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The Influence of Platelet Concentrate on the Development of Cattle Embryos in an *In Vitro* System

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Abstract. There are many studies on the improvement of the conditions for cultivating bovine embryos *in vitro*. However, the issue of co-cultivation of platelets and cow embryos is understudied, although platelet activation leads to the release of a considerable amount of biologically active substances into the culture medium and their co-cultivation with embryos can positively affect the growth and development of the latter. Therefore, the purpose of this study was to investigate the effect of different platelet concentrations in the *in vitro* culture system on the development of cow embryos. Fertilized zygotes (total number 180) were divided into 5 groups: Group 1 – culture medium without platelet addition (control); Group 2 – medium for cultivation with the addition of 10×10^6 platelets/cm^3; Group 3 – final concentration in the system is 20×10^6 platelets/cm^3; Group 4 – 50×10^6 platelets/cm^3; Group 5 – 100×10^6 platelets/cm^3. According to the results, it was found that co-cultivation of embryos with platelets in the *in vitro* system is effective. Thus, a correlation was found between improved embryo development indicators and an increase in platelet concentration. The optimal platelet concentration was 50×10^6/cm^3, which allowed obtaining a 13.9% higher level of blastulation, 15.7% higher average embryo size, and 2.5% higher average number of cells in the blastocyst compared to the control. At the same time, the platelet concentration of 100×10^6/cm^3 led to a significant decrease in the indicators under study, compared with the group without platelets. Therefore, co-cultivation of cow embryos with platelets is advisable since it allows improving the development indicators of cow embryos. The data analysed and presented in this paper will increase the efficiency of cultivation of bovine embryos for both scientific and industrial purposes

Keywords: biotechnology of ruminant reproduction, growth factor, division, blastocyst

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INTRODUCTION

Despite the widespread study of methods used for the cultivation of bovine embryos, there are still ways to improve them. Optimisation of cultivation parameters *in vitro* allows increasing the percentage of embryos suitable for transplantation. Therefore, scientists continue to work on improving the protocols of assisted reproductive technologies in cattle breeding.

In veterinary embryology, the use of blood platelets or biologically active substances released during the cultivation of bovine embryos is still understudied. This study is the first to analyse the effect of diverse platelet concentrations on the percentage of blastulation, cell number, and size of cow blastocysts *in vitro*.

Bovine embryo transplantation is a biotechnological method used for programs of accelerated reproduction of highly productive animals and herd improvement by actively using the genetic potential of females (Dochi, 2019). In cattle breeding, there are 2 methods of obtaining embryos: *in vivo* and *in vitro*. Until 2016, *in vivo* cattle embryo production was dominant (Viana, 2020). However, with the development of the branch, obtaining oocytes by ovum pick up (OPU) allowed obtaining a more considerable number of embryos produced *in vitro* per individual animal, compared with indicators after multiple ovulation embryo transfer (MOET) (Hasler *et al*., 2003; Ferré *et al*., 2020). Notably, the technologies of genomic selection used by selective breeding companies additionally increase the demand for embryos obtained from genetically desirable animals obtained *in vitro* since the use of this technique allows reducing the intervals between generations and increasing genomic growth (Aardema *et al*., 2022).

In addition, *in vitro* embryo production technology has made great strides since the first extracorporeal fertilisation calf was born at the University of Pennsylvania in 1981 (Brackett *et al*., 1986). Considering the above, the ratio of bovine embryos obtained *in vitro* (IVP) to embryos obtained *in vivo* has gradually changed (1 million to 378,769 embryos in 2019, respectively) (Viana, 2020). Today, biotechnology of cow reproduction is a profitable business of international level, which tends to increase the percentage of obtaining embryos by *in vitro* fertilisation (O'Neill, 2005).

However, despite noteworthy progress in the development of techniques for obtaining embryos *in vitro*, the ratio of embryos suitable for transplantation to used ova is still quite low. Thus, Ferré *et al*. (2020) indicate that only 20-40% of fertilized eggs reach the blastocyst development stage on Day 7. Lonergan & Fair (2008) states that only 30-35% of zygotes can develop into characteristics when their transfer to recipient cows is successful. Inaba *et al*. (2016) noted the effect of sperm on the further development of embryos, and the percentage of blastocysts obtained in their study was within 22-44%. By investigating the kinetic patterns of embryos reaching the blastocyst stage, Oliveira *et al*. (2019) obtained 29.9% blastocysts, and in the studies of Sirard *et al*. (1988), the percentage of obtaining embryos suitable for transplantation was 28%.

Given the low level of cattle embryos reaching a stage sufficient for transplantation, scientists continue to work on perfecting cultivation conditions. The production of bovine embryos *in vitro* involves achieving the closest parameters of the oviduct environment (Besenfelder *et al*., 2020). Clearly, the conditions of cultivation are still unable to provide all the advantages of development in the reproductive organs of the female. However, several culture systems and environments have been proposed that allow approaching physiological conditions. Thus, Lopera-Vásquez *et al*. (2016) used a conditioned medium obtained from bovine oviduct epithelial cell lines and noted its positive effect on the quality of embryos obtained *in vitro*. Goovaerts *et al*. (2009) indicate an increase in the percentage of blastocysts during the co-cultivation of embryos and cumulus cells. Chen *et al*. (2017) proposes to culture zygotes on mammalian oviductal epithelial cells, which generate oviductal fluid surrogates, to the blastocyst stage without the addition of embryo culture medium. Leivas *et al*. (2011) and Soto-Moreno *et al*. (2021) recommend adding foetal bovine serum to the culture medium, while Rizos *et al*. (2003) indicate its lack of effectiveness and negative impact on cryotolerance of embryos. Cañón-Beltrán *et al*. (2021) demonstrate a positive effect of nobiletin on the pre-implantation development of bovine embryos *in vitro* and improvement of the quality of produced blastocysts.

As the data given above suggest, there is currently a large amount of information on the improvement of the conditions for the cultivation of bovine embryos *in vitro*, but it is extremely diverse and often the conclusions of scientists are contradictory. That is why, *the purpose of this study* is to investigate the effect on the development of cattle embryos of co-cultivation with different concentrations of platelets in the commercial environment Minitube (Germany) and practical implementation of the obtained results.

MATERIALS AND METHODS

The study was conducted during 2020-2022 based on the Educational and Scientific Laboratory "Centre for Animal Reproductology with Sperm and Embryo Bank" of the National University of Life and Natural Sciences of Ukraine. Animal experiments were conducted in compliance with the requirements of the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (Article 230 of 2006), "General Ethical Principles of Experiments on Animals", approved by the National Congress on Bioethics and agreed with the provisions of the "European Convention for the Protection of Vertebrates Used for Experiments and Other Scientific Purposes" (Strasbourg, 1986).

Production of zygotes *in vitro*

1.1. Maturation

Ovaries from clinically healthy cows were selected in slaughterhouses and delivered to the laboratory in a thermos at 30-33°C no more than 3 hours after sampling. In the laboratory, the ovaries were washed 4 times in a sterile phosphate-salt solution of Dulbecco (Sigma, USA) with the addition of 0.075 mg/cm^3 kanamycin sulphate (Sigma, USA) (solution temperature 37-38°C). The cumulus cell-oocyte complexes (COCs) from the antral follicles (2-8 mm in size) of the ovaries of cows were removed in a laminar box by dissecting the follicles with a safety razor blade in an oocyte collection medium consisting of 5 cm^3 TL HEPES (Minitube, Germany) with the addition 30 mg of bovine serum albumin (BSA) (Sigma, USA). Removal of COCs, their selection and setting for maturation, fertilisation, and subsequent cultivation were also performed in a sterile box. After evaluation under a stereomicroscope SZ51 (Olympus, Japan), COCs of 120-130 μm with solid dense cumulus, intact transparent shell and homogeneous unvacuolized ooplasm of regular rounded shape were selected, without visible morphological signs of atresia. The extracted COCs were washed 6 times in an oocyte collection medium. The COCs were selected and washed on a heating table at 37°C. Oocytes were matured *in vitro* for 22-24 hours in 4-well plates (Oosafe, USA). 300 mm^3 of medium was added to each well, which included 4.5 cm^3 of the initial solution of maturation medium TCM 199 (Minitube, Germany), 0.5 cm^3 of bovine estrous serum, 0.125 IU. FSH (follicle-stimulating hormone) and 0.125 IU of luteinising hormone (LH) (50 mm^3 "Pluset" (Laboratories Calier S.A., Spain)) and 0.125 IU of FSH ("FSH-super" (Agrobiomed, RF)) and 50 mm^3 of an antibiotic-antimycotic (Sigma, USA) were covered with mineral oil (Origio, Denmark), 25 COCs were added and cultivated in a CO_2 incubator at 38.5°C and 6% CO_2 and 5% O_2.

1.2. Preparing sperm for fertilisation

Bull sperm was prepared for fertilisation using density gradients "Origio Gradient Series" (Origio, Denmark) and sperm capacitation medium (Minitube, Germany). The components of the gradient were heated to room temperature (20-25°C), all other reagents were equilibrated in a CO_2 incubator at 38.5-39.0°C and 5% CO_2 for at least 2 hours. The gradient was prepared by carefully layering 1 cm^3 of "Origio Gradient 40" on 1 cm^3 of "Origio Gradient 80", after which sperm previously thawed in a water bath was carefully introduced. The resulting system was centrifuged at a centrifugal force of 300 g for 20 minutes. The supernatant was removed, and the sediment was transferred with a new sterile nozzle into a test tube with 2 cm^3 of medium for sperm preparation and capacitation, which included 5 cm^3 of basic solution for capacitation (Minitube, Germany), 30 mg of BSA (Sigma, USA), 0.55 mg of sodium pyruvate (Sigma, USA) and 50 mm^3 of antibiotic-antimycotic (Sigma, USA) and centrifuged at a centrifugal force of 300 g for 5 minutes, most of the supernatant was removed. The procedure was repeated twice. After washing, the sediment was transferred to the bottom of a test tube with 1 cm^3 of a new portion of medium for the preparation and capacitation of spermatozoa. The mobile sperm fraction was obtained using the swim-up method described by Parrish *et al.* (1986). Incubation for 1 hour was sufficient for mobile spermatozoa to rise to the upper layers of the medium, while dead and pathological ones stayed at the bottom of the test tube. Motile spermatozoa were capacitated in the medium for preparation and capacitation for 4 hours of exposure to heparin (Sigma, USA) at a concentration of 20 μg/cm^3 in a CO_2 incubator at 38.5°C with 6% CO_2 and 5% O_2. After capacitation, the spermatozoa were centrifuged at a centrifugal force of 200 g for 5 minutes. The supernatant was removed and 1 cm^3 of fertilisation medium was added, and the concentration of spermatozoa was counted in the Goryaev chamber.

1.3. Co-cultivation of oocytes with spermatozoa

After maturation, the oocytes were co-cultured with spermatozoa. Oocytes were fertilised in 4-well plates (Oosafe, USA) in 300 mm^3 of medium, which included 5 cm^3 of fertilisation medium (Minitube, Germany), 30 mg of BSA, 0.11 μg of sodium pyruvate, 0.2 mg of heparin, and 50 mm^3 of an antibiotic-antimycotic (Sigma, USA). The oocytes were covered with mineral oil (Origio, Denmark) for 18 hours after the addition of capacitated spermatozoa (at the rate of 1×10^6 motile spermatozoa/cm^3). The number of oocytes in the well ranged from 5 to 10.

2. Obtaining bovine platelets

Blood was obtained from the jugular vein from 8 clinically healthy cows that had not received medication in the preceding two months. Preliminarily, surgical treatment of several centimetres of skin near the jugular vein was performed. From each cow, 20 cm^3 of blood was collected by a standard method into sterile Vacutainer Plus vacuum tubes with EDTA (BD, USA). The test tubes were transported at 4°C to the laboratory no more than 2 hours after selection. All stages of obtaining platelets from whole blood were carried out in a laminar box under aseptic conditions. The blood was transferred to sterile centrifuge tubes and subjected to centrifugation at 100 g within 30 minutes, which caused the blood to divide into three layers: red blood cells at the lowest level, white blood cells at the middle level, and platelet-rich plasma (PRP) at the top. The top layer was selected, transferred to new sterile test tubes, and the platelet count in the suspension was calculated using a Goryaev chamber. Platelet-rich plasma was re-centered at 300 g for 15 minutes, after which the sediment was selected. 1 cm^3 of embryo culture medium was carefully added to the platelet sediment and centrifuged at 300 g for 10 minutes. The sediment was selected, and a medium for embryo culture was added to the sediment at the rate of 1×10^9 platelets/ml.

3. Embryo cultivation

After co-culture, bovine oocytes with loose enlarged cumulus were released from cumulus cells by gentle pipetting in 0.1% hyaluronidase solution (Sigma, USA). Then the oocytes were washed from the enzyme in 5-6 drops of TL HEPES (Minitube, Germany) and transferred to a culture medium comprising 5 cm^3 of culture medium with pyruvate (Minitube, Germany), 0.5 cm^3 of bovine estrous serum, 200 mm^3 of essential amino acids (Sigma, USA), 50 mm^3 of substitute amino acids and 50 mm^3 of antibiotic-antimycotic (Sigma, USA). Fertilized oocytes were discovered by division from the 2- to 8-cell stage 48 hours after contact with spermatozoa. 48 hours after fertilisation, the zygotes were randomly divided into 5 groups and platelet concentrate was added in different amounts. Embryos were cultivated in microdrops of 100 mm^3 (6 embryos per drop) under a layer of mineral oil (Origio, Denmark) in culture dishes (Oosafe, USA) in a CO_2 incubator at 38.5°C with 6% CO_2 and 5% O_2.

4. Embryo development evaluation

Embryo quality was evaluated on Day 7 under a Zeiss Axio Observer A inverted microscope (Carl Zeiss, Germany), photos and size measurements were made using the Octax NaviLase laser system (OCTAX Microscience GmbH, Germany).

The number of nuclei in the embryo was counted under a DMR microscope (Leica, Germany) at a magnification of ×400, ×1000, and photographed with a Canon DS126291 camera (Canon, Taiwan). To visualize the nuclei, the embryo was stripped of its transparent shell using a laser system, after which it was transferred to superfrost slides (Thermo Scientific, USA) in a drop with a solvent (0.2% Tween20 (Sigma, USA) in 0.01 m HCl (Sigma, USA), ph 2.0) for lysis of the blastomere envelope. The process was monitored under an inverted microscope, adding a new portion of solvent if necessary. After lysis of the blastomere shell, the solvent was selected as much as possible and the nuclei were sequentially washed with a phosphate buffer solution (Sigma, USA), 70% ethyl alcohol (Sigma, USA) and 100% ethyl alcohol (Sigma, USA). Glass samples were air-dried and stained with an industrial smear kit "Leucodif 200" (Erba Lachema, Czech Republic), according to the manufacturer's instructions.

5. Statistical processing

The reliability of the results was evaluated according to the criteria proposed by Brzhevska *et al.* (2019). Statistical processing of the obtained results of experimental studies was performed according to N.A. Plokhinsky (1970) and E.V. Montseviciute-Eringen (1964) using the Microsoft Excel data analysis package (Lesnikova & Kharchenko, 2002). Arithmetic mean values and their errors were found, and the probability of difference between parallel data sets was figured out. In all cases, the difference was considered reliable at $P<0.05$.

RESULTS AND DISCUSSION

The clinical benefit of platelet concentrates has been reported to be fourfold higher than that of whole blood (Marx, 2001) (platelet concentrations in bovine blood range within 160-800×10^6/ml (Roland *et al.*, 2014)), but strong scientific evidence that preparations with a platelet content below these are less effective (Cole *et al.*, 2010). Considering the specific cultivation conditions, where the presence of platelets is atypical, it was decided to create systems for culturing bovine embryos using a lower platelet concentration than in whole blood. Thus, 48 hours after fertilisation, the zygote (total number 180) was randomly divided into 5 groups (the study in each group was repeated three times, (n=3):

Group 1 – culture medium without platelet addition (control).

Group 2 – the cultivation system included 90 mm^3 medium for cultivating embryos with the addition of 11×10^6 platelets/cm^3 and 10 mm^3 medium where cattle embryos were cultivated for 48 hours to activate platelets with clot formation.

Group 3 – final concentration in the system is 20×10^6 platelets/cm^3;

Group 4 – final concentration in the system is 50×10^6 platelets/cm^3;

Group 5 – final concentration in the system is 100×10^6 platelets/cm^3 (Table 1).

***Table 1**. The influence of the concentration of platelets in the culture system on the development of bovine embryos in vitro (M±m, n=3)*

Group	Platelet count, platelets/cm^3	Number of zygotes, pcs	Blastocysts, %	Number of cells in the blastocyst, pcs	Blastocyst size, μm
1	No platelets (control)	36	38.8±2.4	173.3±10.3	195.0±3.4
2	10×10^6	36	41.6±3.6	201.1±3.4***	203.2±2.2**
3	20×10^6	36	50.0±3.6 *	215.6±2.7***	213.2±2.3***
4	50×10^6	36	52.7±2.4**	224.4±4.8***	225.6±4.8***
5	100×10^6	36	33.3±0.0*	153.1±4.2***	188.4±2.2*

***Note:** * – P<0.05, ** – P<0.01, *** – P<0.001 compared to the control group (medium without platelet addition)*
***Source:** compiled by the authors*

McCarrel & Fortier (2009) indicated that platelet degranulation begins within 10 minutes after exposure to clotting factors. Therefore, in this study, embryos were introduced into the culture medium 30 minutes after the start of aggregation to prevent the embryo from being involved in the clot formation. Aggregated platelets were left in the culture system (Fig. 1) because the data of McCarrel & Fortier (2009) indicate that the main secretion of growth factors occurs within the first hour, but their constant release is noted throughout the entire period of viability of platelets (7 days).

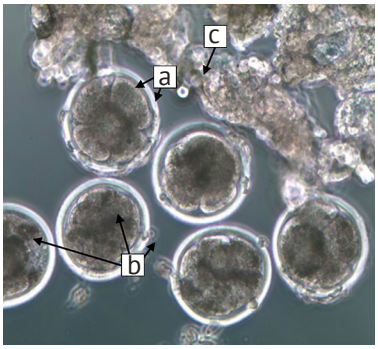

Figure 1. *Culture system with the addition of allogeneic platelets (second group, third day after fertilisation)*
Note: *a) 4-cell embryo; b) 8-cell embryo; c) aggregated platelets. Native preparation, Mag.: ×200*

The effect of platelets on the development of bovine embryos was assessed comprehensively, the percentage of blastulation, and the number of cells (Fig. 2) and blastocyst sizes (Fig. 3) were found.

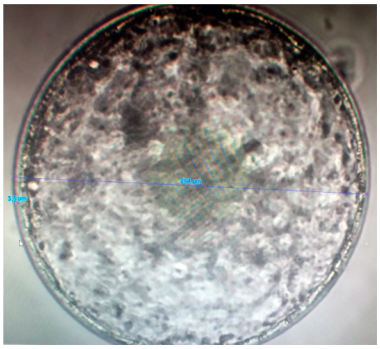

Figure 2. *Bovine blastocyst*
Note: *Cycle Day – 7, Stage Code – 7, Quality Code – 1 (according to the classification proposed by Bó & Mapletoft (2013))*

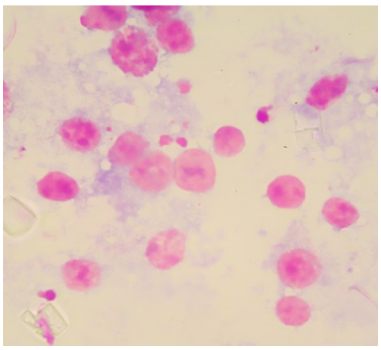

Figure 3. *Embryo nuclei are visualized after blastomere lysis*
Note: *Stained with "Leukodif 200". Mag.: ×1000*

When studying the influence of the concentration of platelets in the culture system on the development of bovine embryos *in vitro*, differences were found. Thus, the addition of 20×10^6 and 50×10^6 platelets/cm^3 contributed to a significant increase in the number of blastocysts by 11.2% ($P<0.05$) and 13.9% ($P<0.01$), respectively, compared to the control (38.8±2.4%). While the addition of 100×10^6 platelets/cm^3 resulted in a significant decrease in blastulation percentage on Day 7 by 5.5% ($P<0.05$) compared to the control.

In addition, in the groups, the authors noted a correlation between the percentage of blastulation on Day 7, the size and number of cells in the embryo. Thus, adding 10×10^6 platelets/cm^3 to the culture system increased the average number of cells in the blastocyst by 16.0% ($P<0.001$), 20×10^6 platelets/cm^3 – by 24.4% ($P<0.001$), 50×10^6 platelets/cm^3 – by 29.5% ($P<0.001$), compared to the control (173.3±10.3 cells). A similar pattern was noted when measuring blastocyst sizes, e.g., the addition of 10×10^6 platelets/cm^3 increased the size of the embryo by 4.2% ($P<0.01$), 20×10^6 platelets/cm^3 – by 9.3% ($P<0.001$), 50×10^6 platelets/cm^3 – by 15.7% ($P<0.001$), compared to the control (195.0±3.4 μm). An increase in the concentration of platelets in the culture system to 100×10^6/cm^3 led to the opposite effect. Thus, the average number of cells in the blastocyst decreased by 11.7% ($P<0.001$), and its average size decreased by 3.4% ($P<0.05$), comparing with control (173.3±10.3 cells and 195.0±3.4 μm, respectively).

Therefore, adding platelets to the culture medium for cow embryos is effective. The concentration of 50×10^6 platelets/cm^3 is best and allows maximising the number and improving the quality of bovine embryos produced *in vitro*.

Platelets are small disc-shaped non-nuclear cells formed by bone marrow megakaryocytes and circulating in the blood, playing a crucial role in supporting vascular integrity and regulating haemostasis (Ghoshal & Bhattacharyya, 2014; Bos-Mikich *et al*., 2019). They are the second most common component of blood after red blood cells, their size ranges from 2 to 5 μm (Michelson *et al*., 2019). Platelets hold three distinct types of granules: α-granules, dense or δ-granules, and lysosomes (Koupenova *et al*., 2018; Morrell *et al*., 2014; Semple *et al*., 2011). Platelet α-granules hold proteins, chemokines, cytokines, and growth factors collected in platelets by megakaryocytes and are necessary for normal platelet function. Δ-granules hold small molecules such as ADP, serotonin, polyphosphates, glutamate, histamine, and calcium necessary for haemostasis (Koupenova *et al*., 2017; Morrell *et al*., 2014). Platelet lysosomes hold glycohydrolases and enzymes that break down glycoproteins, glycolipids, and glycosaminoglycans (Ciferri *et al*., 2014; Morrell *et al*., 2014).

