguez-Perez *et al.* (2018) compared ordinary least squares regression (OLSR) and functional linear regression (FLR) models to predict LWC using reflectance data and wavelengths in grapevines. T. Zhao *et al.* (2020) used hyperspectral imaging to evaluate water status in tomato leaves and computed vegetation indices (VIs) to assess LWC in different parts of the plants. These studies highlight the potential of advanced techniques in monitoring PWS and health for improved crop management. Table 5 presents the above data in a summarized form.

Table 5. *Examples of RS crops on leaf level*

Source	Sensor	Crop	Estimation	Results
(Dhillon, 2015; Dhillon *et al.,* 2019)	Thermal infrared sensor for continuously monitor the temperature of leaves, as well as the ambient temperature, relative humidity, photosynthetically active radiation (PAR), and wind speed	Almond and walnut	PWS	In almond: MCWSI and DSWP linearly related, r^2=0.67; In walnuts, MCWSI and DSWP in quadratic relationship R^2=0.75
(Madhavi *et al.,* 2022)	RGB camera with a pixel resolution of 5472 × 3648	Strawberry	Leaf Colour Using RGB Mean Values Based on Soil Physicochemical Parameters Using Machine Learning Models	GBR model, utilizing RGB mean values throughout the growth stage, demonstrated a good fit with R^2 and RMSE values of (R=0.77, 7.16, G=0.72, 7.37, and B=0.70, 5.68), respectively. On the other hand, the MLR model performed moderately, with R2 and RMSE values of (R=0.67, 8.59, G=0.57, 9.12, and B=0.56, 6.81), when predicting RGB mean values consecutively in strawberry leaves
(Hahn *et al.,* 2021)	Inductive and Capacitive Sensors	Mango	Leaf Monitoring	The capacitance and Hall effect sensors can produce signals that can be used for scheduling irrigation based on predetermined thresholds.
(Skoneczny *et al.,* 2020)	Non-invasive proximal hyperspectral RS	Apple	Hyperspectral Analysis of Healthy, Infected and Dry Leaves	The 1450 nm band in the SWIR range effectively separates infected (I) and healthy (H) leaves, while the 1900 nm SWIR band distinguishes all three leaf types. Pearson correlation tests revealed that ARI, MSR, and QFI showed the strongest correlations with the progression of infection

Table 5, Continued

Source	Sensor	Crop	Estimation	Results
(Rojo *et al.,* 2016)	Continuous WSN leaf temperature monitoring system and soil and pressure sensors, latching solenoid valves	Almond orchards and Vineyards	PWS	In grapes: MCWSI and DSWP are linearly related, with R^2=0.70; In almonds, CWSI and DSWP strongly correlated with a second-order relationship and R^2=0.78
(Rodriguez-Perez *et al.,* 2018)	Hyperspectral	Four grape varieties	LWC expressed as equivalent water thickness	The most accurate model is achieved by utilizing functional linear regression (FLR) within the spectral range centred around 1465 nm, resulting in a coefficient of determination (R^2) of 0.70 and a percentage root mean squared error (%RMSE) of 8.485
(Zhao *et al.,* 2020)	Portable hyperspectral camera	Tomato	Leaf Water Status	The most successful regression model for evaluating water content (WC) is obtained by utilizing TBI regression with DIFF data at 1410 nm and 1520 nm wavelengths. Similarly, the most efficient regression model for determining moisture content (MC) is achieved through NDVI regression with RAW data at 1300 nm and 1310 nm wavelengths. The models used for the MC assessment showed better performance compared to those used for the WC assessment

Source: *developed by the author*

CUTTING-EDGE TECHNOLOGIES

Industries are moving towards smart factories, and the agricultural sector should also embrace the concept of smart farms. Unmanned ground vehicles (UGVs) play a vital role in the development of smart farms (Gonzalez-De-Santos *et al.,* 2020). While there are similarities between smart factories and smart farms, specific research is needed to address the unique characteristics of UGVs designed for outdoor agricultural tasks. Multi-drone systems consisting of smaller or medium-sized drones can perform tasks with greater accuracy, tolerance, and safety compared to larger machines. Autonomous drones, whether operating alone or in fleets, are essential for the precise application of herbicides and fertilizers. This approach reduces chemical usage, resulting in benefits such as lower costs, improved operator safety, enhanced community health, and higher food quality with reduced toxicity.