The activation of platelets leads to morphological changes caused by the reorganisation of the cortical actin cytoskeleton from a disk-like shape to a significantly enlarged spherical shape (Bender & Palankar, 2021). Exocytosis of granules is initiated by the activation of surface receptors, which leads to an increase in the level of intracellular Ca^{2+} and activation of phosphokinase C (PKC) (Manne *et al*., 2017). After stimulation, platelets free the contents of their granules releasing growth factors such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), epithelial growth factor (EGF), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF 1), fibroblast growth factor (FGF), haepatocyte growth factor (HGF) (Drago *et al*., 2013), which can increase the proliferative activity of cells (Mazurkevych *et al*., 2021). B_1 and β_3 integrins: e.g., $\alpha_2\beta_1$ (GPIa/IIa) and $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) (Ludwig *et al*., 2022). Seven antimicrobial peptides: Platelet Factor 4 (PF-4), RANTES, Connective Tissue Activating Peptide 3 (CTAP-3), Platelet Basic Protein, Thymosin β-4 (Tβ-4), fibrinopeptide B (FP-B), fibrinopeptide A (FP-A) (Tang *et al*., 2002) and other molecules. Therefore, apart from their crucial role in coagulation and maintenance of haemostasis after mechanical damage to the vascular system, platelets, due to the many bioactive molecules in their granules and the expression of various receptors on their surfaces, can perform a wide range of other essential functions (Schlesinger, 2018).

There are few clinical reports of platelet use in veterinary medicine. Usually, platelet-rich plasma is used to treat lesions of the musculoskeletal system of horses (Brossi *et al*., 2015; Malyuk *et al*., 2021), skin wounds in dogs Farghali *et al*., 2019), intestinal wounds in pigs (Fresno *et al*., 2010), ovarian hypofunction in cows (Cremonesi *et al*., 2020), etc. This study offers a novel approach to the use of platelet concentrate based on current knowledge about its regenerative effect due to its high content of growth factors and cytokines (Bendinelli *et al*., 2010).

Embryos of all mammalian species studied to date produce and secrete platelet activation factor (PAF) (O'Neill, 2005) at elevated levels of 1 to 100 ng of PAF/embryo in 24 hours (Ammit & O'neill, 1991; Roudebush *et al*., 2002). Co-culture of the embryo with platelets will activate the latter and release biologically active substances into the culture medium, which can positively affect the growth and development of the embryo. However, a sharp decrease in platelet activation factor can adversely affect embryo development since the physiologically important target for PAF released by the embryo is itself (O'Neill, 2005). Therefore, an important task when introducing platelets into the culture system is to find their number to ensure a positive effect on the development of bovine embryos.

In the available literature sources, some publications have been found on the effect of platelet-rich plasma (PRP) on embryo development *in vitro* (Thibodeaux *et al*., 1993; Lange-Consiglio *et al*., 2015; Ramos-Deus *et al*., 2020). A specific feature of these studies is that PRP was used as a substitute for the components of the standard culture medium. Whereas, in the study presented above, platelets were introduced as an added component to the culture system.

Analysing the data obtained during the study, several features were noted. In Group 5 (100×10^6 platelets/cm^3), a decrease in the percentage of blastulation and embryo quality was observed, which is probably caused by a high platelet count in the medium. DeLong *et al.* (2012) claim that excessive platelet counts can lead to cellular apoptosis, suppression, and desensitisation

of growth factor receptors, and have an inhibitory effect. Tang *et al*. (2002) notes that a significant increase in the content of growth factors in the environment also inhibits the development of the embryo. At that time, the quality of embryos in Groups 2, 3 and 4 was higher than in the first (control), which may be related to growth factors released by platelets (platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), epithelial growth factor (EGF), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), fibroblast growth factor (FGF), haepatocyte growth factor (HGF)) (Drago *et al*., 2013). Lange-Consiglio *et al.* (2015) believe that biologically active substances released by platelets during activation can stimulate the development of bovine embryos and the proliferation of trophoblast cells during cultivation *in vitro*. Larson *et al.* (1992) point out that after platelet aggregation, which occurs when they are added to the culture medium, some glycoproteins and fibronectin are released, providing the extracellular matrix necessary for embryo development to the blastocyst stage.

CONCLUSIONS

Studies of the effect of platelets on the preimplantation development of bovine embryos indicate that the latter can also affect the percentage of blastulation and cell proliferation. The authors suggest that blood plates introduced into the culture system before embryos enrich the environment with factors necessary for its development. However, different platelet concentrations in the culture system cause divergent effects. Thus, with the addition of $10\times10^6/cm^3$ platelets to the medium, an increase in the percentage of blastulation by 2.8%, cell proliferation by 16.0% ($P<0.001$), and the size of blastocysts by 4.2% ($P<0.01$) was noted, compared with control. With an increase in the concentration of platelets to $20\times10^6/cm^3$, improvement in the development indicators of bovine embryos was observed, compared with the $10\times10^6/cm^3$ group. The best concentration is 50×10^6 platelets/cm^3, which allows maximising the number and improving the quality of bovine embryos produced *in vitro*. Thus, at the specified concentration of blood plates, the percentage of blastulation increased by 13.9% ($P<0.01$), the number of cells in the blastocyst – 29.5% ($P<0.001$), and the size of blastocysts increased by 15.7% ($P<0.001$) compared to the group without platelet addition. At that time, the addition of 100×10^6 platelets/cm^3 leads to a sharp decrease in all the indicators under study, e.g., the average number of cells in a blastocyst decreased by 11.7% ($P<0.001$), and its average size – by 3.4% ($P<0.05$), compared to control.

This study offers a new strategy for *in vitro* embryo culture and opens the possibility of using platelets in future assisted reproductive technology programs in veterinary medicine as a way to increase the blastulation rate and the quality of the embryos obtained.

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Вплив концентрату тромбоцитів на розвиток ембріонів великої рогатої худоби у системі *in vitro*

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Анотація. Існує велика кількість досліджень щодо удосконалення умов культивування ембріонів великої рогатої худоби *in vitro*. Проте, питання співкультивування тромбоцитів та ембріонів корів мало вивчене, хоча активація тромбоцитів призводить до вивільнення у культуральне середовище значної кількості біологічно активних речовин і їх співкультивування з ембріонами може позитивно вплинути на ріст і розвиток останніх. Тому, метою роботи було дослідження впливу різних концентрацій тромбоцитів у культуральній системі *in vitro* на розвиток ембріонів корів. Запліднені зиготи (загальна кількість 180) поділили на 5 груп: 1 група – середовище для культивування без додавання тромбоцитів (контроль); 2 – середовище для культивування з додаванням 10×10^6 тромбоцитів/см^3; 3 – кінцева концентрація у системі 20×10^6 тромбоцитів/см^3; 4 – 50×10^6 тромбоцитів/см^3; 5 група – 100×10^6 тромбоцитів/см^3. За результатами встановлено, що співкультивування ембріонів з тромбоцитами у системі in vitro є ефективним. Так, відмічали кореляцію покращення показників розвитку ембріонів зі збільшенням концентрації тромбоцитів. Оптимальною концентрацією тромбоцитів виявилася 50×10^6/см^3, що дозволило отримати на 13.9% вищий рівень бластуляції, на 15.7% – середній розмір ембріонів і на 2.5% – середню кількість клітин в бластоцисті, порівнюючи з контролем. У той час концентрація тромбоцитів 100×10^6/см^3 призвела до достовірного зниження досліджуваних показників, у порівнянні з групою без тромбоцитів. Отже, співкультивування ембріонів корів з тромбоцитами є доцільним, адже дозволяє покращити показники розвитку ембріонів корів. Проаналізовані та представлені в статті дані дозволять підвищити ефективність культивування ембріонів великої рогатої худоби як в наукових, так і виробничих цілях

Ключові слова: біотехнологія відтворення жуйних, фактор росту, ділення, бластоциста

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Exterior of Cows of the Ukrainian Black-Spotted Dairy Breed, Obtained under Various Selection Options

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Abstract. Analysis of the effectiveness of evaluating inline and inter-line variants of combinations by type and finding their best variants in practical selective breeding is a relevant task that allows obtaining offspring of the desired quality. The purpose of this study is to figure out the specific features of the body structure of the firstborn cows of the Ukrainian black-spotted dairy breed of the western inbred type, depending on their origin, as well as to establish the type of inheritance of these indicators in line crosses based on the results of the evaluation by type. The study found the types of selection through genealogical analysis of pedigrees, linear evaluation of cows by type according to two evaluation systems – linear description of individual articles of the exterior on a 9-point scale and evaluation of complexes of exterior features of the animal type on a 100-point scale, obtained results were evaluated according to Student's t-test. Using the scheme, it was found that the firstborn cows obtained as a result of the inline selection are characterized by optimal scores by type. However, animals from the Valianta 1650414 line received the highest ratings for linear traits of height at the withers, width of chest and rear, body depth. The study analysed the evaluation of firstborn cows by type, obtained in the interline selection, and it was found that the indicators of linear evaluation by type of cross lines correspond to and prevail over the average values for the breed. Cross cows ♂Valianta 1650414 x ♀Eleveishna 1491007 received the highest score for milk type on a 100-point scale. It was proved that most of the evaluated traits in line crosses were inherited according to the intermediate type. However, in the cross ♂Chifa 1427381 x ♀Eleveishna 1491007, the evaluation of individual traits (height, chest width, angle of withers, rear attachment, and depth of udder) statistically probably exceeded the values of the corresponding evaluations of the firstborns of the paternal and maternal lines, and the dominance of the maternal line was observed in the cross-breed cows ♂Chifa 1427381 x ♀Valianta 1650414 according to the value of the assessment of individual udder traits. The obtained results will allow performing optimal selection of parent pairs from animals evaluated by type for selection and breeding work with cattle herds to consolidate them by type

Keywords: cows, firstborn cows, exterior assessment, breeding selection, cross lines, inheritance

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INTRODUCTION

Currently, the main development vector in the field of dairy cattle breeding is the creation and consolidation of highly productive breeding herds of intensive type, suitable for keeping in the conditions of industrial technology. To solve the set tasks successfully, apart from breeding for increasing the amount of milk yield and milk quality, it is important to evaluate the exterior as a complex of economic and useful features directly related to the level of milk productivity. Presently, the exterior in Ukraine is assessed based on methodological guidelines for the linear classification of dairy and milk-meat cows by type, developed by Ukrainian scientists-breeders, per the ICAR requirements (Khmelnychyi *et al*., 2016).

When breeding the Western inbred type, bulls of American and Canadian breeding, as well as Ukrainian breeding, belonging to different genealogical lines and having different proportions of Holstein blood were used. As a result, there is a certain level of phenotypic variability in both the exterior and productivity of cows in breeding herds. Analysis of the effectiveness of various selection options, considering the linear affiliation of parent pairs, is a relevant condition for building a strategy to further improve breeding and productive qualities of the Western inbred type of the Ukrainian black-spotted dairy breed.

In cattle breeding, one of the important methods is the breeding of animals along lines, which aims to consolidate and reproduce valuable traits of outstanding breeding bulls in the offspring. For this purpose, various selection options are used, considering the lineal affiliation of the animals, namely: inline selection (parental pairs belong to the same line) and inter selection or line crossing (parental pairs belong to different lines). Each of these methods has its own particular tasks, which are planned to be achieved by breeders, and is based on the assumption that the inheritance of the majority of economically useful traits in dairy cattle breeding occurs because of the additive effect of genes. Currently, a considerable number of studies have been conducted to figure out the productivity and reproductive capacity of cows of Ukrainian black- and red-spotted dairy breeds obtained from separate variants of selection of pairs by belonging to traditional lines of the Holstein breed (Khmelnychyi & Salogub, 2019; Khmelnychyi & Loboda, 2019; Khmelnychyi & Bondarchuk, 2019). The analysis of the obtained data suggests that the effectiveness of such methods in individual herds may differ substantially. Therefore, it is necessary to constantly monitor the effectiveness of using certain selection options to achieve the tasks set in each particular case. Having identified successful combinations, one can use their best options in further breeding work.

The use of linear evaluation by type is a valuable tool of breeders, which allows clearly formulating and purposefully influencing the formation of the desired animal type and the consolidation of breeding herds, lines, breeds, and inbred types (Boiko *et al*., 2017; Khmelnychyi, 2017; Pomitun *et al*., 2020). The indicators of linear type assessment depend on the assessed breed, the farm where such assessment was conducted, the qualifications and experience of the expert conducting such assessment (Khmelnychyi *et al*., 2019; Loboda & Bardash, 2019; Palii *et al*., 2020). The effect of heredity on the results of a linear type assessment is evidenced by the fact that its results can depend both on paternal origin and on belonging to a certain genealogical line (Iliashenko, 2017; Hetia *et al*., 2020). In the vast majority, the results of the assessment of descriptive traits are marked by probable inheritance coefficients (Salohub *et al*., 2018; Hakim *et al*., 2020; Karpenko, 2020; Ismael *et al*., 2021). It is established that many of the evaluated exterior traits are positively correlated with productivity and longevity indicators (Alimzhanova *et al*., 2018; Cherniak & Honcharuk, 2018; Iliashenko, 2018). A positive relationship between udder parameter assessment indicators and milk yields is found especially often (Djedović *et al*., 2020). To varying degrees, the evaluated features also correlate with each other and have high variability (Rovegliaa *et al*., 2019; Török *et al*., 2021; Khmelnychyi & Kagrepko, 2021).

The purpose of this study: using the method of linear evaluation by type, to figure out the external characteristics of the firstborn cows of the Ukrainian black-spotted dairy breed of the Western inbred type, obtained by inline and interline selection and to establish the type of inheritance of these indicators in line crosses.

MATERIALS AND METHODS

The study was conducted on cows of the Western inbred type of the Ukrainian black-spotted dairy breed in the Radekhivske Breeding Plant of the Radekhiv District of the Lviv Oblast. The authors used information from pedigree records provided in individual cards of breeding cows (Form 2-MOL) and their research. Using cards of breeding cows, the pedigrees of animals of various combinations were analysed and, accordingly, the types (variants) of selection were determined by pertinence to genealogical lines. Types of selection were figured out by genealogical analysis of animal pedigrees for 4 rows of ancestors. Selection methods were grouped according to the following scheme: inline selection – father and mother of the cow pertained to the same genealogical line; interline selection – father and mother of the cow pertained to different lines.

Linear assessment of firstborn cows by type was performed following the instructions of the method of linear classification of cows of dairy and dairy meat breeds by type (Khmelnychyi *et al*., 2016). According to the method, cows were evaluated at 2-4 months of the first lactation, on a 9- and 100-point scale. According to the 9-point scale, 17 exterior articles were evaluated, where the animal received 5 points for the average

expression of the trait. For deviations towards minimal development, the score was reduced to one point, with the maximum expression of the trait, a score of 9 points is possible.

According to the 100-point classification system, four sets of selection traits were considered, which characterize: milk type pronouncement, body development, the condition of the limbs and the morphological qualities of the udder. A 100-point score is completely subjective. According to the requirements specified in the method, the highest score for firstborn cows can be 89 points. Each group of exterior articles (complex) is evaluated independently of the others. Weight coefficients in the overall assessment of complex traits are as follows: milk type – 15%, trunk – 20%; limbs – 25%, udder – 40%. Based on the assessed features of the type, the overall rating is found according to the formula: OR=(MT·0.15)+(B·0.20)+(L·0.25)+(U·0.40), where OR is the overall rating of complex (group) type features, MT – score for milk type, B – score for body, L – score for limbs and legs, U – score for udder. Therewith, when evaluating the dairy type of animals, attention should be paid to the proportionality of the development of individual parts of the body. The cow should have a pronounced milk type (light head, long neck, flat, long ribs). When evaluating the torso, its strength, height, depth, and length are considered. When evaluating limbs and hooves, the condition of the forelimbs and hind limbs, the strength of the hock joint, and the condition of the hooves are considered. When evaluating the udder, the shape of the udder, the pronouncement of the milk veins, and the size, shape, and diameter of the teats are considered. The probability of the results obtained is estimated based on the student's t-test, where * – $p<0.05$, ** – $p<0.01$, *** – $p<0.001$.

RESULTS AND DISCUSSION

The results of studies on the indicators of linear assessment of firstborn cows by type obtained upon inline selection are presented in Table 1.

***Table 1**. Linear assessment of milk type, torso, limbs, and fatness in firstborn cows with inline selection, points (M±m)*

Indicators	Lines		
	Chifa 1427381	Valianta 1650414	Eleveishna 1491007
Number of animals, heads	32	37	42
Height	5.8±0.09	6.2±0.13	6.0±0.13
Chest width	6.4±0.10	7.0±0.11	6.5±0.11
Body depth	6.3±0.12	6.9±0.10	6.6±0.10
Milk type (angularity)	6.8±0.08	7.1±0.07	6.9±0.08
Rear tilt	5.2±0.11	5.0±0.08	5.3±0.11
Rear width	5.6±0.07	5.8±0.10	5.5±0.07
Pelvic limb angle	5.0±0.09	5.1±0.09	5.2±0.12
Pelvic limb posture	5.6±0.11	5.5±0.09	5.5±0.11
Hoof angle	5.0±0.10	5.2±0.11	5.1±0.10
Fatness	5.0±0.10	4.9±0.10	5.3±0.09

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

The analysis of the indicators presented in Table 1 suggests that during the inline selection of the firstborn of the Valiant 1650414 line, the highest evaluations of growth, chest width, body depth, milk type, and rear width were noted. According to these indicators, they outnumbered the firstborn from the Chifa 1427381 line by 0.4, 0.6, 0.6, 0.3, and 0.2 points, respectively. The first four differences are statistically significant ($p<0.05$–0.001), and the last difference is within the statistical error range. Compared to cows of the Eleveishna 1491007 line, they had a substantial predominance in terms of chest width (0.5 points, $p<0.01$), body depth and rear width (0.3 points each, $p<0.05$). According to the posterior slope assessment, the difference between the firstborn Valiant 1650414 and Eleveishna 1491007 was 0.3 points in favour of the latter ($p<0.05$). Scores of the angle and posture of the pelvic limbs, as well as the hoof angles, did not significantly differ in firstborn cows of the Chifa 1427381, Valiant 1650414, and Eleveishna 1491007 lines obtained upon inline selection. The highest fatness rating was given to cows of the Eleveishna 1491007 line, which outnumbered cows of the Chifa 1427381 and Valianta 1650414 lines by 0.3 and 0.4 points, respectively ($p<0.05$ and $p<0.01$).

The results of the evaluation of firstborn cows by type in Chifa 1427381, Valianta 1650414, and Eleveyshna 1491007 lines, obtained upon inline selection, suggest that certain exterior features inherent in each of the lines were discovered. According to these indicators, the firstborns of the Valianta 1650414 line have features

inherent in animals of the intensive milk type – they are tall, have wide chests and a deep body, which indicates a good development of internal organs (heart, lungs, digestive tract) and the ability to consume, assimilate, and process into milk sufficient amounts of bulk feed. They are not prone to the deposition of subcutaneous fat, as evidenced by the assessment of fatness. No significant differences were found between the animals of the Chifa 1427381 and Eleveishna 1491007 lines in terms of the magnitude of the exterior assessment.

In general, with inline selection in the Chifa 1427381, Valiant 1650414, and Eleveishna 1491007 lines, all firstborn cows in terms of height, chest width, body depth, milk type pronouncement, rear tilt and width, angle and posture of pelvic limbs and hoof angle received positive ratings that correspond to the average level (5 points) or predominate it.

Udder assessment is one of the key elements of linear classification of cows by type in the dairy and dairy-meat areas of productivity. Based on the characteristics of the development of the dairy system, it is possible to figure out the suitability of a cow for operation in the conditions of an industrial milk production system, the ability to produce large volumes of milk, and the tendency to injury and morbidity of the udder. The results of evaluation of the udder of firstborn cows obtained upon inline selection are presented in Table 2.

***Table 2**. Linear udder assessment in firstborn cows upon inline selection, points (M±m)*

Indicators	Lines		
	Chifa 1427381	Valianta 1650414	Eleveishna 1491007
Number of animals, heads	32	37	42
Anterior udder attachment	5.4±0.13	6.7±0.07	6.0±0.07
Posterior udder attachment	5.2±0.06	5.6±0.10	5.2±0.09
Central ligament	5.3±0.08	6.4±0.12	5.4±0.08
Udder depth	5.2±0.09	6.6±0.06	6.5±0.06
Placement of anterior teats	5.2±0.11	5.0±0.08	5.1±0.04
Placement of posterior teats	5.1±0.10	5.0±0.11	5.0±0.03
Length of teats	5.6±0.10	5.5±0.03	5.4±0.03

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

It was found that firstborn cows of the Valiant 1650414 line were characterized by the highest udder evaluation indicators, namely: according to the assessment of anterior udder attachment and central ligament, they outnumbered cows of the Chifa 1427381 and Eleveishna 1491007 lines by 1.3 and 0.7, and 1.1 and 1 points, respectively, in all cases $p<0.001$; according to the assessment of posterior udder attachment, their advantage was 0.4 points in both cases, $p<0.01$; according to the udder depth score, cows of the Chifa 1427381 line predominate with hight significance (1.4 points, $p<0.001$).

Furthermore, the firstborn of the Chifa 1427381 line had lower scores of anterior udder attachment and its depth than the peers of the Eleveishna 1491007 line. The differences were 0.6 and 1.3 points, respectively, $p<0.001$.

The results of estimating the udder depth indicate that in cows of the compared groups, this indicator was optimal for suitability for machine milking. Thus, firstborn cows from the Valiant 1650414 line, being the tallest, had a more elevated udder from the level of the hock joint. They have better developed chest and rear width, body depth, and a well-pronounced milk type.

The assessment of complex (group) type traits upon inline selection (Table 3) showed that according to all group articles and general assessment, the animals corresponded to the "good" and "good plus" classes per the international requirements.

***Table 3**. Linear assessment of complex (group) characteristics of experimental cows by type upon inline selection, points (M±m)*

Indicators	Lines		
	Chifa 1427381	Valianta 1650414	Eleveishna 1491007
Number of animals, heads	32	37	42
Milk type	79.1±0.15	82.2±0.11	82.0±0.08
Body	83.5±0.12	83.2±0.09	82.8±0.15
Limbs and hooves	84.0±0.10	82.0±0.11	82.0±0.16
Udder (milk system)	81.5±0.13	83.0±0.13	83.6±0.14
Overall rating	82.1±0.12	82.6±0.12	82.7±0.11

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

The score of complex traits of the dairy type was highest in cows from the Valiant 1650414 line, which according to this indicator statistically significantly outnumbered cows of the Chifa 1427381 line (p<0.001). Compared to the animals of the Eleveishna 1491007 line, their advantage was not significant. Group body traits in cows of the compared lines were rated "good plus", but the highest score was given to firstborn cows from the Chifa 1427381 line. They statistically significantly prevailed in this indicator of cows of the Eleveishna 1491007 line (p<0.01). The condition of the pelvic and thoracic limbs was assessed with higher scores in cows from the Chifa 1427381 line. Their superiority over their peers of the Valiant 1650414 and Eleveishna 1491007 lines was statistically highly significant (p<0.001). The highest udder score was given to cows from the Eleveishna 1491007 line, they outnumbered the peers of the Chifa 1427381 and Valiant 1650414 lines by 2.1 and 0.6 points, respectively (p<0.01). The overall score was lowest for cows of the Chifa 1427381 line, they were inferior to cows of the Valiant 1650414 and Eleveishna lines 1491007, respectively, by 0.5 and 0.6 points (p<0.01). Indicators of linear assessment of firstborn cows obtained as a result of crossing different lines are presented in Table 4.

***Table 4**. Linear assessment of milk type, body, limbs, and fatness in firstborn cows upon inline selection, points (M±m)*

Indicators	Line combination				
	♂Chifa 1427381 x ♀Eleveishna 1491007	**♂Chifa 1427381 x ♀Starbaka 352790**	**♂Chifa 1427381 x ♀Valianta 1650414**	**♂Valianta 1650414 x ♀Eleveishna 1491007**	**♂Starbaka 352790 x ♀Valianta 1650414**
Number of animals, heads	70	82	94	114	68
Height	6.4±0.03	5.8±0.06	5.9±0.08	6.3±0.09	6.5±0.04
Chest width	6.8±0.10	6.4±0.12	6.6±0.11	6.8±0.12	6.9±0.07
Body depth	6.6±0.09	6.2±0.04	6.4±0.13	7.0±0.06	7.2±0.10
Milk type	6.6±0.11	7.0±0.03	7.0±0.07	7.2±0.05	6.9±0.09
Rear tilt	5.2±0.06	5.1±0.11	4.9±0.05	5.2±0.10	5.0±0.11
Rear width	5.8±0.09	6.0±0.11	6.2±0.06	6.0±0.11	6.1±0.10
Pelvic limb angle	5.1±0.08	5.4±0.10	5.5±0.11	5.0±0.08	5.3±0.02
Pelvic limb posture	5.6±0.06	5.5±0.08	5.8±0. 02	5.6±0.09	5.7±0.09
Hoof angle	5.4±0.08	5.5±0.11	5.6±0.05	5.3±0.13	5.1±0.07
Fatness	4.9±0.09	4.8±0.10	4.8±0.04	5.0±0.11	5.0±0.05

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

It was found that cows obtained upon inline selection also have quite positive indicators of exterior assessment. All evaluated firstborns are tall. The greatest height in the sacrum is found in descendants from the combination of the lines ♂Starbaka 352790 x ♀Valiant 1650414, ♂Chifa 1427381 x ♀Eleveishna 1491007, and ♂Valianta 1650414 x ♀Eleveishna 1491007. Differences in this indicator with cows originating from the crosses of the lines ♂Chifa 1427381 x ♀Starbaka 352790, ♂Chifa 1427381 x ♀Valianta 1650414 are statistically significant, p<0.05. Evaluated cows have a deep body with a wide chest. The greatest chest width rating was given to cows from ♂Starbaka 352790 x ♀Valianta 1650414, ♂Valianta 1650414 x ♀Eleveishna 1491007, and ♂Chifa 1427381 x ♀Elewayshna 1491007 crosses, and the lowest – from the ♂Chifa 1427381 x ♀Starbuck 352790 crosses. Similar differences are observed in the body depth. Furthermore, in the evaluated firstborns, obtained during interline selection, the milk type (angularity) of body structure forms is well-pronounced. Its rating is slightly higher than the average value. It is the lowest in cows of the ♂Chifa 1427381 x ♀Eleveishna 1491007 cross and is 6.6 points, in other groups of animals under study it was within 6.9-7.2 points. The tilt and width of the rear are generally evaluated as the best option in all cases. As for the limbs, they are postured straight with the optimal angle when viewed from the side. In cows of all the compared groups, the score of limbs, including the hoof angles, has average values with small deviations towards increase. The fatness rating is within 4.8-5.0 points.

In terms of the strength of attachment of the anterior part of the udder (Table 5), the highest rating was given to cows from the ♂Chifa 1427381 x ♀Valianta 1650414 and ♂Starbaka 352790 x ♀Valianta 1650414 crosses. According to this indicator, they outnumbered cows from other crosses by 0.3-0.7 points (p<0.01). A similar pattern is observed in the assessment of attachment of the posterior part of the udder. It was found that cows of the ♂Chifa 1427381 x ♀Valianta 1650414 cross received 6.1 points for central ligament, which is 0.2-0.9 points more than those of the same age as other crosses, or 3.4-17.3%, at p<0.01.

***Table 5**. Linear udder assessment in firstborn cows upon interline selection, points (M+m)*

Indicator	Line combination				
	♂Chifa 1427381 x ♀Eleveishna 1491007	♂Chifa 1427381 x ♀Starbaka 352790	♂Chifa 1427381 x ♀Valianta 1650414	♂Valianta 1650414 x ♀Eleveishna 1491007	♂Starbaka 352790 x ♀Valianta 1650414
Number of animals, heads	70	82	94	114	68
Anterior udder attachment	5.9±0.12	6.2±0.12	6.6±0.04	6.1±0.10	6.5±0.04
Posterior udder attachment	5.6±0.11	5.8±0.08	6.0±0.04	5.7±0.11	5.7±0.02
Central ligament	5.2±0.08	5.6±0.06	6.1±0.03	5.8±0.11	5.9±0.03
Udder depth	6.7±0.05	6.8±0.08	6.6±0.12	6.5±0.05	6.6±0.04
Placement of anterior teats	5.1±0.05	5.2±0.09	5.0±0.12	5.3±0.06	5.2±0.04
Placement of posterior teats	4.9±0.03	5.0±0.04	5.0±0.11	5.1±0.06	5.1±0.09
Length of teats	5.0±0.04	4.9±0.05	4.9±0.07	5.0±0.06	5.0±0.02

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

The bottom of the udder is raised quite high above the hock joint in animals of all crosses, as evidenced by the results of assessing the depth of the udder. According to this indicator, the daughters of bulls of the Chifa 1427381 line from cows of the Starbaka 352790 and Eleveishna 1491007 lines received the highest score. Evaluation of the placement and length of teats indicates the optimal values of these indicators in cows of the compared groups and their good adaptability to machine milking.

Animals of the ♂Chifa 1427381 x ♀Valianta 1650414 cross were distinguished by the highest comprehensive score (Table 6) of the "good plus" class, which exceeded the animals of other groups by 1.4-3.4 points ($p<0.001$). Cows of the ♂Chifa 1427381 x ♀Eleveishna 1491007 cross had the lowest overall score. They were 0.9-3.4 points inferior in this indicator to their peers derived from other interline selection variants, all differences are statistically significant, $p<0.001$.

***Table 6**. Linear evaluation of complex (group) traits of experimental cows by type upon interline selection, points (M±m)*

Indicators	Line combination				
	♂Chifa 1427381 x ♀Eleveishna 1491007	♂Chifa 1427381 x ♀Starbaka 352790	♂Chifa 1427381 x ♀Valianta 1650414	♂Valianta 1650414 x ♀Eleveishna 1491007	♂Starbaka 352790 x ♀Valianta 1650414
Number of animals, heads	70	82	94	114	68
Milk type	79.5 ± 0.11	83.5 ± 0.14	83.6 ± 0.11	84.3 ± 0.13	84.0 ± 0.16
Body	78.8 ± 0.12	80.0 ± 0.12	85.1 ± 0.14	80.0 ± 0.14	84.4 ± 0.15
Limbs and hooves	79.0 ± 0.10	79.5 ± 0.12	80.0 ± 0.10	81.0 ± 0.11	80.0 ± 0.14
Udder (milk system)	79.5 ± 0.08	81.2 ± 0.07	82.4 ± 0.12	78.4 ± 0.12	79.0 ± 0.13
Overall rating	79.1 ± 0.12	80.8 ± 0.10	82.5 ± 0.13	80.0 ± 0.10	81.1 ± 0.12

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

In general, the results of linear assessment of complex traits of firstborn cows by type according to the 100-point system found that in experimental animals obtained with both interline and inline selection options, the average score level was 82.6-83.9 points, which corresponds to the "good plus" criterion. Therewith, firstborn cows received an average score of 82.6-84.3 for milk type, body – 83.2-85.0, limbs and hooves – 82.0-83.0, udder or dairy system – 83.0-84.0 points.

The phenotypic manifestation of exterior characteristics of cows is formed as a consequence of the interaction of hereditary and paratypical factors and is mainly controlled by additive genes. Proceeding from this, one can expect that upon crossing lines, the indicators of evaluating cows by type will have values equal to the half-sum of the values of the parent lines. However, the factual assessment of some traits in cows originating from cross lines does not always follow this rule. Figure 1 shows the deviation of the assessment of the firstborns of the line crosses under study by type from the average indicator of peers of the corresponding lines, obtained upon inline selection in percentage.

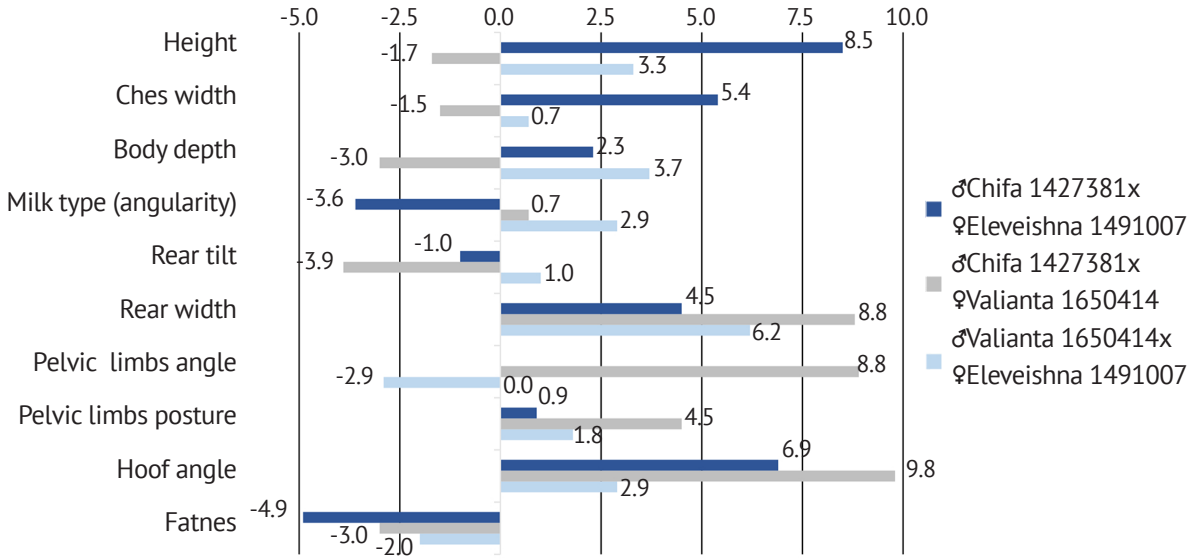

***Figure 1**. The correlation of scores for the milk type, body, limbs, and fatness in firstborn cows upon interline selection and an intermediate indicator of the peers of parental lines, %*
***Source:** compiled by the authors*

The analysis of the data presented in Figure 1 suggests that the firstborn offspring from the ♂Chifa 1427381 x ♀Eleveyshna 1491007 cross, in terms of growth, outweigh the value of the half-sum of the score of peers from inline selection in Chifa 1427381 and Eleveyshna 1491007 lines by 8.5%. Furthermore, their superiority in factual values over the firstborns of the parental lines is statistically significant (Chifa 1427381 – $p<0.001$; Eleveishna 1491007 – $p<0.01$). By analogy, statistically significant differences are observed in the scores of chest width and hoof angle ($P< 0.05$-0.01). According to the rear width score, the advantage is significant only over the peers of the Eleveishna 1491007 line ($p<0.05$), and the latter substantially outweigh them in the fatness score ($p<0.01$). Scores of other animal traits originating from the ♂Chifa 1427381 x ♀Eleveishna 1491007 cross differ from the half-sum values of the parent lines not significantly, i.e., they are inherited according to an intermediate type.

In firstborns from the ♂Chifa 1427381 x ♀Valiant 1650414 cross, the assessment of such features as the rear width, the angle and posture of the pelvic limbs, the hoof angle is more than the half-sum of the ratings of peers of the parental lines by 8.8, 8.9, 4.5, and 9.8%, respectively. According to the factual scores of the rear width, the angle of the pelvic limbs and hooves, they statistically significantly predominate cows of the Chief 1427381 and Valiant 1650414 lines ($p<0.01–0.001$), according to the assessment of the posture of the hind limbs – cows of the Valiant 1650414 line ($p<0.01$). All other differences are not significant.

Firstborns from the ♂Valianta 1650414 x ♀Eleveishna 1491007 cross have the highest score from the half-sum of ratings of peers of parental lines only based on the rear width, which is 6.2%. When comparing factual scores, they statistically significantly predominate the cows of the Eleveishna 1491007 line in this feature ($p<0.01$). Scores of all other traits deviate slightly from the intermediate value of the parent lines.

The results of the udder assessment (Fig. 2) suggest that firstborns of the ♂Chifa 1427381 x ♀Eleveishna 1491007 cross received a higher score for posterior attachment and udder depth, respectively, by 7.7% and 14.5% than the half-sum of similar scores of peers in the parent lines. According to these indicators, they were statistically significantly superior to cows from inline selection in both the Chifa 1427381 line and the Eleveishna 1491007 line ($p<0.05$-0.001). The firstborn cows of ♂Chifa 1427381 x ♀Valianta 1650414 cross, according to the assessment of the front and rear attachment of the udder, as well as its depth, exceeded the average values of the parent lines by 9.1%; 11.1%, and 11.9%, respectively. Therewith, this excess over the factual scores of these features over the firstborns of the Chifa 1427381 line was statistically significant in all three cases ($p<0.01$-0.001), over the firstborns of the Valianta 1650414 line – only in relation to the assessment of posterior udder attachment ($p<0.01$). Cows of ♂Valianta 1650414 x ♀Eleveishna 1491007 cross, based on the evaluation of the rear attachment of the udder and placement of the front teats, prevailed over the average indicator of the parental forms by 5.6% and 5.0%, respectively. There was a statistically significant difference only in the assessment of posterior udder attachment with cows of the Eleveishna 1491007 line ($p<0.001$). According to the estimation of udder length, the firstborns from all evaluated variants of inline selection significantly outnumbered cows obtained upon crossing lines ($p<0.01–0.001$).

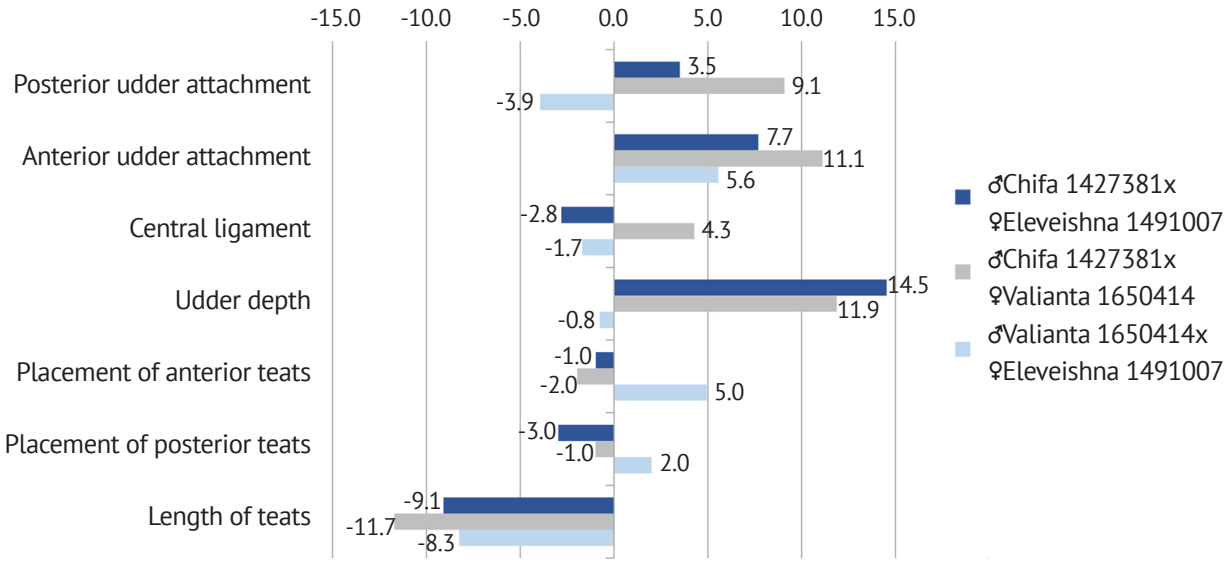

***Figure 2**. The correlation of udder scores in firstborn cows of inline selection and an intermediate indicator of the peers of parental lines, %*

Cows of the Valianta 1650414 line obtained upon inline selection were marked by the highest scores of udder attachment (anterior and posterior), central ligament, and udder depth compared to the same cows of the Chifa 1427381 and Eleveishna 1491007 lines. In cows of ♂Chifa 1427381 x ♀Valianta 1650414 cross, the value of these udder indicators shows a deviation from the intermediate nature of their inheritance and a certain level of dominance of the maternal line.

Pertinence of cows to a certain line of origin substantially affects the level of phenotypic variability of exterior features, which is manifested in the results of linear assessment of cows by type. The influence of lineal pertinence on the development of individual articles of the body of firstborn cows is substantial and predominantly reliable, it ranges within 3-36% (Bazyshyna, 2017; Khmelnychyi, 2017; Hladii & Polupan, 2018). This explains the differences between the results of linear evaluation of firstborns by type, obtained with different options of selection – inline and line crosses.

Similar results regarding the linear evaluation by type were obtained in studies conducted on firstborn cows of the Sumy inbred type of the Ukrainian black-spotted dairy breed. It was found that the highest score for both complex and descriptive traits was in cows of the Valianta 1650414 line, obtained during inline selection and Valianta 1650414 x S.T. Rokita 252803 and Valianta 1650414 x Kheneve 1629391 crosses (Khmelnychyi & Bondarchuk, 2019). The firstborns of these lines were marked by the highest coefficients of phenotypic consolidation according to the descriptive characteristics of body depth, angularity, rear width, posture of the hind limbs and attachment of the front lobes of the udder (Khmelnychyi *et al*., 2019). In this study, the positive effect of pertinence to the Valianta 1650414 line on the evaluation results was observed both upon inline selection and upon crossing lines.

The analysis of the results of evaluation by type for the cows of the Ukrainian black-spotted dairy breed of lines Chifa 1427381, Eleveyshna 1491007, Starbaka 352790, and Valianta 1650414, obtained upon inline and interline selection, revealed that the best scores according to group and descriptive traits were obtained when selecting Starbaka 352790 x Starbaka 352790, Eleveishna 1491007 x Starbaka 352790, Valianta 1650414 x Starbaka 352790. The offspring of the Chifa 1427381 line had the lowest scores both in inline selection and in all cross variants (Kochuk-Iashchenko, 2017). Similarly, the results presented by the authors of this paper show that the offspring from inline selection in the Chifa 1427381 line and the Chifa 1427381 x Eleveishna 1491007 cross had the lowest results for some complex and descriptive traits.

The necessary materials for a comparative analysis of the results regarding the nature of the inheritance of the scores of descriptive traits of firstborn cows were not found in available scientific publications. There are published research results that establish that 65.3% of Holstein cows had an intermediate type of inheritance of breeding value based on milk yield. Upon comparing inline and interline selection, it was found that 56.7% and 70.6% of cows had an intermediate type of inheritance, respectively. Upon the inline selection, parental heredity dominated in 20.7% of animals, and upon the interline selection – only 1.9%. The authors concluded that upon inline selection, the influence of parental heredity is higher than upon crossing lines (Babenko & Klopenko, 2017). An analysis of the specific features of inheritance of breeding value based on the milk yield of Holstein bulls found that 82.2% of the bulls had

a phenotypic manifestation of the additive form of inheritance (Kruhliak & Kruhliak, 2021). In the presented studies, it was proven that the intermediate type of inheritance was present in 65% of the descriptive traits upon crossing lines ♂Chifa 1427381 x ♀Eleveyshna 1491007, 75.6% – ♂Chifa 1427381 x ♀Valianta 1650414, and 94.1% upon crossing lines ♂Valianta 1650414 x ♀Eleveishna 1491007.

CONCLUSIONS

Upon inline selection, cows of the Valianta 1650414 line had the highest scores of height, chest width, body depth, milk type pronouncement, front and rear attachment of the udder, central ligament and udder depth, and were significantly superior in these features to the cows of the Eleveyshna 1491007 and Chifa 1427381 lines.

The highest values of growth, chest width, and body depth were observed in cows of the ♂Starbaka 352790 x ♀Valianta 1650414, ♂Chifa 1427381 x ♀Eleveishna 1491007, and ♂Valianta 1650414 x ♀Eleveishna 1491007 crosses, the best score for milk type was given to cows of ♂Chifa 1427381 x ♀Starbaka 352790 cross.

The highest score for udder attachment (front and rear) and central ligament was obtained by firstborn bulls of the Chifa 1427381 and Starbaka 352790 lines with cows of the Valianta 1650414 line, and the highest score of udder depth was obtained by daughters of bulls of the Chifa 1427381 line from cows of the Starbaka 352790 and Eleveishna 1491007 lines. The placement and length of the teats had optimal values in cows of all the compared groups.

In the firstborn cows obtained with all the compared selection variants, the average level of the linear evaluation of complex traits by type according to the 100-point system was 82.6-83.9 points, which corresponds to the "good plus" criterion. The vast majority of evaluated traits (64.7-94.1%) in line crosses had an intermediate nature of inheritance.