The Research Centre for High Technology Greenhouse Plant Production at Ehime University is working on a project called "Intelligent Greenhouse Systems," in which the main concept is the "Speaking Plant" approach (Hiroshige, 2015). The SPA approach suggests that the best way to grow crops is by considering the plants' physiological status. To implement SPA, a chlorophyll fluorescence imaging drone is developed to measure the photosynthetic activity of tomato plants in greenhouses. This drone is designed to be low-cost, easy to use, and suitable for commercial tomato production. The researchers also studied how storage temperature affects tomato fruit colour using multiple regression analysis to improve postharvest management.

This study (Kalaitzoglou *et al.,* 2021) aims to investigate how blue light affects plant growth by measuring light absorption and photosynthesis in tomato plants. The experiment involved growing tomato plants in six different combinations of artificial solar and blue LED light, with varying levels of blue light. The results showed that increasing the blue light fraction led to a decrease in plant growth, which is related to a more compact plant morphology and lower light absorption. However, the blue light did not affect the plants' maximum photosynthetic capacity. The study suggests that increasing blue light in a solar light environment can hinder plant growth, but further research is needed to confirm this in high-light growth environments typically used in tomato production.

A digital twin refers to a virtual representation of a farm that offers benefits such as increased productivity, improved efficiency, and reduced energy consumption and losses. A. Nasirahmadi and O. Hensel (2022) presented a comprehensive overview of digital twin concepts and technologies in the field of agriculture. It covers various aspects including soil management, irrigation, robotics, farm machinery, and post-harvest processing. The review explores data recording, modelling, artificial intelligence, big data, simulation, analysis, prediction, and communication aspects of digital twin systems in agriculture. By continuously monitoring the farm in real time and updating the virtual model to reflect changes in the physical environment, digital twins provide valuable support to farmers. This technology represents the cutting-edge advancement in the digital transformation of the agricultural sector.

C. Pylianidis *et al.* (2021) used a combination of qualitative and quantitative methods to explore the advantages of digital twins in the agricultural domain. The study begins with a comprehensive literature review focusing on digital twins in agriculture between 2017 and 2020. By examining 28 use cases and comparing them with examples from other fields, the researchers assess the extent of digital twin implementation in

agriculture. They analysed reported benefits, service categories, and technology readiness levels to evaluate the adoption of digital twins in the agricultural sector. Furthermore, the study identifies specific characteristics of digital twins that can be beneficial in agriculture and proposes a roadmap for their implementation, considering increasing levels of complexity. Ultimately, the research underscores the distinctive attributes of digital twins that make them particularly valuable in the context of agriculture.

"Agri-Food 4.0" is a term used to describe the latest developments in the agriculture sector, which includes the integration of digital technologies and the Internet of Things. Similar to the concept of "Industry 4.0", Agri-Food 4.0 aims to optimize efficiency and productivity through the use of these technologies. While the agriculture sector has already incorporated electronic controls and data collection methods through sensors and drones, there is still a need to improve supply chain performance and decision-making processes. This survey (Lezoche *et al.,* 2020) reviews over a hundred papers on new technologies and supply chain methods to understand the future of Agri-Food 4.0.

According to the United Nations Development Programme (2021), global agriculture and food systems are under constant enormous pressure. It is estimated that by 2050 the world population will grow to almost 10 billion people and to feed it, current food production must be increased by 59-98% (Domingues *et al.,* 2022). It is also estimated that agriculture uses over 70% of global freshwater supplies, with around half of this being lost and wasted. Globally, about one-third of the food produced is wasted or thrown away. Agri-food is at the heart of the United Nations 2030 Agenda and impacts all 17 Sustainable Development Goals.