Inheritance of scores of height, chest width, hoof angle, rear attachment and depth of udder in ♂Chifa 1427381 x ♀Eleveishna 1491007 cross, scores of rear width, angle of pelvic limbs and hooves and rear attachment of udder in ♂Chifa 1427381 x ♀Valianta 1650414 cross according to the heterosis type were statistically significantly ($p<0.05$-0.001) higher than the factual scores in parental lines. In the ♂Chifa 1427381 x ♀Valianta 1650414 cross, the score of udder attachment (anterior and posterior), central ligament, and udder depth indicates the dominance of the maternal line in terms of these traits.

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Екстер'єр корів української чорно-рябої молочної породи, отриманих за різних варіантів підбору

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Анотація. Аналіз ефективності оцінки внутрішньолінійних та міжлінійних варіантів поєднань за типом та виявлення найкращих їх варіантів у практичній селекційно-племінній є актуальним завданням, що дозволить отримувати нащадків бажаної якості. Мета роботи – на підставі результатів оцінки за типом корів-первісток української чорно-рябої молочної породи західного внутрішньопородного типу української чорно-рябої молочної породи визначити особливості будови їх тіла залежно від походження, а також встановити тип успадкування цих показників при кросах ліній. При проведенні досліджень визначали: типи підбору шляхом генеалогічного аналізу родоводів, лінійну оцінку корів за типом за двома системами оцінки – лінійний опис окремих статей екстер'єру за 9-бальною шкалою та оцінювання комплексів екстер'єрних ознак типу тварин за 100-бальною шкалою, отримані результати оцінювали за Стьюдентом. Використовуючи схему, було встановлено, що корови-первістки, отримані в результаті внутрішньолінійного варіанту підбору характеризуються оптимальними оцінками за тип, проте тварини з лінії Валіанта 1650414 отримали найвищі оцінки за лінійні ознаки висоти в крижах, ширину грудей та заду, глибину тулубу. Було проаналізовано оцінку за типом у корів-первісток, отриманих при міжлінійному варіанті підбору і встановлено, що за показниками лінійної оцінки за типом кроси ліній відповідають та переважають середні значення для породи. Корови кросу ♂Валіанта 1650414 х ♀Елевейшна 1491007 отримали найвищий бал за молочний тип по 100-бальній шкалі. Було доведено, що більшість із оцінюваних ознак при кросах ліній успадковувалися за проміжним типом, однак при кросі ♂Чіфа 1427381 х ♀Елевейшна 1491007 оцінка окремих ознак (ріст, ширина грудей, кут ратиць, заднє прикріплення та глибина вимені) статистично вірогідно перевищувала значення відповідних оцінок первісток батьківської і материнської лінії, а у корів кросу ♂Чіфа 1427381 х ♀Валіанта 1650414 за величиною оцінки окремих ознак вимені спостерігалося домінування материнської лінії. Отримані результати дозволять проводити оптимальний добір та підбір батьківських пар з оцінених за типом тварин, для проведення селекційно-племінної роботи із стадами ВРХ з метою консолідації їх за типом

Ключові слова: корови-первістки, оцінка екстер'єру, племінний підбір, крос ліній, успадкування

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Indicators of Immunity in Associated Mycotoxicosis of Cows

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Abstract. The issue of cattle reproduction was and still is one of the main tasks in cattle breeding. Losses of farms from infertility of cows are quite significant and range within 3.19-5.41 per 1 day of infertility. Mycotoxins produced by fungi of the *Fusarium* family, namely deoxynivalenol (DON) and zearalenone (ZEN) adversely affect not only the functioning of all organs and systems of the cow's body, but also produce an immunosedative effect. The purpose of this study was to establish the effect of the DON and ZEN complex on the main indicators of the immune response of cows and its correction in a comparative aspect using a feed additive based on zeolite and organic acids and recombinant α-, g-interferons. The study material was the blood of cows (serum and stabilized) sick with mycotoxicosis caused by the association of DON and ZEN. Methods used: photonephelometric using *E. coli* test culture, spontaneous rosette formation with sheep red blood cells according to M. Jondal, modified method of rosette formation according to M. Wansbrough-Jones, R. Limatibul's method, simple radial immunodiffusion in gel according to G. Mancini, precipitation in a polyethylene glycol solution according to M. Digeon. Experimental studies were performed on black-spotted cows in farms of the Sumy Oblast. The dynamics of the immune indicators of cows during the development of mycotoxicosis and upon treatment with products zeolite-based, organic acids, and an aqueous solution of recombinant α-, g-interferons were studied. It was found that the indicator of bactericidal, lysozyme, complementary, and phagocytic activity of cow blood serum under treatment increased to the indicator inherent in healthy animals. The dynamics of immunoglobulins in the treatment with zeolite and organic acids and recombinant α-, g-interferons was investigated, and an increase to the level of intact cows was established. It was proved that the indicators of the immune response of cows upon using a feed additive based on zeolite and organic acids at a dose of 2.5 kg per tonne of fodder and a preparation based on an aqueous solution of recombinant α-, g-interferons at a dose of 3 ml per animal were significantly higher

Keywords: cows, mycotoxicosis, zearalenone, deoxynivalenol, cow immunity, feed additives

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INTRODUCTION

Animal husbandry is currently facing such a phenomenon as mycotoxin damage to fodder in many regions of Ukraine. The economic losses suffered by milk production farms are quite significant and, according to the American method of determining the economic effect, range within $3.19-5.41 per day (Kibar *et al*., 2018). Improving existing and developing new methods for intensifying the reproductive capacity of cows is an urgent issue. It is known that mycotoxins, which are produced by fungi of the *Fusarium* family, such as deoxynivalenol and zearalenone, have a toxic effect on the body of cows (Gupta *et al*., 2022). These toxins are described by destructive changes in the intestine and suppress the body's immune reactions (Bulgaru *et al*., 2021). However, the effect on the organs of the immune system is insufficiently described.

The phenomenon is quite widespread in the world and scientists (Kemboi *et al*., 2020; Santos Pereira *et al*., 2019) are inclined to believe that the damage to feed by mycotoxins ranges within 42-65%. Nevertheless, the author (Bailey *et al*., 2019) points out damage to wheat and barley, but damage to feed mixture made from maize is not covered in this study. In the production of livestock products, it is mycotoxins that cause considerable economic losses, which can include reduced productivity of cows, insufficient production of offspring (Faisal *et al*., 2018) and premature culling of cows (Nichea *et al*., 2015). However, the research of the above-mentioned authors was conducted on Simmental and red-spotted cows, and other breeds of cows in other climatic zones can have quite substantial features of the course of mycotoxicosis. Researchers (Wang *et al*., 2019) developed a method for detecting mycotoxins in fodder and food products, which is based on the use of specific extremely sensitive monoclonal antibodies and solid-phase enzyme immunoassay. In addition, the studies (Wang *et al*., 2022; Wang *et al*., 2021) developed an express analysis based on two parallel specific immunochromatographic tests for the simultaneous detection of aflatoxin B1 (AFB1) and zearalenone (ZEN) in fodder.

Therewith, researchers (Clin *et al*., 2016; Barański *et al*., 2021) indicate that under the action of mycotoxins, the overall resistance of the body decreases, which leads to a violation of the reproductive ability of the body and infertility. Several *Fusarium* mycotoxins can alter various intestinal defence mechanisms, such as epithelial integrity, cell proliferation, mucus layer, immunoglobulin uptake, and cytokine production. Recently, the emergence of new and disguised *Fusarium* mycotoxins in farm animals has been of concern, which may contribute to toxic health effects (Ekwomadu *et al*., 2021), although the metabolic fate of mycotoxins is still a matter of scientific debate.

Acute aflatoxicosis leads to the death of the animal, while chronic aflatoxicosis leads to immune suppression and other chronic pathologies of internal organs and pathological conditions (Zahran *et al*., 2019). However, the authors cite data related to deoxynivalenol damage in animals, and the effect of assimilated exposure to mycotoxins still requires further investigation. It was found that aflatoxins temporarily reduced the phagocytic activity of leukocytes in cows. Furthermore, the ability to induce T-cell proliferation in mycotoxicosis decreased (Toutounchi *et al*., 2021). Therewith, the observation described by the authors was over 60 days of age and requires clarification of the generation of these cells directly in the thymus.

Under the action of deoxynivalenol, the regulation of antigen-presenting ability is disrupted, which may explain the immunotoxicity of this mycotoxin (Toutounchi *et al*., 2021). However, the author focuses on the formation of antigen-receptor bonds but does not describe exactly how mycotoxins affect the synthesis of distinct types of lymphocytes.

Immune responses are variable. Aflatoxin B1-induced immunosuppression has been demonstrated in various livestock species (e.g., turkeys, chickens, and pigs) and in laboratory animals (mice, guinea pigs, and rabbits). The response of bovine lymphocytes to aflatoxin in vitro is similar to that of other laboratory animals. Trichothecenes are powerful immunosuppressants that directly affect immune cells, as well as alter immune responses because of tissue damage elsewhere. Sheep and calves treated with *fusarium* T-2 toxin develop leukopenia and the functioning of peripheral lymphocytes decreases. Immunosuppressive effects of ochratoxin A, rubratoxin B, and patulin have been reported. Citrinine caused lymphopenia, but stimulated responses against antigens (Sun *et al*., 2022). Antibodies against mycotoxins conjugated with proteins are formed for analytical purposes. Therewith, it is necessary to clarify the mechanism of action of zearalenone on the immune response.

The studies of Roberts *et al.* (2021) described that a considerable increase in the percentage of CD4-CD8+ T-cells was observed in bulls treated for 2 weeks and characterized by a decrease in the ratio of CD4:CD8 T-cells ($p \leq 0.10$). Cattle at the final stage of fattening are susceptible to immunotoxic and inhibitory transcripts of the effects of deoxynivalenol and fumonisins. However, the coverage of similar mechanisms in dairy cows requires attention.

Considering the above, *the purpose of this study* was to figure out the dynamics of changes in immunity indicators in the dynamics under the action of mycotoxins according to the classical scheme of prevention of mycotoxicosis and compare with experimental treatment.

MATERIALS AND METHODS

The study was conducted in the conditions of farms of LLC "Marivske" and LLC "Vitchyzna" of the Sumy Oblast on cows of black-spotted breed. The animals were aged

from 3 to 5 years, weighing 550-580 kg. Therewith, cows were divided into 4 groups according to the analogue method. Animals of Group 1 (n=25) – healthy cows that have not been diagnosed with mycotoxisosis. Animals of Groups 2-4 were fed fodder containing zearalenone and deoxynivalenol at concentrations above 0.5 mg/kg. Cows of Group 2 (n=27) were not tested during the experimental period. Animals of Group 3 were given a feed additive based on zeolite and organic acids at a dose of 2.5 kg per tonne of fodder. Cows of Group 4 were given a feed additive based on zeolite and organic acids at a dose of 2.5 kg per tonne of fodder and a preparation based on an aqueous solution of recombinant α–, g-interferons at a dose of 3 ml per animal.

The presence of mycotoxins in concentrated fodder was found. European standards EN 15891:2011 (SIST 1) and EN 15850:2010 (SIST EN 15850:2010…, 2010) were used for this.

Research material: cow blood taken from the tail vein in an amount of 12 ml on Day 0, 10, 30, 60, and 125 of the research, in sterile polystyrene syringes. After sampling, the blood was divided into 2 parts, one part was placed in the refrigerator for 1.5 hours at 3°C to form blood serum, while the other was stabilized according to the EDTA method.

Bactericidal and lysozyme activity was found in blood serum by photonephelometric method using the *E. coli* test culture. Neutrophil phagocytic activity was found in stabilized blood using the *E. coli* test culture.

The number of T-lymphocytes was found according to the "rosette formation" method. The total number of T-lymphocytes was found according to M. Jondal's method of spontaneous rosette formation with sheep red blood cells (Jondal *et al.*, 1993). The content of early lymphocytes was investigated according to M. Wansbrough-Jones's modified method of rosette formation (Wansbrough-Jones *et al.*, 1979). The number of theophylline-sensitive and theophylline-resistant T-cells was found according to S. Limatibul's method (Limatibul *et al.*, 1978). The immunoregulatory index was calculated as the ratio of T-helper cells to T-suppressors. The number of B-lymphocytes was found according to N.F. Mendes (Mendes *et al.*, 1973).

Immunoglobulins of the main classes (A, M, G) in blood serum were investigated according to G. Mancini's method of simple radial immunodiffusion in a gel (Mancini *et al.*, 1965). The total level of circulating immune complexes (CEC) and their fractional composition by molecular weight were investigated according to M. Digeon's method of precipitation in a polyethylene glycol solution (Digeon *et al.*, 1977).

Statistical data were processed according to the Student's t-test of variational statistics for research in biology. The data are presented as an average value for groups with a square deviation error (M±m), the critical significance level was 0.05 (P). When calculating statistical data, the authors of this study used the Statistica 7.0 software.

All experimental studies were conducted per the corresponding requirements and standards that meet the requirements of DSTU ISO/IEC 17025:2005, IDT. Animal husbandry and all manipulations were performed according to the provisions of the Procedure for conducting tests and experiments on animals by scientific institutions (Law of Ukraine No. 249, 2012) of the European Convention for the protection of vertebrates used for experimental and other scientific purposes (European Convention…, 1986).

RESULTS AND DISCUSSION

Natural resistance and its correction in cows with mycotoxicosis. The bactericidal activity of blood serum of healthy animals during the 125-day experimental period ranged within 44.9±1.31-47.1%. The results of the study of cow blood serum are presented in Table 1.

Table 1. *Change dynamics in the bactericidal activity of cow blood serum, %*

Group of cows		Control – healthy Group 1	Control – sick with mycotoxicosis Group 2	Experimental Group 3	Experimental Group 4
Experimental period, day	0	46.8±1.14	33.65±1.03 *	34.36±1.1 *	33.3±1.26 *
	10	47.1±1.22	33.64±1.29 *	37.94±1.26*	37.1±1.36 *
	30	44.9±1.31	29.36±1.62 *	38.91±1.31 *	41.4±1.35
	60	46.2±1.83	29.67±1.33 *	39.64±1.26 *	44.3±1.39
	125	46.3±1.76	27.35±1.06 *	41.29±1.98	46.7±1.97

Note: **P⩽0.05*
Source: *compiled by the authors*

The indicator of bactericidal activity (BA) in sick cows (with mycotoxicosis) was reduced by 1.4-1.6 times. The value of BA in the blood serum of animals of Group 2 during the studies was 1.4 times lower than the control values by Day 10 of the experiment, by Day 30 – by 1.5 times, by Day 60 – by 1.6 times, by Day 125 – by 1.70 times.

The bactericidal activity of blood serum of animals of Groups 3 and 4 during the experiment changed upwards, but at contrasting times and in separate groups

this indicator did not change equally. The greatest value of bactericidal activity was achieved in the blood serum of animals of these groups by Day 125 of experiments. At that time, this indicator increased in Group 3 by 1.2 times, and in Group 4 – by 1.4 times. Therewith, the bactericidal activity of the blood serum of cows of Group 3 was 1.15 times lower than that of healthy animals, and in Group 4 it practically did not differ from that of healthy animals.

Dynamics of lysozyme activity in cow blood serum. Lysozyme plays an important function as a biomarker produced by the cow's body (Chia *et al.*, 2019). The lysozyme activity (LA) of the blood serum of cows of the control Group 1 practically did not alter during the experiment period – 21.0-22.5%. LA of the blood serum of sick animals at the beginning of the experiment was lower than in healthy animals and amounted to 14.9±1.24%, 15.3±0.95% in cows of Group 3, 14.95±0.98% in cows of Group 4.

Analysing the dynamics of this indicator, it is worth pointing out that unreliable fluctuations were observed in healthy cows during the entire research period. The authors of this paper believe that this is explained by the influence of external factors, such as high ambient temperature, crowded housing. This is confirmed by the study (Toutounchi *et al.*, 2021).

Lysozyme activity in cows with mycotoxicosis was reduced during the entire follow-up period and not significantly ranged within 13.8±1.67–15.1±1.28%. The decrease in this indicator is conditioned upon the effect of mycotoxins on the body of cows, which leads to a decrease in the activity of white blood cells. Comparable results were obtained by researchers (Zahran *et al.*, 2021; Cheng *et al.*, 2016). The results of the study of lysozyme activity of cow blood serum are presented in Table 2.

Table 2*. Change dynamics in the lysozyme activity of cow blood serum, %*

Group of cows		Control – healthy Group 1	Control – sick with mycotoxicosis Group 2	Experimental Group 3	Experimental Group 4
Experimental period, days	0	21.3±1.25	14.9±1.24*	15.3±0.95*	14.95±0.98*
	10	22.1±1.27	15.1±1.28*	17.64±0.65*	18.82±0.65*
	30	21.95±1.62	14.95±1.39*	20.6±0.78	20.94±0.94
	60	21.76±0.95	13.9±1.25*	21.4±1.32	21.22±0.84
	125	22.01±0.97	13.8±1.67*	21.75±1.22	22.47±0.93

Note: **P⩽0.05*
Source: *compiled by the authors*

The dynamics of lysozyme activity in the blood serum of cows of Group 3 began to increase on Day 10 of research, and on Day 30 – did not differ from a similar indicator of healthy animals. The best result was obtained in Group 4, where a significant increase in lysozyme activity was recorded on Day 10 of the study.

Dynamics of complementary activity of cow blood serum. The complementary activity of the blood serum of cows of the control Group 1, for 125 days of experiments, practically did not change and was within 22.86±1.28–23.96±1.35. Data from the study of the dynamics of complementary activity of cow blood serum are presented in Table 3.

Table 3*. Change dynamics in the complementary activity of cow blood serum, units*

Group of cows		Control – healthy Group 1	Control – sick with mycotoxicosis Group 2	Experimental Group 3	Experimental Group 4
Number of animals		25	27	20	20
Experimental period, days	0	23.31±1.04	12.92±1.86*	13.4±0.44*	13.39±0.35*
	10	23.56±1.09	13.27±0.25*	15.82±0.4*	16.6±0.65*
	30	23.98±1.35	13.81±0.36*	17.27±0.27*	18±1.91*
	60	23.96±0.36	13.37±0.51*	19.98±0.46*	19.48±0.95*
	125	23.32±0.92	14.14±1.66*	20.25±0.57	21.02±1.2

Note: **P⩽0.05*
Source: *compiled by the authors*

The complementary level in the blood serum of cows with mycotoxicosis was 1.17-1.28 times lower than the physiological norm before the start of experiments. The same indicator in the blood serum of cows of Group 2 was even lower compared to the background and control values by 1.2 times by Day 30 of the experiment, by Day 60 – by 1.4 times, and by Day 125 – by 2.3 times.

Complementary activity in the blood serum of cows of Groups 3 and 4 increased significantly before the end of the experiments, and, depending on the group, changed differently: in Group 3 in relation to Day 0, on Day 10 – by 1.09 times, on Day 30 – by 1.22 times, on Day 60 – by 1.20 times, on Day 125 – by 1.15 times. However, the animals of the control group were 1.20, 1.15, 1.14, and 1.15 times inferior in this indicator, respectively. The same indicator of blood serum of cows of Group 4 increased by 1.08, 1.17, 1.15, and 1.20 times, respectively, compared to the initial value on Days 10, 30, 60, and 125 of the experiment. However, it was respectively 1.12, 1.10, 1.20, and 1.10 times lower than control in the same experimental periods.

Phagocytosis and its correction in cows with mycotoxicosis. The initial value of phagocytic activity in the blood of animals of Groups 2-4 was lower by an average of 1.4-1.5 times (by 12.5-16.0%) (Table 4).

Table 4*. Change dynamics in the phagocytic activity of cow blood serum, %*

Group of cows		Control – healthy Group 1	Control – sick with mycotoxicosis Group 2	Experimental Group 3	Experimental Group 4
Experimental period, days	0	45.51±1.41	26.32±1.03*	24.21±1.18*	25.3±0.93*
	10	45.42±1.15	26.46±1.08*	28.55±1.11*	28.39±1.44*
	30	45.46±1.12	25.34±1.66*	30.21±1.12*	31.98±1.81*
	60	46.1±0.59	26.34±0.64*	32.33±0.78*	35.41±2.02*
	125	45.31±1.24	25.39±0.72*	34.53±1.38*	43.18±0.87

Note: **P⩽0.05*
Source: *compiled by the authors*

The phagocytic activity of leukocytes in the blood of cows of Groups 3-4 was reduced and amounted to 26.32±1.03% in Group 2; 24.21±1.18% in – Group 3, and 25.0±0.93% – in Group 4. Subsequently, in animals of Group 2, the indicator of phagocytic activity of leukocytes did not change significantly, and at the end of the studies was 26.14±1.06%.

A similar indicator in cows of Group 3 had substantial dynamics on Day 10 and amounted to 28.55±1.11%, on Day 30 – 30.21±1.12%, on Day 60 – 32.33±0.78%, and at the end of the experimental period was 34.53±1.38%, which is 1.4 times more than the initial value. However, this indicator in Group 3 stayed significantly lower (P<0.01) compared to the same indicator in healthy animals. The phagocytic activity of leukocytes of cows in Group 4 increased, starting from Day 10, and at the end of studies amounted to 43.18±0.87%, which did not significantly differ from healthy cows.

In case of mycotoxicosis of cows caused by zearalenone and deoxynivalenol from *Fusarim* and *Aspergillus fumigatus* toxin, all elements of the natural resistance system are suppressed, which is manifested in a decrease in the bactericidal, lysozyme, and complement activity of blood serum (Knutsen *et al*., 2017).

Indicators of T- and B-immune systems and their correction in cows with mycotoxicosis. The results of studies of the dynamics of the content of T-E-ROC lymphocytes in the blood of cows are presented in Table 5.

Table 5*. Content dynamics of T-E-ROC lymphocytes in the blood of cows, %*

Group of cows		Control – healthy Group 1	Control – sick with mycotoxicosis Group 2	Experimental Group 3	Experimental Group 4
Experimental period, days	0	42.02±1.14	26.15±0.74*	25.95±0.55*	27.03±1.15*
	10	42.35±1.41	26.44±0.42*	27.67±0.8*	30.18±1.03*
	30	42.37±1.6	26.56±0.11*	30.97±1.12*	36.17±1.17*
	60	41.64±1.01	26.04±1.05*	33.72±0.62*	39.54±1.82*
	125	42.12±1.07	25.88±0.38*	37.34±0.7*	40.34±1.45

Note: **P⩽0.05*
Source: *compiled by the authors*

The content of T-lymphocytes in the blood of cows that received balanced mineral composition had a value at the level of 41.87±1.34 - 42.37±1.6%. The level of T-E-ROC lymphocytes in the blood of cows of Groups 2-4 at the beginning of the experiment was 1.55-1.62 times less than in healthy cows. In cows of Group 2, no increase in the values of this indicator was noted throughout the experiment. The indicator of blood T-cells of cows of this group on Day 10 of the experiment tended to increase, but they were 1.53 times lower than the same indicator in healthy animals. On Day 30, this ratio was 1.37, on Day 60 – 1.23, and on Day 125 – 1.13 times.

The level of T-cells in the blood of Group 4 in relation to the indicator of healthy animals was 1.55 times lower on Day 0, 1.14 times lower on Day 10, and 1.17 times lower on Day 30. However, starting from Day 60 of the experiment, this indicator did not significantly differ from that in healthy cows.

Content dynamics of T-helpers in the blood of cows. The blood of cows of Group 1 contained 20.51-21.67% of T-helpers. This indicator in the blood of cows of Groups 2-4 was 1.5 times lower before the start of experiments than in Group 1. Data from the study of the dynamics of T-helpers in the blood of cows are presented in Table 6.

Table 6. *Dynamics of T-helpers in the blood of cows, %*

Group of cows		Control – healthy Group 1	Control – sick with mycotoxicosis Group 2	Experimental Group 3	Experimental Group 4
Experimental period, days	0	21.67±1.14	14.19±0.1*	14.37±0.88*	14.49±0.93*
	10	20.51±1.39	13.49±0.53*	15.78±1.29*	18.32±0.35*
	30	21.15±0.51	13.77±1.21*	17.26±0.19*	19.06±0.65*
	60	20.97±1.11	13.86±1.29*	18.65±0.06*	19.52±0.54
	125	21.45±0.06	14.06±0.95*	18.61±0.42*	20.59±0.6

Note: **P⩽0.05*
Source: *compiled by the authors*

The level of T-helpers in the blood of Group 2 cows was stable throughout the experiment. The content of T-helpers in the blood of cows of Group 3 slightly increased compared to the background: up to Day 10, on Days 30, 60, and 125 – by 1.09, 1.20, 1.3, and 1.27 times, respectively, and was inferior to the control values. The indicator of T-helpers in the blood of cows of Group 4 increased by 1.29 times by Day 10 of the experiment, by 1.3 times – by Day 30, by 1.35 times – by Day 60, by 1.42 times – by Day 125, not significantly differing from the same indicator in healthy cows.

Content dynamics of T-suppressors in the blood of cows. The results of the study of the dynamics of T-suppressors in the blood of cows are presented in Table 7.

Table 7. *Content dynamics of T-suppressors in the blood of cows, %*

Group of cows		Control – healthy Group 1	Control – sick with mycotoxicosis Group 2	Experimental Group 3	Experimental Group 4
Experimental period, days	0	18.06±0.57	23.51±1.39*	23.39±0.6*	23.4±1.84*
	10	18.56±0.2	23.19±0.87*	22.13±1.15*	22.39±0.33*
	30	19.05±1.38	22.37±1.05*	21.46±0.47*	18.32±0.55
	60	18.75±0.72	22.76±1.52*	20.01±0.33*	18.02±1.29
	125	18.42±0.86	22.57±1.28*	20.01±0.35*	18.12±1.61

Note: **P⩽0.05*
Source: *compiled by the authors*

Normalization of suppressor reactions was noted in cows of Group 4, since the level of T-suppressors almost corresponded to physiological standards by Day 10 of the experiments. The number of T-suppressors in the blood of cows of Group 1 (control) was stable – 17.9-18.8%. Background indicator of T-suppressors in the blood of cows of Groups 2-4 had greater values (1.18-1.18 times), which was higher by 2.4-3.4%. This trend in cows of Group 2 persisted throughout the experiment. The number of T-suppressors increased by Day 10 compared to the background and control by 1.10 and 1.24 times, by Day 30 – by 1.14 and 1.26 times, by Day 60 – by 1.15 and 1.3 times, by Day 125 – by 1.16 and 1.33 times. The reaction of T-suppressors in the blood of cows of Groups 3 and 4 during the experiment significantly decreased but did not occur in the same way.

The level of T-suppressors in the blood of animals of Group 3 decreased in comparison with the background values: by Day 10, on Days 30, 60, and 125, respectively – by 1.07 times, 1.0 times, 1.06 times, 1.08 times, while exceeding the control figures by 1.10; 1.05; 1.07, and 1.20 times. The maximum decrease in T-suppressors was observed in the blood of cows of Group 4.

The level of T-suppressors was 1.60 times lower than the background by Day 10 of the experiment, 1.10 times lower – by Day 30, 1.09 times lower – by Day 60, and 1.10 times lower by Day 125, which practically corresponded to the control data. Zearalenone is thought to affect the immune response considerably, which is the main defence mechanism against pathogens, toxins, and other antigens in all mammals, with immunostimulating or immunosuppressive results (Bulgaru *et al.*, 2021).

Immunoglobulins and their correction in cows with mycotoxicosis. Studies of the blood serum of cows of Group 1 on IgG in real time showed that their level was within 26.5-28.0 g/l (Table 8).

Table 8. *Dynamics of T-helpers in the blood of cows, %*

Group of cows		Control – healthy Group 1	Control – sick with mycotoxicosis Group 2	Experimental Group 3	Experimental Group 4
Experimental period, days	0	27.12±0.86	17.3±0.53*	17.36±1.16*	17.29±1.1*
	10	27.2±1.07	16.47±0.01*	18.74±0.19*	19.36±0.57*
	30	27.24±0.64	15.72±0.82*	19.44±0.98*	23.23±0.83*
	60	27.16±0.78	14.07±1.9*	19.62±1.51*	24.94±0.71*
	125	27.0±1.13	13.84±0.43*	19.96±1.16*	26.87±0.11*

Note: **P⩽0.05*
Source: *compiled by the authors*

Immunoglobulins are an indispensable indicator, as they indicate the level of antibodies, which generally characterizes the possibility of an immune response to the action of a pathogenic agent (Nagahata *et al*., 2020).

At the same time, under the toxic compounds, namely mycotoxins, a decrease in this indicator was observed, which may indicate a decrease in the synthesizing function of lymphatic cells (Rahman *et al*., 2021). The level of IgG in the blood serum of animals of Group 2 decreased, and during the experiment and up to Day 10 of research, compared to the background indicator, was 1.05 times lower, up to Day 30 – 1.1 times, up to Day 60 – 1.23 times, up to Day 125 – 1.25 times.

The IgG content in the blood serum of cows of Groups 3 and 4 during studies on Day 10 was higher than the initial (background value) by 1.08; and 1.12 times, by Day 30 – by 1.12 and 1.36 times, by Day 60 – by 1.13 and 1.44 times, by Day 125 – by 1.15 and 1.55 times, respectively. This indicates a more effective increase in immunoglobulins when using recombinant α-, g-interferons compared to the use of a feed additive based on zeolite and organic acids through the activation of immunocompetent organs of the body. Similar data were obtained by the authors in a study on calves (Tang *et al*., 2022)

Dynamics of IgA content in cow blood serum. The level of IgA in the blood serum of healthy cows (Group 1) ranged within 2.25-3.11 g/l. This indicator in the blood serum of cows of Groups 2-4 was reduced by 1.4-1.5 times before the start of experiments. The level of serum IgA in cows of Group 2 dynamically decreased, yielding to the background and control levels on Day 10 of the experiment by 1.04 times, on Day 30 – by 1.08 times, on Day 60 – by 1.15 times, on Day 125 – by 1.17 times. The same indicator in the blood serum of cows of Group 3 increased compared to the beginning of the experiment and on Day 10 was 1.6 times higher, on Day 30 – 1.56, on Day 60 – 1.88, and on Day 125 – 1.81 times higher, respectively. In the blood serum of cows of Group 4, the IgA content increased by 1.7, 1.59, 1.6, and 2.45 times, respectively, starting from Day 10. Data on the dynamics of IgA in blood serum of cows are presented in Table 9.

Table 9. *Dynamics of IgA in blood serum of cows, g/l*

Group of cows		Control – healthy Group 1	Control – sick with mycotoxicosis Group 2	Experimental Group 3	Experimental Group 4
Experimental period, days	0	2.91±0.28	1.25±0.16*	1.14±0.61	1.29±0.14
	10	2.63±0.43	1.20±0.57*	1.87±0.17	2.24±0.19
	30	3.11±0.9	1.15±0.23*	1.78±0.06	2.06±0.17
	60	2.61±0.61	1.09±0.23*	2.15±0.26	2.09±0.57
	125	2.25±0.38	1.07±0.35*	2.06±0.38	3.16±0.91

Note: **P⩽0.05*
Source: *compiled by the authors*

The authors also report that the innate immune response is triggered by receptors that recognize the pathogen and activate several signalling pathways that control the immune response. Neutrophils, monocytes/macrophages, and dendritic cells that mediate interaction with pathogens are components of the innate immune system that can form networks, playing a key role in the initial immune response to infection and tissue damage. These are phagocytic cells that, upon stimulation, can produce reactive oxygen species that are important for cell signalling and homeostasis. The indicator of bactericidal activity of blood characterizes

the ability of blood serum complexes to destroy 99.9% of bacteria (Zaghi *et al*., 2020). The data obtained confirm the statement of most studies that under toxic substances, in this case mycotoxins, it leads to a decrease in non-specific immune factors due to inhibition of the work of immunocompetent organs. Rivera notes that a decrease in the bactericidal activity occurs due to the action on the body of exogenous and endogenous microflora that produces toxins (Rivera *et al*., 2020). It is important that mycotoxins exert a negative effect complexly and in a lower concentration, while microorganisms in the early process act in the overwhelming majority in the zone of the inflammatory reaction.

The use of a feed additive (experimental Group 3) in the diets of cows at a dose of 2.5 kg per tonne of fodder contributed to an increase in bactericidal activity due to a decrease in toxic substances in the blood of experimental cows. The mechanism of action of the supplement is based on a synergistic combination of mechanical (adsorption) and detoxification action of organic acids. Organic acids, namely acetic acids, increase the acidity to pH 6.3-6.5 when feeding the additive, which adversely affects the growth and development of microscopic fungi, while not suppressing the viability of scar microflora. Comparable results were obtained by other scientists who investigated the pig livestock, but in their studies more attention was paid to studying the effect of sorbents on the gastrointestinal tract of monogastric animals. Therewith, the level of acidity displacement had only a lower limit sufficient for the destruction of fungi (Dąbrowski *et al*., 2016; Bailey *et al*., 2019).

Furthermore, the toxic effect on the liver and the decrease in the synthesis of immunomodulating proteins is also indicated by Iori *et al*. (2022) who noted transcriptomic changes that showed differences in the expression of genes involved in the inflammatory response, oxidative stress, drug metabolism, apoptosis, and cancer. Cell death associated with necrosis rather than apoptosis was noted. As for the mechanism of toxicity, a molecular pathway linking the inflammatory response and oxidative stress has been postulated. Activation of Toll-Like Receptor 2 (TLR2) by AFB1 triggers an intracellular signalling cascade involving a kinase (p38β MAPK), which enables the nuclear translocation of activator protein-1 (AP-1) and NF-κB, ultimately leading to the release of pro-inflammatory cytokines.

P. Ferraboschi (2021) points out the need to use synthesized lysozyme to protect animal feed from the effects of microscopic fungi and bacteria. The author notes that this increases the level of lysozyme in the blood and positively affects the immunity of animals. This statement correlates with the authors' studies that the action of immunostimulants increases lysozyme activity, but the authors do not agree that exogenous lysozyme can support a high immune response for a long time.

The use of an immunomodulator based on recombinant α- and g-interferons (experimental Group 4) gave the best result. The increase in the bactericidal activity of the blood was caused by the immune system stimulation, based on an increase in the formation of cytokines and blocking of lymphocyte receptors, which is confirmed by the results of studies by Malvandi *et al*. (2022).

R. Falkauskas *et al*. (2022) reported a significant negative correlation (r=−0.540) between urinary beta-zearalenol and beta-zearalanol concentrations and a positive correlation (r=0.826) between serum beta-zearalenol and alpha-zearalanol concentrations ($p<0.05$). During the study, it was found that feeding cows for two weeks with feed without mycotoxins can reduce the concentration of alpha- and beta-zearalenol in body fluids and can reduce the concentration of ZEN in milk but does not reduce the concentration of zearalenone. This is consistent with the results of the present study, although this paper also suggests using sorbent-and interferon-based agents to reduce mycotoxin levels.

An imbalance between the formation of reactive oxygen species and their inefficient elimination leads to a sharp increase in them, which causes cell damage known as oxidative stress (Solhaug *et al*., 2016). Wang *et al*. (2019) reported that zearalenone (5, 10, 20 μm) increases the formation of reactive oxygen species in bovine neutrophils and reduces the activity of antioxidant enzymes, followed by the formation of extracellular neutrophil traps, a network of extracellular DNA fibres that help neutrophil cells kill extracellular pathogens.

However, F.A. Uzal (2016) states that locally generated and systemically circulating Ig can have an effect when vascular permeability and inflammation occur due to its ability to fix complement, promote antibody-dependent cell-mediated cytotoxicity, and opsonize. In most species, IgE-producing plasma cells are present in the lamina propria in small numbers. Its significance may lie in the IgE-dependent cytotoxicity of eosinophils, mast cells and basophils, as well as in mediating immediate type (Type I) hypersensitivity reactions in the intestinal mucosa, which confirms changes in the production of immunoglobulins in toxic conditions, but the author draws attention to IgE, and the dynamics of IgA and IgG stays out of the researcher's attention.

CONCLUSIONS

It was found that the presence of associated forms of mycotoxins in the diet of cows, produced by fungi of the genus *Fusarium*, has an immunosuppressive effect and causes a decrease in the bactericidal activity of blood serum from 23.47% to 29.9%, the lysozyme activity of blood serum of cows from 28.17% to 32 .58%, complement activity of serum from 44.12% to 56.52%, phagocytic activity of serum from 45.12% to 47.48%, blood T-E-ROC lymphocytes from 35.09% to 38.72%, T-helpers from 32.23% to 34.52%, immunoglobulins from 42.23% to 44.52% and an increase in T-suppressors from 16.63% to 23.18%.

The use of a zeolite-based feed additive and organic acids had an effect on the restoration of indicators, namely: bactericidal activity of blood serum – by 16.78%, lysozyme activity of blood serum of cows – by 29.66%, complementary activity of serum – by 37.62%, phagocytic serum activity – by 29.89%, T-E-ROC blood

lymphocytes – by 30.50%, T-helpers – by 22.78%, immunoglobulins – by 51.48%, and T-suppressors – by 14.79%.

The use of a zeolite-based feed additive and organic acids and a product based on an aqueous solution of recombinant α-, g-interferons affected the recovery of indicators, namely: bactericidal activity of blood serum – by 28.69%, lysozyme activity of blood serum of cows – by 20.12%, complementary activity of serum – by 41.52%, phagocytic activity of serum – by 41.41%, T-E-ROC blood lymphocytes – by 32.99%, T-helpers – by 29.63%, immunoglobulins – by 51.48%, and T-suppressors – by 24.17%.

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Показники імунітету при асоційованому мікотоксикозі корів

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Анотація. Питання відтворення великої рогатої худоби було і залишається одним із найголовніших завдань у галузі скотарства. Збитки господарств від неплідності корів досить значні та становлять від 3,19 до 5,41 $ за 1 день неплідності. Мікотоксини, що виробляються грибами родини *Fusarium*, а саме деосиніваленол (DON) і зеараленон (ZEN) негативно впливають не тільки на роботу всіх органів і систем організму корови, а й справляють імуноседативний ефект. Метою досліджень було встановити вплив комплексу DON і ZEN на основні показники імунної відповіді корів та її корекції в порівняльному аспекті з використанням кормової добавки на основі цеоліту і органічних кислот та рекомбінантних α- g-інтерферонів. Матеріалом досліджень була кров корів (сироватка та стабілізована), хворих на мікотоксикоз, спричинений асоціацією деосиніваленолу та зеараленону. Використані методи: фотонефелометричний з використанням тест-культури *E. coli*, спонтанного розеткоутворення з еритроцитами барана за M. Jondal, модифікований метод розеткоутворення за M. Wansbrough-Jones, метод P. Limatibul, простої радіальної імунодифузії в гелі за G. Mancini, преципітації в розчині поліетиленгліколю за M. Digeon. Експериментальні дослідження були виконані на коровах чорно-рябої породи в господарствах Сумської області. Було досліджено динаміку показників імунітету корів при розвитку мікотоксикозу та при застосуванні лікування із застосуванням засобів на основі целеоліту, органічних кислот та водного розчину рекомбінантних α- g-інтерферонів. Встановлено, що показник бактерицидної, лізоцимної, комплементарної та фагоцитарної активності сироватки крові корів під дією проведеного лікування підвищувався до показника у здорових тварин, Було досліджено динаміку імуноглобулінів в процесі лікування при застосуванні цеоліту та органічних кислот і рекомбінантних α- g-інтерферонів, встановлено підвищення до рівня інтактних корів. Було доведено, що показники імунної відповіді корів при застосуванні кормової добавки на основі цеоліту та органічних кислот у дозі 2,5 кг на тону корму та препарату на основі водного розчину рекомбінантних α- g-інтерферонів у дозі 3 мл на тварину були достовірно вищими

Ключові слова: корови, мікотоксикози, зеараленон, деосиніваленол, імунітет корів, кормові добавки

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Bioecological Features, Biochemical and Physicochemical Parameters of Grain of New Genotypes

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Abstract. The presented study allowed for better coverage of the features of new varieties of soft winter wheat and winter triticale, demonstrating the bioecological potential of their crops and the biochemical and physico-chemical parameters of grain, which is a relevant matter. This paper presents the results of selection work on winter triticale, highlights the parameters of productivity, the biochemical composition of grain of the new Myronosets variety. The purpose of this study was to create new genotypes of winter triticale and soft winter wheat and investigate them in terms of bioecological potential and biochemical, physico-chemical parameters of grain. The study involved biometric, physico-chemical, biochemical, and mathematical research methods. The Myronosets variety has a high grain yield (6.8-7.2 t/ha), a high protein content (⩾14.2%) and a composition of essential amino acids, which confirms its nutritional and consumer value. It is recommended to use flour from this variety to produce functional bakery products. For the first time, it was found that Myronosets triticale variety is superior (4919 mg/100 g DM) to Ariivka wheat variety (3977 mg/100 g DM) and Borotba rye variety (3241 mg/100 g DM) in total amino acid content. It was found that triticale grain is inferior to wheat grain in terms of tryptophan and isoleucine content. Rye is inferior to wheat grain in all essential amino acids except leucine, and to triticale – in all amino acids except tryptophan. The most represented amino acids in triticale grain were as follows: leucine (1442 mg/100 g), valine (733 mg/100 g), phenylalanine (720 mg/100 g) and isoleucine (510 mg/100 g), in rye – leucine (1343 mg/100 g), valine (481 mg/100 g) and phenylalanine (396 mg/100 g). Further broad targeted introduction of the new variety in agroecosystems will increase the volume of grain of valuable both animal feed and to produce functional bakery products

Keywords: essential amino acids, ecological and adaptive properties, bioecological potential, grain quality

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INTRODUCTION

Currently, the use of intergenerational synthetic hybrids is relevant, since the latter combine important complementary features of both parent species. Triticale is one of such selection developments, which is endowed with high yield and quality of wheat and stress resistance of rye. It is known that triticale (x *Triticosecale* Wittmack ex A. Camus) is the first successful synthetic amphiploid cereal crop, which originated in 1874 as a result of hybridisation of hexaploid wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) (Ayalew *et al.*, 2018; Liubych *et al.*, 2020). The production area under this grain crop has grown significantly in the world, as triticale compromises the beneficial agronomic properties of the parent forms (Moskalets *et al.*, 2016; Liubych *et al.*, 2020). In this regard, triticale grain production doubled – from six million tonnes in the 1990s to almost 13 million tonnes in 2020 on a planetary scale. Triticale is the leader in Poland in terms of cultivation worldwide. Thus, in Ukraine, the area under triticale is five times smaller than in Germany and three times smaller than in Belarus or China (FAOSTAT, 2019). Presently, triticale plants are used in many ways, mainly as a grain intended for feed production (Goral *et al.*, 2021). Furthermore, in spring, the land cultivated for triticale is used for pastures, as fresh livestock feed, or for hay and silage. Triticale is also cultivated for bioethanol (Randhawa *et al.*, 2015; Liubych *et al.*, 2020), as well as to produce food products such as functional bakery products (Liu *et al.*, 2017).

The first barren triticale was bred in 1875 in Scotland. Later, in 1888, Rimpau (Germany) crossed hexaploid wheat and rye to produce the first viable hybrid by spontaneous chromosome doubling (Würschum *et al.*, 2014; Liubych *et al.*, 2020). European countries were newcomers both to the creation of this essential species and to the cultivation of a new crop adapted to different environmental conditions. The first variety was created in Hungary in 1968 (Blum, 2014). Selective breeding of triticale in North America officially began in 1954 at the University of Manitoba in Canada, from where the industrial variety (Rosner) was bred in 1969 (Ayalew *et al.*, 2018).

Triticale fields occupy over 4 million hectares of land, with an average annual yield of nearly 18.4 million tonnes of grain in 2019 (FAOSTAT, 2019). According to the same data, Poland, Germany, Belarus, and France were and still are the leading triticale producing countries, accounting for 73% of global production. Germany and France showed the highest performance, followed by Poland and Belarus (Randhawa *et al.*, 2015; FAOSTAT, 2019).

Since triticale contains wheat (diploid, tetraploid, and hexaploid), rye, and various forms (primary, secondary, and substituted) of triticale as its genetic resource base, genetic variations can be continuously created (by crossbreeding) to enrich the genetic pool (Würschum *et al.*, 2014). However, the optimal use of triticale depends on the exact combination and use of germ plasma by applying various breeding and genetic tools to accumulate the desired genes in culture. Crop selection benefits from using both conventional and molecular selective breeding methods.

Global climate change requires a reassessment of the structure of acreage and species and varietal diversity of winter crops (Intergovernmental Panel..., 2014). This is because winter crops, including triticale, that are the leading link in ensuring sustainable production of food grains, since they are endowed with the highest yield potential with genetic resistance to limiting environmental factors of a particular region, pests, and pathogens, which is the main thing in adaptive agriculture (Sabagh *et al.*, 2021; Kim *et al.*, 2017). Targeted introduction of particular varieties is another way to regulate the grain productivity of triticale (Li *et al.*, 2013). Therefore, considering the specific features of soil and climatic conditions of a particular region upon introducing a particular variety allow fully coverage its bio-potential in terms of grain yield and quality.