In 2019, the twenty-six EU member states signed a declaration of cooperation to support the successful digitization of agriculture and rural areas in Europe. In it, they recognize the potential of digital technologies to help tackle important and pressing economic, social, climate and environmental challenges facing the EU's agri-food sector and rural areas. In 2022, FAO calls for an end to soil degradation. Up to 95% of the world's food production depends on soil. As a result of unsustainable farming practices, overexploitation of natural resources and a growing population, one-third of soils have already been degraded, and experts estimate that soil erosion could lead to 10% crop loss by 2050. Soils are also full of life and contain approximately 25% of the world's biodiversity (FAO, 2023).

The introduction of so-called precision agriculture (PA) and digital technologies in the field of agriculture are ways to optimize and improve the processes in it. New digital technologies make farming more accessible, using a combination of different technologies working together to improve the precision of farming, including high-speed internet (5G) mobile phones, high-resolution RS technologies through images from satellites and unmanned aerial vehicles (UAVs), sensors connected to the Internet of Things (IoT), wireless sensor networks (WSN), robotics and 3D printing (Kalaitzoglou *et al.,* 2021).

The use of PA is primarily driven by WSN, which consists of interconnected wireless nodes that monitor environmental conditions (Shafi *et al.,* 2019). These nodes are equipped with components, such as transmitters, microcontrollers, sensors, antennas, and electronic circuits to collect and transmit data to a gateway. Nodes are categorized as either data sources or receivers/gateways, with receivers having more computing power. In agriculture, different plants have varying needs even within the same farmland, and sensors are employed to monitor the changing behaviour of plant parameters, including soil properties, plant characteristics, weather conditions, fertilization options, and water requirements. The most commonly used wireless communication protocols in agricultural IoT applications are Cellular, 6LoWPAN, ZigBee, RFID, Wi-Fi, and LoRaWAN (Avşar & Mowla, 2022).

The combination of ML, HPC, and big data technologies opens up new opportunities for data-intensive science in the interdisciplinary field of agrotechnologies. ML algorithms applied to sensor data transform farm management systems into real-time AI-driven programs that offer valuable recommendations to support farmers' decision-making (Liakos *et al.,* 2018). ML approaches were successfully applied in various areas, such as classifying plant diseases from plant images using CNNs for different plant species and diseases, detecting insects on leaves through object segmentation and deep learning techniques (Domingues *et al.*, 2022), and predicting soil moisture levels (Atanasov *et al.,* 2023). In a global context, RS has been supporting decision-making in agriculture for over 45 years (Ammoniaci *et al.,* 2021; Domingues *et al.,* 2022).

CONCLUSIONS

Remote sensing technologies offer valuable insights for improving agricultural productivity and efficient water resource management. Soil moisture sensors provide accurate and consistent data, enabling objective observations of plant water status, although they have limitations in capturing field-wide moisture variations. Handheld remote sensing devices offer better temporal, spectral, and spatial resolution for on-site monitoring, but their effectiveness is limited for evaluating larger areas compared to sensors on planes and satellites. Proximal sensors require location-specific calibration and may pose data analysis challenges. Leaf physiology methods provide objective assessments of crop water stress but are time-consuming, destructive, and not suitable for real-time monitoring. Modern imaging techniques such as fluorescence, thermography, and multispectral imaging enable fast and non-destructive

phenotyping for early detection of water stress. Satellite-based remote sensing has significant potential but can be constrained by spatial and temporal resolution limitations and cloud cover. To overcome these challenges, unmanned aerial vehicles (UAVs) equipped with remote sensing sensors offer a cost-effective alternative, providing high-resolution imagery with flexibility in flight and mission timing. UAVs surpass satellite resolution and offer quick and repeatable deployment. Combining multispectral and hyperspectral images enhances the comprehensive understanding of crop conditions. By integrating these technologies, agriculture can benefit from improved practices and sustainable resource management.