The purpose of this study was to create a new genotype of winter triticale and to investigate it in terms of productivity, yield and quality of grain, resistance to lodging, shedding, brittleness of the ear, germination of grain in the ear, comprehensive resistance to pathogens and damage by the Hessian fly, frost, winter, and drought resistance.

MATERIALS AND METHODS

During 2008-2020, research was performed to create a new variety of winter triticale (Mironosets), which was investigated by genotypic and phenotypic characteristics in the conditions of Polissia (Nosivska Selection and Experimental Station of the National Academy of Agrarian Sciences of Ukraine) and Forest-Steppe (V.M. Remeslo Myronivskyi Institute of Wheat of the National Academy of Agrarian Sciences of Ukraine (MIW NAAS)). Triticale grain and flour was evaluated by biochemical and technical indicators at the V.Ya. Yuriev Institute of Crop Production of the National Academy of Agrarian Sciences of Ukraine in the grain quality laboratory. Morphological parameters of plants (leaves, bush, ear, grain) and stages of ontogeny were studied. Biometric plant parameters included: plant height, main ear length, number of spikelets in the main ear, number of grains in the main ear, grain weight from the main ear, thousand-kernel weight, and yield from an area of 10 m^2.

The dry weight of one grain and the water content of the grain were found by drying 100 g of grain at 80°C for 48 hours and re-weighing the sample at zero water content. Grain samples (30 g) were ground on LMT-2 equipped with a 1 mm mesh. Wholemeal flour samples (1 g) were dried in an oven at 80°C for 48 hours, then 5 mg of dry flour was weighed in tin capsules and the total N concentration was figured out

according to Dumas burning using a DA 7440 infrared express analyser. The gluten content was found using the Inframatic 8600 device, and dietary fibre (cellulose) was found on the FIWE-3 analyser.

Amino acid composition was found by hydrolysis of flour samples using 6 n. HCL containing 0.1% phenol in an oven at 110°C for 24 h. Amino acids were found using mercaptopropionic acid, O-phthalaldehyde. Chromatographic separation was performed using an ODS2 Waters Spherisorb column. The analysis was performed using 20 mmol/L phosphate (potash) buffer (pH 6.48) as a solvent. A standard mixture of amino acids was prepared by mixing 8 different essential amino acids in 0.1 N HCL and included threonine (Thr), methionine (Met), phenylalanine (Phe), valine (Val), leucine (Leu), isoleucine (Ile), lysine (Lys), and tryptophan (Trp).

Data on grain yield, protein concentration, and gluten were presented as the average ± standard deviation of five biological repeats. Differences between treatments and varieties were separated by two-way analysis of variance performed in SPSS version 13.0. To compare the obtained data, the student's t-test was calculated using Delta 2D software at a significance level of $p \leq 0.05$.

RESULTS

Based on the results of selective breeding work performed during 2004-2017, several new genotypes were created. Special attention should be paid to some of the best varieties in terms of economic characteristics – soft winter wheat Ariivka and winter triticale Myronosets. Ariivka wheat variety was created using the method of intervarietal hybridisation by repeated individual selection from hybrid populations obtained from the crossing of ♀Donska semi-dwarf x ♂ Line K-6477/91. And the Myronosets variety is a method of intervarietal hybridization from crossing ♀ (♀Augusto x ♂Jaguar) x ♂K9844/93 (Fig. 1).

Figure 1. *Winter triticale of Myronosets variety*
Note: *a, b, c – crops, d, e – removal of stamens from triticale spikelets, f, g – ear, h – grain*
Source: *photographed by the authors*

It is known that the growing season duration (GSD) and the phenological phases of plant development are genetically determined parameters (Alipour *et al.*, 2021), which vary in insolation, precipitation, and temperature conditions, which inherently determines plant productivity (Heimler *et al.*, 2010; Dennett *et al.*, 2013; Kim *et al.*, 2017). Therefore, the resistance of triticale Myronosets plants was evaluated in the main phases of plant development, depending on the place of cultivation. A sharp reduction in the duration of the passage of plant development phases affected the structure of the crop, which affected the overall productivity of crops. In this regard, a study of the adaptability of plant varieties for their further cultivation in particular places was conducted.

It was found that for plants of the above-mentioned triticale variety, there is a slow development of the ground part in autumn and spring, compared with plants of the rye varieties under study. This was noted in the tillering phase during 2017 and 2018. Therewith, the average weight of the plant's roots was 0.24 g, and

the ground part was 1.9 g (in the Forest-Steppe). But in the stem elongation phase, the difference between both triticale and rye and wheat plants levelled off and further significant differences (R⩽0.05) is not observed between the dry matter accumulation indicators of the ground part and roots (Table 1).

***Table 1**. Features of dry matter accumulation by grain plants (2017-2018), n=27*

Organogenesis, stage	Weight, 10 plants, g DM	Variety, culture					
		Myronosets (triticale)		Ariivka (wheat)		Borotba (rye)	
		Forest-Steppe	Polissia	Forest-Steppe	Polissia	Forest-Steppe	Polissia
II	Roots	2.3±0.18*	2.5±0.11*	1.7±0.14	2.0±0.09	3.0±0.30	3.2±0.10
	Ground part	1.8±0.10*	2.1±0.15*	1.8±0.19	1.9±0.24	3.3±0.21	3.4±0.14
III	Roots	3.2±0.28*	3.4±0.27	2.8±0.22	3.3±0.29	4.7±0.45	5.2±0.23
	Ground part	2.5±0.26*	3.4±0.30	3.1±0.45	3.0±0.10	4.8±1.04	5.4±0.33
V-VI	Roots	15.7±2.06	19.3±2.00	13.5±2.05	15.0±1.21	18.8±3.20	22.4±2.15
	Ground part	19.5±1.80	20.1±2.16	15.0±2.58	16.5±1.80	20.2±3.76	19.4±2.06

***Note:** * – P⩽0.05 relative to the control (standard); SDx –the standard mean deviation. The differences between the study variants differ statistically at ⩽0.05*
***Source:** compiled by the authors*

The result of successful ecological testing of plants lies in the specific features of their reproductive biology. Both in the conditions of Polissia and in the conditions of Forest-Steppe, plants of the Myronosets variety form the largest number of flowers in the inflorescence (96-121 pcs.), seeds in an ear (73-77 pcs.), compared to wheat (62-70 and 56-59 pcs.) and rye (76-83 and 58-65 pcs.) (Table 2), which indicates a high biological yield potential in triticale.

***Table 2**. Elements of grain plant productivity, Forest-Steppe (2017-2020), n=27*

Total number	Variety, culture					
	Myronosets (triticale)		Ariivka (wheat)		Borotba (rye)	
	Forest-Steppe	Polissia	Forest-Steppe	Polissia	Forest-Steppe	Polissia
Flowers in one inflorescence, pcs.	96±4.0	121±3.0	70±3.0	62±2.0	83±3.0	76±2.0
Spikelets in the main ear, pcs.	32±2.0	31±2.0	22±1.8	23±2.0	28±2.0	29±2.0
Seeds in the main ear, pcs.	77±2.0	73±2.0	56±2.0	59±2.0	65±2.0	58±2.0
Grain weight from the main ear, g	3.6±2.0	2.8±2.0	1.6±2.0	1.1±2.0	2.7±2.0	2.4±2.0
Seed productivity of one plant	12.8±2.0	9.1±2.0	11.2±2.0	7.6±2.0	6.2±2.0	7.7±2.0

***Note:** * – P⩽0.05 relative to the control (standard); SDx – the standard mean deviation. The differences between the study variants differ statistically at ⩽0.05*
***Source:** compiled by the authors*

According to the duration of androgametogenesis and pollen viability in Myronosets plants (unlike rye and wheat), seeds are formed and ripen from the centre to the periphery of the ear. This feature in triticale is important in the formation of highly viable seeds in this part of the ear, and therefore, drought-resistant plant populations can produce conditioned seeds even under limiting factors. Notably, in triticale Myronosets, regardless of the year of research and weather conditions, the grain yield was no less than 8.0 points.

Tests of the new variety have shown that in terms of the number of spikelets in the ear, the number of grains from the main ear, the Myronosets variety is at the level of the best varieties (Ladne, AD 256, Dorena, Slavetne, etc.). And reliable differences obtained in terms of ear density (28.5 spikelets per 10 cm of rachilla) indicate that it forms a denser ear, which is characterised by a better harvesting index. On average, for 2008-2019, the LCH/97 line provided a yield of 6.17 t/ha, which is 0.15 t/ha less than for the AD 256 variety. It was found out that the high grain productivity of the variety is formed due to an increased number of productive stems per 1 m^2 (523 pcs.), a well-seeded dense ear, etc. The amount of protein in triticale grain, its fractional

composition, the presence of essential amino acids, the amount and quality of gluten is an important technological criterion for the nutritional value of protein and grain quality (Rombouts *et al.*, 2009; Xu *et al.*, 2020).

According to 4-year studies, some triticale genotypes, such as rarity (national standard), Myronosets, can produce high stable yields of high-quality grain in Polissia and Forest-Steppe conditions. The data in Table 1 shows that the above-mentioned genotypes have high baking indicators, e.g., the grain character is 690-720 g/l and 35-44% vitreous (flour quality indicators) (Table 3). Analysis of technological indicators of grain showed that the new variety forms grain, with 14.5% protein, 26.8% gluten. The flour from the new winter triticale variety under study is first-class due to its gluten content >14%.

***Table 3**. Indicators of grain properties of winter triticale varieties, average for 2017-2020 (n=4)*

Cultivar	Grain indicators					
	TKW, g	Grain unit, g/l	Protein content, %	Raw gluten content, %	Fat content, %	Starch content, %
Rarytet (control)	47.4±0.9	717.5±2.1	13.5±0.5	23.4±0.3	2.0±0.1	62.0±0.2
Myronosets	46.2±1.5	690±1.5*	14.5±0.2*	26.8±0.2*	2.0±0.0	63.1±0.3*
Woltario	45.0±1.0*	720±2.4	12.9±0.3	22.5±0.3*	2.1±0.0	61.8±0.4
Pawo	44.3±0.8*	704±1.8*	13.6±0.7	26.5±0.2*	1.9±0.0	63.4±0.7*
Ariivka (wheat)	50.3±1.2*	786±2.1*	14.6±0.4*	29.7±0.5*	2.2±0.0	59.5±0.8*
Borotba (rye)	42.0±1.1*	680±0.9*	10.8±0.9*	11.3±0.7*	2.0±0.0	70.3±0.6*

***Note:** * – grain unit; ** – P≤0.05 relative to control (standard); SDx – standard mean deviation. The differences between the study variants differ statistically at p≤0.05*
***Source:** compiled by the authors*

Analysis of the fractional composition of triticale grain proteins of the Myronosets variety showed the superiority of spare proteins gliadins and glutenins (49.3%) over water-soluble proteins – albumins and globulins (36.4%) (Table 4), which is reflected in the higher quality of gluten, dough, bread, and overall bakery score (8.7 points). Rye has lower average and low baking properties (bread volume – 305 ml, total baking score 3.8 pts) caused by a low content of water-insoluble proteins (37.3%). The role of the ratio of gliadins to gluten (Gli/Glu) was established in determining the quality of gluten triticale of the variety under study with high baking properties of the Rarytet variety, which has a Gli/Glu ratio of 0.9, and which together with wheat have higher quality indicators.

***Table 4**. Ratio of protein fractions and baking properties of triticale, wheat and rye, Forest-Steppe, average for 2019-2020 (n=4)*

Cultivar	Grain indicators						
	Content, %			Gli + Glu	Gli/Glu	Bread volume, ml	Overall baking score, pts
	albumin + globulin	gliadin (Gli)	glutenin (Glu)				
Rarytet (control)	36.4±0.3	24.5±0.3	26.7±0.5	51.2	0.9	512±7.5	9.0
Myronosets	34.9±0.2*	22.8±0.4	26.5±0.3	49.3	0.9	520±5.1*	8.7
Woltario	43.5±0.6	20.7±0.3	18.3±0.2	39.0	1.1	440±3.0*	6.5
Pawo	41.7±0.5	21.0±0.4	19.1±0.4	40.1	1.2	407±5.4*	6.2
Ariivka (wheat)	34.6±0.5*	26.2±0.7	29.4±0.1	55.6	0.9	608±12.8*	8.4
Borotba (rye)	50.5±0.2*	20.8±0.3	16.5±0.6	37.3	1.3	305±8.2*	3.8

***Note:** * – P≤0.05 relative to the control (standard); SDx – the standard mean deviation. The differences between the study variants differ statistically at ≤0.05*
***Source:** compiled by the authors*

It is known that the combination of increased plant productivity with low growth and high baking properties in one variety while maintaining complex resistance to adverse environmental factors is important for strengthening its competitiveness (Martiniello, 2011; Liu *et al.*, 2017). In the Myronosets variety, this was achieved. According to the results of hybridological analysis, in crosses of Myronosets plants with tall-growing varieties in the first

generation, heterosis is observed towards reducing the stem of hybrids compared to the parent forms based on "stem height". However, there is a different type of inheritance among hybrid combinations involving the newly created variety (depression, partial negative inheritance, intermediate inheritance, and partial positive dominance). Phenotypic changes in traits in F1 hybrids indicate that genetically, indicators for the "stem height" trait are dominated not only by the additive type of gene interaction, but also by negative dominance and super-dominance.

The quality of triticale grain depends not only on the gluten content and quality, but also on the carbohydrate-amylase complex of the grain (Alijošius *et al.*, 2016). The study of the features of starch content in grain and the carbohydrate-amylase complex allowed figuring out that the starch content in grain of these varieties and crops is quite high and amounts to 64.0-68.8% (Table 1). Starch is the main component of grain, so its content, condition, and properties always affect the rheological properties of the dough, and therefore the quality of the final products. Notably, triticale grain contains a considerable total amount of sugars and has a lower starch gelatinisation temperature, as well as highly active α-amylase (Guzmán *et al.*, 2011; Goral *et al.*, 2021).

Protein-amino acid composition is one of the most complex properties of grain composition. Amino acid composition determines the biological value of food and food products (according to the total amount of non-essential amino acids) as their biochemical criterion (Jaśkiewicz & Szczepanek, 2018). The analysed soft wheat genotypes differ significantly ($P \leqslant 0.05$) in terms of ash, moisture, protein, and starch content. Significant differences between the analysed genotypes were found in the lipid content ($P \leqslant 0.05$). All wheat genotypes were characterised by high protein content and optimal starch and lipid levels. Figure 2 shows a qualitative analysis of essential amino acids in some grain genotypes.

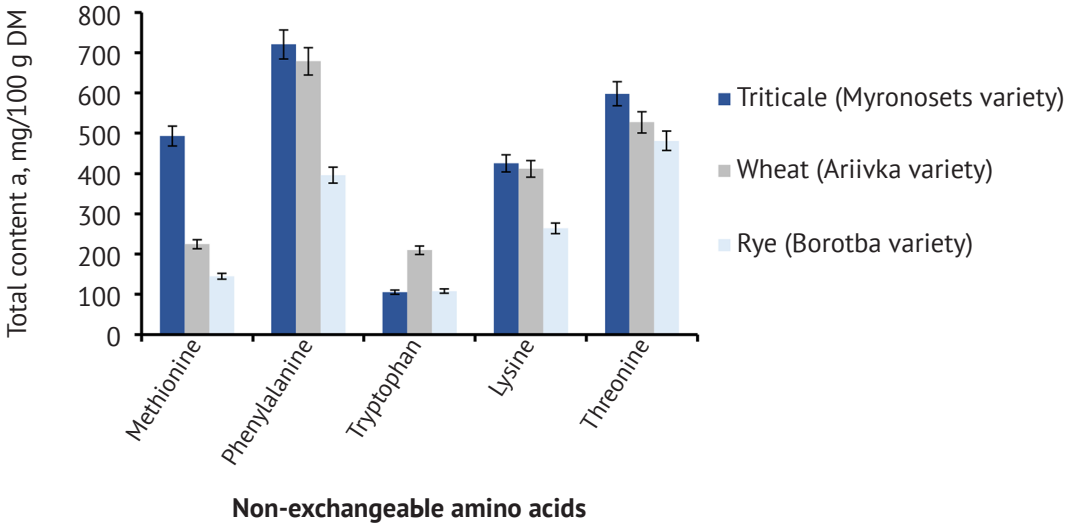

Figure 1. *Content of essential amino acids in triticale, wheat, and rye grains*
Source: *compiled by the authors*

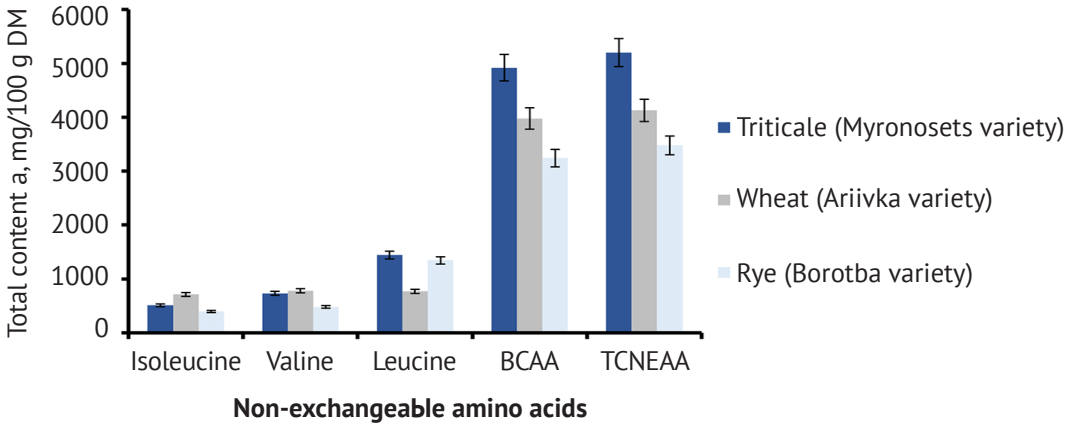

Figure 2. *Content of essential amino acids in triticale, wheat, rye grains, BCAA, TCNAA index*
Note: *(BCAA – aminoacids with branched side chains; TCNAA – total content of non-replaceable amino acids)*
Source: *compiled by the authors*

Figures 2 and 3 show that different genotypes of cereals showed different content of essential amino acids. It was found that the content of such amino acids as leucine (1442 mg/100 g), valine (733 mg/100 g), phenylalanine (720 mg/100 g) and isoleucine (510 mg/100 g) is most pronounced in triticale grain. While in rye grain, the content of which was lower than in triticale and amounted to 1343 mg/100 g for leucine, 481 mg/100 g for valine, and 396 mg/100 g for isoleucine. The triticale of Myronosets variety is superior (4919 mg/100 g DM) to Ariivka wheat variety (3977 mg/100 g DM) and Borotba rye variety (3241 mg/100 g DM) in total amino acid content. However, it is somewhat inferior to wheat grain in terms of tryptophan and isoleucine content. In general, the triticale grain of the Myronosets variety is characterised by a high content of protein and amino acids, which indicates its high nutritional value.

Regarding the pathogens of leaf diseases, the most widespread on grain crops, the following were found: *Blumeria graminis (DS)f.sp. tritici Speer, Puccinia recondite Rob ex Desm f.sp. Septoria tritici Rob. et Desm*. In the conditions of Polissia and Forest-Steppe, the ecological test of the triticale of Myronosets variety allowed estimating the resistance of plants during 2017-2020 and compared with other varieties (Table 5) in terms of the degree of damage by powdery mildew, septoria, brown rust, root rot, and ear fusarium.

***Table 5**. Resistance of triticale plants of different varieties to diseases, pts (2017-2020), n=4*

Disease	Year	Rarytet (control)		Myronosets		Woltario	
		Forest-Steppe	Polissia	Forest-Steppe	Polissia	Forest-Steppe	Polissia
Powdery mildew	2017	9	9	9	9	9	9
	2018	9	8	9	8	9	9
	2019	9	9	9	8	9	9
	2020	9	9	9	8	9	9
	mean	9.0	8.8	9.0	8.3	9.0	9.0
Brown rust	2017	8	7	9	8	9	9
	2018	8.8	8	9	7.5	9	9
	2019	8.5	8.5	9	8	8	9
	2020	7.5	8.0	9	9	8	9
	mean	8.0	8.0	9.0	8.5	8.5	9
Septoria	2017	7.0	8	9	8	9	9
	2018	8.0	8.8	9	9	9	8
	2019	8.0	8.5	9	8	9	7.5
	2020	8.5	7.5	9	9	9	8
	mean	8.0	8.0	9.0	8.5	9.0	8.0
Root rot	2017	8	8	9	8	9	8
	2018	8	8	9	8.0	9	7.5
	2019	8	8	9	8.5	9	7.0
	2020	8	8	9	7.5	9	8
	mean	8.0	8.0	9	8.0	9.0	8.0
Fusarium head blight	2017	8	8	9	8	9	8
	2018	8	8	9	7.5	9	7.5
	2019	8	8	9	8	9	7
	2020	8	8	9	9	9	7
	mean	8.0	8.0	9.0	8.5	9.0	7.5

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

As data from Table 5 shows, the Myronosets variety showed the greatest resistance during the study period to powdery mildew (8.9 points), septoria (8.2 points), its values did not significantly exceed the control-rarity variety. Thus, it is advisable to use the new triticale variety in the selection breeding process for immunity, namely as a source of complex resistance to pathogens.

DISCUSSION

Triticale is the first artificially created grain crop that can to a certain extent predominate in terms of economically valuable characteristics of wheat and rye (Blum, 2014; Jaśkiewicz *et al.*, 2019). Presently, triticale culture has extensive breeding, industrial, and consumer opportunities (Gao *et al.*, 2013; Liubych *et al.*, 2020; Goral *et al.*, 2021). The triticale of Myronosets variety can take its rightful place among grain crops since it has high grain quality indicators, productive potential, and stable yields on an annual basis.

Triticale has a very narrow genetic basis (Blum, 2014; Kim *et al.*, 2017), since most of its germplasm was obtained from only a few materials that were crossed

with each other (Ayalew *et al.*, 2018). Crossbreeding of improved wheat and rye varieties allows getting the best genetic combination to take advantage of the genetic benefits from the selection breeding capabilities of both parent species (Moskalets *et al.*, 2016; Blount *et al.*, 2017).