Prospects for future research in state-of-the-art technologies for remote sensing of crop water status and nutrients in agriculture include advancing sensor technologies for improved accuracy and resolution, integrating data from multiple sources for comprehensive analysis, and exploring the integration of remote sensing with precision agriculture techniques for site-specific management.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

The author has no conflict of interest to declare.

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Сучасні технології дистанційного зондування водного режиму та поживних речовин у сільському господарстві: огляд

Светослав Атанасов
Аспірант, магістр комп'ютерних систем та технологій
Університет Тракія
6015, Студентське містечко, м. Стара Загора, Болгарія
https://orcid.org/0000-0002-2658-1611

Анотація. Актуальність дослідження зумовлена необхідністю впровадження ефективних методів та інструментів моніторингу водних ресурсів та відстеження рівня поживних речовин у ґрунті для покращення сільськогосподарського виробництва та сталого використання природних ресурсів. Метою дослідження є надання комплексного огляду новітніх технологій та методів, що використовуються в ДЗЗ для точної оцінки стану водних ресурсів та рівня поживних речовин у сільськогосподарських культурах з метою підвищення продуктивності та сталості сільського господарства. Були вивчені останні досягнення в методах дистанційного зондування, які дозволяють проводити точний моніторинг і оцінку рівнів води і поживних речовин у посівах, що має вирішальне значення для оптимізації сільськогосподарських практик. Дослідження літератури проводилося шляхом адаптації методів систематичних оглядів і мета-аналізу, яким надається перевага при складанні звітів. У цьому дослідженні представлено огляд технології ДЗЗ з особливим акцентом на визначенні водного та поживного статусу сільськогосподарських культур у сільському господарстві. Також було проведено ретельний огляд досліджень, присвячених застосуванню та технологіям ДЗЗ у сільському господарстві, з використанням підходу «від широкого до вузького». Проаналізовані наукові дослідження свідчать про наступне: ДЗЗ на великомасштабному рівні, ДЗЗ на рівні поля, ДЗЗ на рівні теплиць, ДЗЗ на рівні рослин та ДЗЗ на рівні листків. Також представлені сучасні передові технології. Результати цього дослідження можуть бути корисними для тих, хто займається питаннями сталого сільського господарства, таких як дослідники, викладачі та студенти-початківці

Ключові слова: огляд; точне землеробство; точне зрошення; розумне землеробство; дистанційне зондування; моніторинг посівів; реакція рослин на воду; неінвазивний; неруйнівний

Журнал
«НАУКОВІ ГОРИЗОНТИ»

Том 26, № 9
2023

(Англійською мовою)

Редагування англомовних текстів:
С. Воровський

Відповідальний редактор:
Д. Кокра

Редагування бібліографічних списків:
Д. Кокра

Комп'ютерна верстка:
М. Кришталь

Підписано до друку з оригінал-макета 27.09.2023
Ум. друк. арк. 20,9

Видавництво: Поліський національний університет
10008, б-р Старий, 7, м. Житомир, Україна
Тел. (0412) 22-04-17
E-mail: info@sciencehorizon.com.ua
www: https://sciencehorizon.com.ua

Journal
"SCIENTIFIC HORIZONS"

Volume 26, No. 9
2023

Editing English-Language Texts:
S. Vorovsky

Managing Editor:
D. Kokra

Editing Bibliographic Lists:
D. Kokra

Desktop Publishing:
M. Kryshtal

Signed to the print with the original layout 27/09/2023
Conventional Printed Sheet 20.9

Publisher: Polissia National University
10008, 7 Staryi Bvld., Zhytomyr, Ukraine
Tel. (0412) 22-04-17;
E-mail: info@sciencehorizon.com.ua
www: https://sciencehorizon.com.ua/en