Back in 1937, Blakeslee & Avery noted that crossing wheat as the parent plant and rye as a pollen donor most often resulted in sterile hybrids. However, when colchicine usage induced polyploidy, they concluded that the chemical doubled the number of chromosomes and produced fertile offspring (Blum, 2014). Based on this knowledge, in 1937, the scientist Pierre Civodron in France improved the technique of obtaining reproductive offspring by crossing wheat and rye. Applying new selection methods, he created many industrial species with good production qualities (Blum, 2014; Moskalets *et al.*, 2016; Parent *et al.*, 2017; Ogbonnaya *et al.*, 2017; Laze *et al.*, 2019; Pradhan *et al.*, 2020).

Triticale refers to carbohydrate, energy-concentrated nutrients (Wang *et al.*, 2020). Recently, apart from corn, it is one of the most important nutrients and energy sources in the nutrition of domestic and farm animals in Ukraine (Sikora *et al.*, 2019). Grains, as well as by-products obtained from triticale processing, are extremely suitable well-digested nutrients for domestic and farm animals, as they contain a large amount of starch and are relatively poor in raw fibre (Makowska *et al.*, 2020). The importance of triticale in direct human nutrition or indirectly as food is great (Zhu, 2018). However, global production of triticale and other cereals is not proportional to the increase in the global population (Glamoclia *et al.*, 2017).

This artificial species is of interest due to its nutritional value, which in many ways exceeds the value of wheat and rye (Tricker *et al.*, 2016; Sheteiwy *et al.*, 2018). The nutritional value of triticale grain is conditioned upon its high content of protein, essential amino acids, minerals, vitamins B, PP, E, and carotenoids. As noted, (Shishlova, 2016; Gao *et al.*, 2016; Jaśkiewicz & Szczepanek, 2018), triticale culture is not very discriminatory in terms of growing conditions and is the most capable among other crops in the zone of weak realisation of biological potentials (Alijošius *et al.*, 2016). Currently, there are several varieties of winter and spring triticale with high productivity, grain quality and stable expression of economic characteristics (Blum, 2014; Würschum *et al.*, 2014). Not all varieties have valuable economic characteristics that meet the requirements of baking and alcohol use (Blount *et al.*, 2017; Glamoclia *et al.*, 2018). Therewith, the expansion of the triticale gene pool with such properties is important for national food safety (Moskalets *et al.*, 2016).

Essential and non-essential amino acids play a significant role in the human diet (Wan *et al.*, 2017; Yasir *et al.*, 2019). Due to their branched-chain structure, the essential amino acids – valine, leucine, and isoleucine – are collectively referred to as branched-chain amino acids (BCAAs). These amino acids play a crucial role in skeletal muscle, not only as a major component of proteins, but also as an energy source, especially during exercise. BCAAs are also involved in the regulation of protein metabolism in skeletal muscle cells (Tsunekawa *et al.*, 2021).

Branched chain amino acids (BCAAs), such as leucine, isoleucine, and valine, are essential for healthy functioning of cells and organs (Almeida *et al.*, 2020). Many authors (Kihlberg & Ericson, 1964; Morey *et al.*, 1983; Dennett *et al.*, 2013; Tsunekawa *et al.*, 2021) proved that high dietary intake of BCAAs prevent the prevalence of overweight and obesity in adults. The BCAA index of triticale grain is 1.4 times higher than that of Ariivka wheat grain and 1.5 times higher than that of rye, which confirms its nutritional and consumer value, from the flour of which it is advisable to produce functional bakery products.

Most of the amino acids present in the analysed genotypes of triticale, rye, and wheat are leucine (636-1442 mg/100 g), phenylalanine (396-720 mg/100 g), valine (291-733 mg/100 g), lysine (264-425 mg/100 g), isoleucine (222-715 mg/100 g), and threonine (481-598 mg/100 g). Gluten protein consists of the monomers gliadin and polymer glutenin, which are recognised as the main reserve proteins of wheat, rich in asparagine, glutamine, arginine, and proline (Rombouts *et al.*, 2009; Brzozowski & Stasiewicz, 2017), but very low in such nutritionally important amino acids as lysine, tryptophan, and methionine (Byrne *et al.*, 2012; Glamoclia *et al.*, 2018). Humans and animals can synthesise nine essential amino acids from 20 amino acids. The remaining amino acids, which are considered essential amino acids, must come from food. These include cysteine (as well as tyrosine), a semi-essential amino acid, since it can only be synthesised from methionine and phenylalanine (Kihlberg & Ericson, 1964; Aho & Koivistoinen, 2009). The amino acid composition is important for determining the nutritional value of cereals for human and animal nutrition (Mosaddek *et al.*, 2013; Penuelas *et al.*, 2020).

Elevated levels of lysine depend on biosynthesis, in which the enzymes aspartokinase (AK) and dihydrodipicolinate synthase (DHPS) play an important role (Postles *et al.*, 2016). An increase in lysine synthesis can be achieved by expressing enzymes (AK, DHPS) that are insensitive to lysine feedback inhibition. This is achieved in grain seeds with a high lysine content (Kondratenko *et al.*, 2015; Xu *et al.*, 2020). Tryptophan acts a precursor of the neurotransmitter serotonin and the pineal hormone melatonin. The tryptophan content obtained in the analysed wheat genotypes was lower than the content reported by Gafurova *et al.* (2002) (1.8-2%), which is higher than that reported by other researchers (Kihlberg & Ericson, 1964; Aho & Koivistoinen, 2009) (0.92-1.0%), but similar to the content reported by Kondratenko *et al.* (2015) (on average 1.07%). According to some authors (Aho & Koivistoinen, 2009; Ackah *et al.*, 2021), cereals contain the amino acid methionine in minimal amounts. Phenylalanine is essential for the growth

and development of young children, and therefore it is enough to consume it in lesser amounts (Singh *et al*., 2012; Alijošius *et al*., 2016).

CONCLUSIONS

A study of the possibilities for agricultural reform has resulted in the following conclusions. As an analysis of global agricultural experience showed, the effective functioning of the agricultural sector could not be achieved without appropriate regulation of tax mechanisms. In each country, the taxation system has its own specific features, which reflect the current state and trends in agricultural development. Primarily, the income tax system is applied, while land taxation has the function of levelling the conditions of agricultural activity.

Each country adapts its agricultural taxation system according to the current economic situation and specific historical background of the state. There are two main approaches to setting up a taxation system for agricultural producers: unified and specialised. In Azerbaijan, the tax legislation classifies the simplified tax as a state tax, although in terms of content and essence it can be considered a special tax regime. The government provides a large number of benefits for enterprises that are involved in the agricultural sector. Yet, the incentive mechanisms must be used rationally, as poor management will not only fail to achieve the long-term goals but will also deteriorate the overall situation in a particular area.

Based on a system-structural approach, a system of definite steps that would optimise the agricultural taxation system in Azerbaijan has been developed. These can be divided into two key areas: the design of a coherent and transparent system of taxation of agribusinesses and the establishment of a system to monitor the effectiveness of enterprises in utilising the various tax incentives.

The modernisation and efficiency of the tax system and the use of new approaches to taxation have always been and will be of great interest to entrepreneurs and ordinary citizens alike. Efforts towards the improvement of the tax system must be sustained at all times. This issue should always be on the national government's agenda. In summary, the findings of this investigation and the conclusions drawn from it can be used as an effective scientific basis for further studies on the prospects for amending the Tax Code in the agricultural sector. This study may prompt other researchers to approach the issue from a new perspective.

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Біоекологічні особливості, біохімічні та фізико-хімічні показники зерна нових генотипів

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Анотація. Представленні дослідження дозволили більше розкрити особливості нових сортів пшениці м'якої озимої та тритикале озимого, демонструючи біоекологічний потенціал їх посівів та біохімічні та фізико-хімічні показники зерна, що має актуальний характер. У статті надані результати з селекційної роботи тритикале озимого, висвітлено параметри продуктивності, біохімічний склад зерна нового сорту Мироносець. Метою досліджень було створити нові генотипи тритикале озимого і пшениці м'якої озимої та вивчити їх за біоекологічним потенціалом та біохімічними та фізико-хімічними показниками зерна. В роботі використані біометричні, фізико-хімічні, біохімічні та математичні методи досліджень. Сорт Мироносець має високу урожайність зерна (6,8-7,2 т/га), високий вміст білку (⩾14.2%) і склад незамінних амінокислот, що підтверджує його харчову та споживчу цінність, з борошна якого доцільно виробляти функціональні хлібобулочні вироби. Вперше визначено, що сорт тритикале Мироносець за вістом загальних амінокислот переважає (4919 mg/100 g DM) пшеницею сорту Аріївка (3977 mg/100 g DM) і жито сорт Боротьбу (3241 mg/100 g DM). Встановлено, що зерно тритикале поступається зерну пшениці за вмістом триптофану і ізолейцину. Жито поступається зерну пшениці за усіма незамінимими амінокислотами окрім лейцину, а перед тритикале усіма амінокислотами, окрім триптофану. Найбільше представленими амінокислотами в зерні тритикале були: Лейцин (1442 мг/100 г), Валін (733 мг/100 г), Фенілаланін (720 мг/100 г) і Ізолейцин (510 мг/100 г), у жита – Лейцин (1343 мг/100 г), Валін (481 мг/100 г) і Фенілаланін (396 мг/100 г). Подальша широка адресна інтродукція нового сорту в агроекосистемах дозволить збільшити обсяги зерна цінного як корму для тварин, так і для виготовлення функціональних хлібобулочних виробів

Ключові слова: незамінні амінокислоти, еколого-адаптивні властивості, біоекологічний потенціал, якість зерна

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Effectiveness of Different Groups of Preparations for Pre-Sowing Treatment of Winter Wheat Seeds

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Abstract. The first stage of modernizing cultivation of the main grain crops lies in the exogenous use of natural growth regulators and nanofertilisers for pre-sowing seed treatment. Special attention is paid to the quality of winter wheat seed material, given the high probability of seed germination in stressful climatic conditions and the need to form winter-hardy crops. Therewith, this study is relevant because modern requirements for agronomic biofortification of plant products make provision for the introduction of environmentally safe, effective, and cost-effective measures in the cultivation of agricultural crops. The use of preparations based on fulvic acids and growth regulators is an effective way to regulate the morphogenesis and productivity of winter wheat. The purpose of this paper was to investigate the effect of pre-sowing treatment of winter wheat seeds with such preparations as Fulvohumin (chelated fertiliser), 1-naphthyl-acetic acid (auxin) and succinic acid (biogenic growth stimulator, adaptogen) on germination and biometric parameters of seedlings. The study involved the use of laboratory and statistical methods. It was found that pre-sowing soaking in 1% Fulvohumin solution increased the energy of seed germination, seedlings contained a large proportion of dry matter, compared to seed treatment with 0.025% succinic acid solution or 1-naphthyl-acetic acid. In terms of germination energy and raw root mass of 7-day shoots, a combination of Fulvohumin and 1-naphthyl-acetic acid was effective. The largest total length and crude weight of 7-day seedlings were after joint seed treatment with Fulvohumin and succinic acid. According to the totality of seedling formation data, complex pre-sowing treatment of winter wheat seeds with Fulvohumin is recommended together with succinic acid and 1-naphthyl-acetic acid. Treatment exclusively with 1-naphthyl-acetic acid was ineffective. These studies are promising for programming field seed germination and mathematical modelling of winter crop growth at the initial stages of ontogenesis in the field

Keywords: Fulvohumin, succinic acid, 1-naphthyl-acetic acid, seed germination, wheat seedlings

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INTRODUCTION

The productivity of field crops, including winter wheat, is dictated by a complex of agrobiological conditions, namely the optimal state of crops. A prerequisite for the formation of high-quality seedlings is the high biological value of seeds, their rapid and uniform germination. The sowing qualities of seeds determine the resistance of seedlings to adverse factors, the intensity of plant development and crop formation. Therefore, pre-sowing laboratory testing of seed material is an essential concept of agricultural technologies for strategically important agricultural crops. Only certified seed material is used for the cultivation of wheat. Batches of this material are diagnosed for germination energy, laboratory germination, purity, and sowing suitability. Industrial processing to produce high-quality commercial seeds involves the removal of chemical germination inhibitors, which is necessary to increase germination, obtain uniform seedlings, and achieve a prominent yield potential. Seeds of the main agricultural crops undergo several stages of pre-sowing treatment (Ignatz *et al*., 2019). Conventional methods of increasing the germination rate of wheat seeds mainly involve chemical treatment, which can be environmentally dangerous. Therefore, new methods of physical influence on seed germination are being investigated. For instance, the study (Wang *et al*., 2022) notes that water activated by plasma in experiments with wheat improved germination.

Presently, the most common means of modifying pre-sowing seed treatment are growth stimulators with microfertilisers. Their segment is becoming increasingly important in the ecological context of less use of pesticides and aggressive agrochemicals in agriculture. Modern aspects of implementing phytostimulants are focused on achieving commercial product quality standards and improving plant viability. Their practical significance lies in the formation of sustainable agricultural systems that are resistant to biotic and abiotic stresses, especially in the context of climate change (Kisvarga *et al*., 2022).

It is important to replenish the content of nutrients to support the stability of the grain crop, and therefore fulvic acids and microfertilisers include technologies for their cultivation (Pashchak *et al*., 2021). Fulvic acids are essential for converting mineral compounds into organic ones that will be absorbed by plants (Kumar & Aloke, 2020). Kumar *et al.* (2020) found that the content of available nitrogen, potassium, and phosphorus increases after the application of fulvic fertilisers. Fulvic acids reduce the pesticide load and are effective when growing in contaminated industrial areas (Braziene *et al*., 2021; Ali *et al*., 2018). These acids also reduce plant uptake of toxic chlorine and cadmium compounds (Yildirim *et al*., 2021).

To date, the problem of resistance of seeds at the germination stage to pesticide stress has been understudied (Kalytka *et al*., 2016). For ecological management of agriculture, the advantages of using phytohormones to intensify plant growth are determined. The role of auxins in inducing root system cell division has been established. Treatment of wheat seeds with indole-3-acetic acid stimulated the growth of the flag leaf, namely its dry weight and area (Aldesuquy, 2011). But a high dose of indolyl-3-acetic acid (50 mg/kg) weakened plant growth. Patented mixtures for pre-sowing seed treatment traditionally include auxins, namely 1-Naphthaleneacetic acid (NAA).

In the state of physiological maturity, seeds are described by low metabolic activity, so to fulfil the productivity potential, it is recommended to treat the seed material with anti-stress growth regulators. In this aspect, succinic acid is considered as an environmentally safe growth regulator. According to Kots *et al.* (2012), in the initial stages of development, seedlings from seeds treated with succinic acid have increased resistance to osmotic shock, high temperature, and pathogens (Kots *et al*., 2012). In general, the stimulating effect of succinic acid on the intensity of respiration of germinating seeds is noted, as well as the mobilization of organic substances and an increase in winter hardiness of plants.

There is no doubt that the use of growth regulators of various mechanisms of action with trace elements for pre-sowing seed treatment is a principal factor in increasing the adaptive capabilities of plants at the initial stages of growth and the formation of their high productivity in general. Activation of growth processes and optimization of seedling nutrition is the main management strategy for efficient cultivation of agricultural crops in various soil and climatic conditions. *The purpose of this study* was to select effective combinations of preparations for pre-sowing treatment of winter wheat seeds and significantly increase the intensity of seedling formation.

MATERIALS AND METHODS

The experiment used winter wheat of the Lazurna variety, seeds of the first reproduction, the originator – the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine. The influence of growth regulators on seed germination energy and morpho-biometric parameters at the shoot level was investigated in the laboratory of the Department of Plant Protection and Quarantine of the Uman National University of horticulture in 2020-2021. The effectiveness of pre-sowing treatment of winter wheat seeds was evaluated after soaking in such test substances as succinic acid (0.025% solution), 1-Naphthaleneacetic acid (NAA, 25 mg/l) and Fulvohumin (1% solution) and their combinations: Fulvohumin+NAA, Fulvohumin+succinic acid; succinic acid+NAA, Fulvohumin+succinic acid+NAA at the rate of 10 litres of spray material per 1000 kg of seed material. A comparison option is pre-sowing treatment with water without preparations.

Such indicators as germination energy, total length of shoots, length of roots and aboveground seedlings, raw and dry mass of seedlings were evaluated. Germination

energy was found as a percentage of the total number of seeds taken for germination, as an average of six repetitions of each variant. Sprouted seeds include seeds that have a normally developed sprout and a primary root that is not shorter than the length of the seed. In an undergerminated seed, the root is underdeveloped, or it is not present, as well as the shoot. To find this indicator, 100 seeds were selected from the treated seed fraction and sprouted in Petri dishes between filter paper. Petri dishes were placed in thermostats, where the temperature was supported at 20°C, humidity – 90%. Seed germination was observed daily for seven days. After three days of germination, the germination energy was found, and after seven days – biometric parameters of sprouts (the length of roots, the length of the aboveground shoot, and the total length of seedlings were measured with a measuring ruler). Using OHAUS Pioneer analytical balances, 100 pieces of the seedling parts under study were weighed with an accuracy of 0.0000 g to find their raw mass. In the root and aboveground (shoots) parts of seedlings, the dry matter content was found according to the thermograviometric method (Hrytsaenko *et al.*, 2003). The attachments of plant samples were weighed (per 100 pcs of roots or shoots) before and after drying (at 100-105°C). Percentage of dry matter content (*DMC*) was calculated according to Equation 1:

$$DMC(\%)=Mraw\times 100/Mdry \quad (1)$$

where *M* is the sample weight, g.

The authors of this study used the State Standard of Ukraine 4138-2002 "Seeds of agricultural crops. Methods of determining quality" and the recommendations of the International Seed Testing Association (ISTA) to follow the methodology for determining the sowing qualities of winter wheat and biometric parameters of seedlings (Seeds of agricultural crops, 2003; International Seed Testing Association, 2011; Kalenska, 2011).

Statistical processing was performed according to the variance method (Ushkarenko, 2013). Correlation analysis and graphical representation of results – using Microsoft Office Excel 7.0 and Statistica 10.0 software. The results were calculated at a significance level of 0.05.

Research objects. Fulvohumin is a solution containing fulvic acids and their salts with trace elements. It is used for pre-sowing seed treatment, stimulating plant growth and development, correcting micronutrient deficiencies, strengthening plant immunity and increasing yield. Content of active substances: N – 0.1-3.0%, P_2O_5 – 0.1-2.5%, K_2O – 0.1-4.0%, C_{gen} – 16.0-18.0%, carbon of humic acids (C_{ha}) – 0.1-4.0%, carbon of fulvic acids (C_{fa}) – 12.0-15.0%, organic matter – 40.0-90.0% (List of pesticides and agrochemicals..., 2020).

Succinic acid ($C_4H_6O_4$) (SA) – (butanedioic acid, ethane-1,2-dicarboxylic acid, $HOOCCH_2CH_2COOH$ – an organic dibasic saturated carboxylic acid. Pure acid is colourless crystals. Solid substance. Highly soluble in water and ethanol (Succinic acid, 2020).

(Alpha-) 1-Naphthaleneacetic acid (NAA) is an auxin, an odourless solid substance, white or colourless, the release form is a water-soluble concentrate. A substituted naphthalene derivative with an attached acetic acid residue, one of two possible isomers of naphthaleneacetic acid. Like many other substituted aromatic compounds of acetic acid, it has an auxin-like effect and is used in crop production as a herbicide and growth stimulator (1-Naphthaleneacetic acid, 2020).

RESULTS AND DISCUSSION

Seeds that have a high germination energy produce friendly shoots that are more resistant to adverse conditions. Soaking wheat seeds in bioregulators activates its membrane and enzymatic activity. A close relationship was established between germination rates and wheat seed germination energy. In the experiments of Tariq *et al.* (2017), the length of the roots of seedlings from seeds treated with a 2% nutrient solution was 8-10 cm, and the length of the roots soaked only in water was 4-6 cm. Humus derivatives increased the germination energy and seed germination (Ahmed & Awad, 2020).

In this experiment, the highest germination energy of winter wheat seeds was obtained after its treatment only with Fulvohumin and in a mixture with NAA – 96% (Fig. 1). The germination energy of seeds treated with a mixture of Fulvohumin, succinic acid and NAA was 95%. Pre-sowing treatment of winter wheat seeds with 0.025% succinic acid solution turned out to be more effective against soaking in NAA. In addition, in the NAA variant of pre-sowing treatment, the germination energy was 6% lower than the control. Pre-sowing treatment of seeds with two preparations was less effective with succinic acid and NAA with a germination energy of 88%.

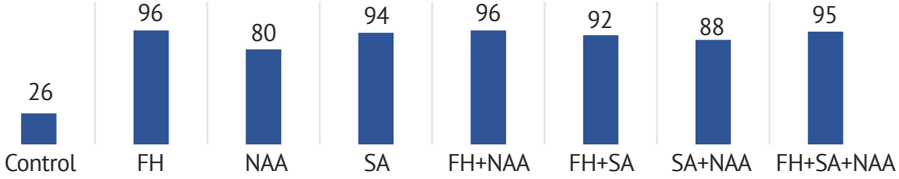

***Figure 1**. Germination energy of winter wheat seeds depending on pre-sowing treatment, %*
***Note:** (FH – Fulvohumin, SA – succinic acid, NAA – 1-Naphthaleneacetic acid) (LSD05 – 6%, V (coefficient of variation) – 7%, experimental average – 91%)*
***Source:** compiled by the authors*

Furthermore, in the Fulvohumin+succinic acid variant, the seed germination energy increased by 2-4%, compared to the use of only one of these preparations. In the experiments of Karashchuk *et al.* (2020) the germination energy of winter wheat seeds treated with Quadrastim with succinic acid increased by 3-8% from the control, field germination was higher by 4-7%. For pre-sowing treatment of tomato seeds, soaking in fulvic acid with a concentration of 80 mg/l is recommended, where germination increased by 13%, and root length – by 32% compared to the control (Zhang *et al.*, 2021).

According to the biometric parameters of wheat seedlings on the 7th day of accounting, among the variants of one-component seed treatment, the advantage of the use of Fulvohumin was noted, where the length of the roots was 6.32 cm, the total length of the seedlings was 12.02 cm, against 5.98 cm and 11.17 cm in the control, respectively (Table 1).

***Table 1**. Biometric indicators of wheat germination depending on pre-sowing treatment, cm*

Variant	Root length	Length of aboveground shoots	Total length of seedlings
Control (water)	5.98	5.19	11.17
Fulvohumin	6.32	5.70	12.02
NAA	4.11	5.61	9.72
Succinic acid	5.38	4.91	10.29
Fulvohumin + NAA	4.62	4.85	9.47
Fulvohumin + succinic acid	6.12	6.25	12.37
Succinic acid + NAA	4.90	5.53	10.43
Fulvohumin + succinic acid + + NAA	5.84	6.36	12.2
Experimental average	*5.41*	*5.55*	*10.96*
LSD_{05}	*0.27*	*0.43*	*0.61*
V, %	*14*	*10*	*11*

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

The length of the roots of wheat seedlings from seeds treated with NAA was the smallest – 4.11 cm, also significantly less than the control after the joint use of NAA with Fulvohumin (by 1.36 cm) or succinic acid (by 1.08 cm).

In experiments with beans, succinic acid did not promote the growth of the root system of seedlings, the length of the main root of shoots was 5% less than the control, despite the higher seed germination (Shevchuk *et al.*, 2019). In this study by the authors, the total length of 7-day-old wheat seedlings after seed treatment with succinic acid was inferior to the control by 0.88 cm. However, the use of two-component treatment Fulvohumin + succinic acid ensured the formation of seedlings of the maximum length – 12.37 cm.

In the variant Fulvohumin + succinic acid+NAA, the seedlings had the largest length of aerial shoots – 23% more than the control, and the total length of the seedlings exceeded the control by 9%. The length of the roots after complex pre-sowing treatment with Fulvohumin+succinic acid+NAA is not significantly greater than the control (by 0.14 cm) but exceeds other applications of NAA. Notably, after NAA treatment, the percentage of length of aboveground shoots is the highest – 58%.

The positive effect of NAA on the formation of the aboveground mass of seedlings is confirmed by the indicators of the raw mass of shoots – 4.612 g/100 pcs. (Table 2). However, the raw mass of the roots was the smallest in the experiment – 3.867 g/100 pcs. According to the indicators of raw root mass, the highest efficiency of two-component pre-sowing treatment of wheat seeds with the preparations under study was noted. Due to the pre-sowing treatment with Fulvohumin + NAA, the raw weight of the roots is 1.4-1.7 times greater, compared to the use of these preparations separately, and 48% greater than the control. The total weight of seedlings in the application of Fulvohumin + NAA was 10.910 g/100 pcs, exceeding control by 25%, and single-component processing options – by 1.2-1.3 times. In addition, when treated with Fulvohumin + NAA, the proportion of raw root mass is the highest in the experiment – 59% of the total.

***Table 2**. Raw weight of wheat seedlings, depending on pre-sowing treatment, g/100 pcs*

Variant	Raw root mass	Raw mass of aboveground shoots	Total raw mass of seedlings	± to control the total raw mass
Control (water)	4.208	4.531	8.739	
Fulvohumin	4.546	4.435	8.981	0.242
NAA	3.867	4.612	8.479	-0.260
Succinic acid	4.295	3.920	8.215	-0.524

Table 2, Continued

Variant	Raw root mass	Raw mass of aboveground shoots	Total raw mass of seedlings	± to control the total raw mass
Fulvohumin + NAA	6.387	4.523	10.910	2.171
Fulvohumin + succinic acid	5.418	6.044	11.462	2.723
Succinic acid + NAA	5.479	5.178	10.657	1.918
Fulvohumin + + succinic acid + NAA	4.673	4.994	9.667	0.928
Experimental average	*4.859*	*4.780*	*9.639*	*1.028*
LSD_{05}	*0.334*	*0.323*	*0.660*	–
V, %	*17*	*13*	*13*	

Source: *compiled by the authors*

Despite the smallest mass of aboveground wheat shoots from seeds treated only with succinic acid – 3.920 g/100 pcs, after its combined use with Fulvohumin, the maximum value of this indicator in the experiment was noted – 6.044 g/100 pcs. Due to the combined treatment with Fulvohumin+succinic acid, the raw root mass increased by 1.210 g/100 pcs from the control, i.e., by 29%. As a result, the total raw mass of seedlings of the Fulvohumin+succinic acid variant was the maximum in the experiment – 11.462 g/100 pcs, exceeding the control by 31%.

In contrast to the elevated indicators of germination energy and seedling length, pre-sowing treatment of wheat with a mixture of Fulvohumin+NAA+succinic acid did not have a unambiguously positive effect on the raw mass of seedlings, yielding to the options of two-component treatments. However, the total mass of seedlings substantially exceeded the control. Upon pre-sowing seed treatment with NAA or succinic acid, the total raw mass of seedlings is insignificantly lower than the control – by 0.260-0.524 g/100 pcs. On average, according to the experiment, the length and raw aboveground mass of shoots on the 7th day of accounting was 50-51% of the total, and a correlation of medium strength was established between these indicators – r=0.73±0.01 (Fig. 2).

Weak correlation between germination energy and total seedling length (r=0.36±0.15) and their total raw mass (r=0.30±0.19) (Figs. 3-4)

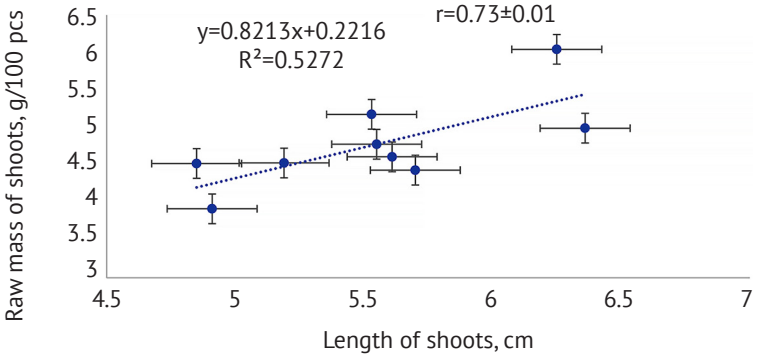

Figure 2*. Correlation of indicators of length and raw mass of aboveground wheat shoots*
Source: *developed by the authors using Excel 2016*

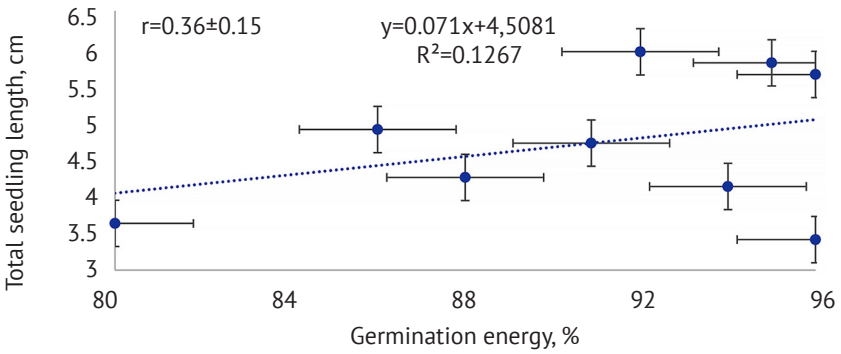

Figure 3*. Correlation of seed germination energy indicators and total wheat germination length*
Source: *developed by the authors using Excel 2016*

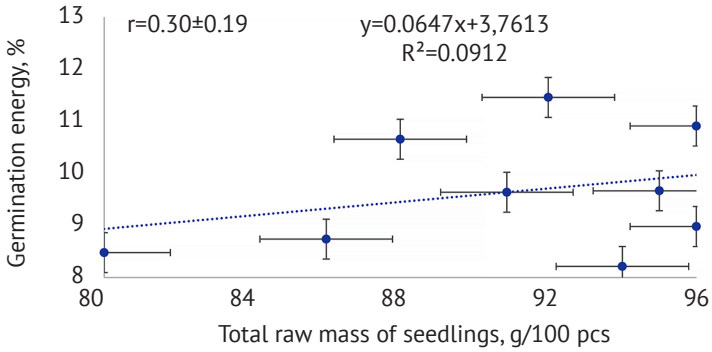

***Figure 4**. Correlation of indicators of the total raw mass of wheat seedlings and seed germination energy*
***Source:** developed by the authors using Excel 2016*

The variation in the dry weight of wheat seedlings after single- and two-component treatments with the preparations under study was significant (Table 3).

Thus, the dry mass of 7-day-old shoots of the succinic acid application options is lower than the control – 0.5904 g/100 pcs, NAA – 0.608 and Fulvohumin + NAA – 0.6109 g/100 pcs. Fulvohumin contributed to the formation of aboveground shoots with the highest dry mass – 0.7445 g/100 pcs, which is 17% of their raw mass (Fig. 5).

***Table 3**. Dry weight of wheat seedlings, depending on pre-sowing seed treatment, g/100 pcs*

Variant	Dry mass of roots	Dry mass of aboveground shoots	Total dry mass of seedlings	± to control, total dry mass
Control (water)	0.5285	0.6359	1.1644	–
Fulvohumin	0.8226	0.7445	1.5671	0.4027
NAA	0.4747	0.6080	1.0827	-0.082
Succinic acid	0.6217	0.5904	1.2121	0.0477
Fulvohumin + NAA	0.7498	0.6109	1.3607	0.1963
Fulvohumin + + succinic acid	0.6896	0.7804	1.4700	0.3056
Succinic acid + NAA	0.6996	0.7290	1.4286	0.2642
Fulvohumin + + succinic acid + NAA	0.9240	0.7766	1.7006	0.5362
Experimental average	*0.6890*	*0.6845*	*1.3733*	–
LSD_{05}	*0.0550*	*0.0498*	*0.0828*	
V, %	*21*	*12*	*15*	

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

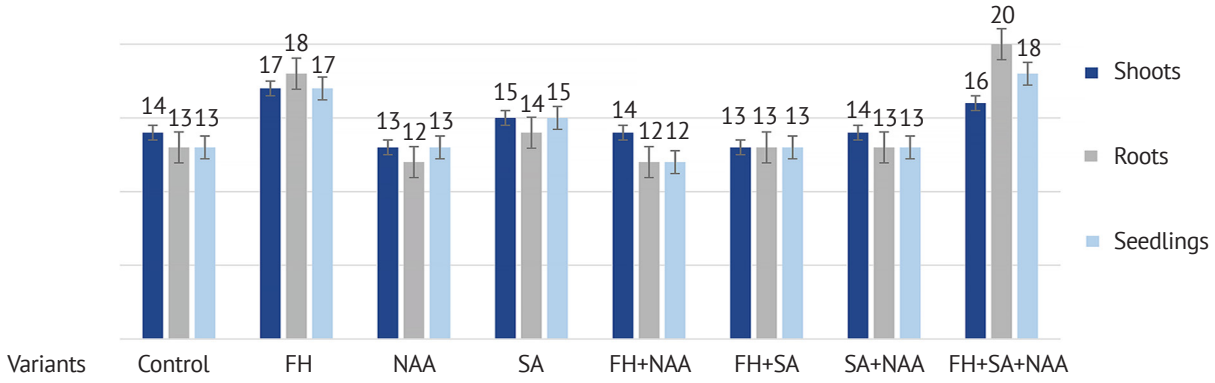

***Figure 5**. The share of dry mass of aerial shoots, roots, and seedlings from their raw mass, %*
***Note:** (FH – Fulvohumin, SA – succinic acid, NAA – 1-Naphthaleneacetic acid)*
***Source:** compiled by the authors*

According to this indicator, the most effective were the combinations of preparations Fulvohumin + Succinic acid – 0.7804 g/100 pcs and Fulvohumin + succinic acid + + NAA – 0.7766 g/100 pcs. The share of dry mass of aboveground shoots of these variants was 13% and 16% of the raw mass, respectively. Complex pre-sowing treatment of wheat seeds with Fulvohumin+succinic acid+NAA provided the maximum dry matter content in the root part of seedlings – 0.9240 g/100 pcs, which is 20% of the raw mass and 1.8 times more than the control. The dry mass of the roots of 7-day-old seedlings after pre-sowing seed treatment with two-component mixtures was 12-13% of the raw mass of the roots and significantly exceeded the control (LSD_{05} – 0.0550 g/100 pcs).

In the control variant, the share of dry mass of aboveground shoots and roots was 13-14% of their raw mass. Dependence between the total dry weight of seedlings (r, g/100 pcs), their length (x, cm) and raw mass (y, g/100 pcs) is calculated using the following regression equation (Eq. 2, Fig. 6):

$$r=0.4899+0.115\times x+0.0623\times y \quad (2)$$

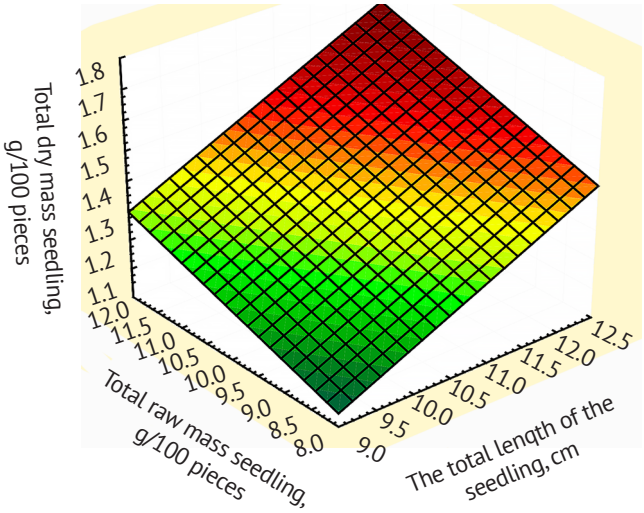

***Figure 6**. Data dependence diagram of total length (x), raw (y) and dry mass of seedlings (z)*
***Source:** developed by the authors using Statistica 10.0.*

There was no positive effect of NAA on the accumulation of dry matter in the root part of wheat seedlings – 0.4747 g/100 pcs, i.e., 10% below the control. The dry mass of roots in wheat seedlings from seeds treated with NAA was 12%, treated with succinic acid – 14%, while upon treatment with Fulvohumin it increased to 18%. The results of other studies show that seed induction with 1-Naphthaleneacetic acid did not contribute to substantial growth of sweet maize, Vigna, and cucumber seedlings (Somtrakoon & Kruatrachue, 2014). At a concentration of 10 mg/l, NAA showed a negative effect on the dry weight of cucumber roots.

It was found that the total dry weight of wheat seedlings strongly depends on the seed germination energy – r=0.69±0.06. The percentage content of dry matter (z, %) in wheat seedlings can be calculated based on the data of total dry mass (x, g/100 pcs) and seed germination energy (y, %) using the following regression equation (Eq. 3):

$$z=3.6525+0.020\times x+6.3721\times y \quad (3)$$

Thus, pre-sowing treatment of winter wheat seeds with Fulvohumin definitely contributes to the accumulation of more dry matter in the aboveground and root parts of seedlings. The total dry weight of seedlings in the variant of pre-sowing treatment with Fulvohumin was 1.5671 g/100 pcs., which is significantly higher than the control. The indicators of the total dry mass of seedlings after treatment with two preparations are higher than the control (1.3607-1.470 g/100 pcs). As a result, wheat seedlings were formed with the highest dry mass after processing with Fulvohumin + succinic acid + NAA – 1.7006 g/100 pcs, which is 46% higher than the control. The total dry mass of seedlings from seeds treated only with Fulvohumin and in the mixture of Fulvohumin + Succinic acid + NAA was 17-18% of the total raw mass, while in the control this indicator was 13%.

Fulvic acids are organic compounds that are common in the soil, but their added application increases crop yields. Therefore, fulvic acids are part of new biofertilisers. The highest efficiency is observed with the complex use of edaphic preparations for seed treatment, soil and foliar fertilization (Marenych *et al.*, 2020). It was experimentally confirmed that humic fertilisers improved root growth, contributed to the formation of high-quality seedlings, and increased the yield of field crops (Savy *et al.*, 2020). According to the experiments of Braziene *et al.* (2021) pre-sowing soaking of wheat, barley, and sugar beet seeds in fulvic acid significantly

increased their germination rate. The height of wheat shoots was greater by 3%, the air-dry mass of roots – by 23%, compared to the control. As a result of the use of fulvic acid, the wheat grain yield increased by 15%. Humic substances investigated by Litvin *et al.* (2020) intensified the growth processes of wheat and simultaneously had a detoxifying effect. The germination rate of wheat seeds after processing was higher than the control by 8-11%, the total length of the roots – by 42-50%.

According to Marenych *et al.* (2019), for foliar feeding of grain crops, fractional application of the humic preparation was more effective than one-time application and gave a yield increase of 16%, compared to the control. Foliar application of fulvic acid eliminates negative conditions after biotic stress of plants. At the rate of application of fulvic acid of 6 mg/l, the highest indicators of wheat plant height (80.89 cm), weight of 1000 grains (33.60 g) and grain yield (4.16 t/ha) were obtained (Al-Haidary & Al-Zubaidy, 2020). The research of Ahmad *et al.* (2018) covered the development of an effective organo-mineral fertiliser. The soil fertiliser contained humic acid, while the leaf fertiliser included fulvic acids and gibberellic acids. Their use increased the yield of wheat.

The most active component that begins to be synthesized by plants under stressful conditions and effectively optimizes their metabolism is succinic acid (Paramo *et al.*, 2020). As an adaptogen and growth stimulator, succinic acid still has a modest sales market, but with prospects due to its biological safety. It was found that in the spring period, succinic acid intensifies the growth of the root system of winter wheat. The average root length of winter wheat plants treated with succinic acid exceeded the control by 21%, and wheat seedlings also had a 4% higher dry matter content (Pryplavko & Gaviy, 2019).

The combined use of succinic acid with microfertiliser turned out to be more effective. For instance, the seed germination of vegetable plants increased by 3%, the energy of germination increased by 10% (Kuts *et al.*, 2021). Treatment of bean seeds with vegetable aqueous solution of succinic acid increased laboratory germination by 7.7%, the number of shoots – by 5.4%, but practically did not affect the length of the hypocotyl (Shevchuk *et al.*, 2019). In experiments with asparagus seeds, succinic acid increased its germination rate by 14%. In addition, according to Shevchenko *et al.* (2021), asparagus seedlings from succinic acid-treated seeds did not substantially increase root mass, while the height of aboveground shoots was 8-14 cm higher than the control. Pre-sowing treatment of seeds with Quadrostim, which includes succinic acid, increased the germination rate of winter wheat. The length of the shoots was 3.2-3.8 cm, the weight was 6.1-6.9 g/100 pcs, while in the control it was 3.6-3.2 cm and 4.4-6.0 g/100 pcs, respectively (Karashchuk *et al.*, 2020).

Pre-sowing treatment of wheat seeds with exogenous phytohormones affects the growth and development of plants along with the differentiated dynamics of their distribution in shoots and roots. The highest content of indolyl-3-acetic acid was noted in the roots of seedlings (Kosakivska *et al.*, 2022). From the group of auxins, 1-Naphthaleneacetic acid is actively used to enhance growth and increase the yield of grain crops (Basuchaudhuri, 2016; Alam *et al.*, 2002). According to Jahan *et al.* (2019), the use of 1-Naphthaleneacetic acid (25 mg/l) in wheat crops positively affects root growth, overall dry biomass, and yield. In the study by Hanaa & Safaa (2019), the maximum indicators of the height of wheat plants (92 cm), thousand-kernel weight (36.5 g), and grain yield (6.61 t/ha) were obtained after spraying the leaves with indolyl-3-acetic acid.

During the study of the effect of growth stimulants on the germination of *Pongamia pinnata* seeds, it was found that the greatest germination was in the seeds soaked in a solution of indole-3-butyric acid, the seedlings had a longer root length. Gibberellic acid, compared to 1-Naphthaleneacetic acid, more significantly increased the length of shoots, leaf area, and total dry mass (Venkatesh *et al.*, 2000). Treatment of maize seeds with heterocyclic compounds and auxins (indolyl-3-acetic and 1-Naphthaleneacetic acids) resulted in an intensification of seed germination and an increase in the growth parameters of maize shoots compared to untreated seeds, but 1-Naphthaleneacetic acid has a lower stimulating effect (Tsygankova *et al.*, 2016). In other experiments, foliar spraying of wheat with 1-Naphthaleneacetic acid (10 mg/l) increased the thousand-kernel weight and yield by 5.2-8.8% (Sun *et al.*, 2018). In general, pre-sowing treatment of wheat seeds should be complex, with phytohormones and nutrient compounds.

CONCLUSIONS

The problem of reducing germination energy due to the use of chemical protectants is especially relevant in the zones of insufficient and unstable moisture, with a lack of soil moisture during the period of sowing winter cereals. Interest in nanofertilisers and bioregulators that improve the quality of crops is constantly growing, and in the future these preparations will become an essential element of the technology of growing major agricultural plants.

Treatment of winter wheat seeds with Fulvohumin or succinic acid increases the energy of its germination to 94-96%. Among the combinations of the preparations under study, the germination energy after treatment with Fulvogmin + NAA (96%) and Fulvogumin + succinic acid + NAA (95%) is significantly greater. According to statistical analysis of biometric data, only Fulvohumin contributes to a significant increase in the length of the root part of seedlings (by 0.34 cm). Pre-sowing application of Fulvohumin with succinic acid provides the formation of higher wheat shoots (12.37 cm) of a larger raw mass (11.462 g/100 pcs). The total raw mass of wheat seedlings from seeds treated with Fulvohumin exceeded the control by 3%, and the raw mass of roots – by 8%.

Pre-sowing treatment of wheat with NAA at a concentration of 0.025% did not have a significant positive effect on seed germination and seedling formation.

The biological value of plants is estimated by indicators of their dry weight. The maximum values of the dry mass of roots and seedlings in general were observed after seed treatment with Fulvohumin and in a mixture of Fulvohumin+succinic acid+NAA – 17-20%. Total seedling dry mass is highly correlated with seed germination energy (r=0.69±0.06), while raw mass is weakly correlated (r=0.30±0.19). Evaluating the seed germination energy, the length of seedlings, their raw and dry mass, pre-sowing treatment of wheat with Fulvohumin and a combination of this preparation with succinic acid and 1-Naphthaleneacetic acid is recommended as the most effective.

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Ефективність різних груп препаратів для передпосівної обробки насіння пшениці озимої

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Анотація. Початковим етапом модернізації вирощування основних зернових культур є екзогенне використання природних регуляторів росту та нанодобрив для передпосівної обробки насіння. Якості посівного матеріалу пшениці озимої приділяється особлива увага, зважаючи на високу ймовірність проростання насіння у стресових кліматичних умовах та необхідність формування зимостійких посівів. Поряд з цим, сучасні вимоги до агрономічної біофортифікації рослинної продукції передбачають впровадження екологічно-безпечних, дієвих та економічно вигідних заходів під час вирощування сільськогосподарських культур, що зумовило актуальність дослідження. Застосування препаратів на основі фульвокислот та регуляторів росту – ефективний спосіб регуляції морфогенезу і продуктивності пшениці озимої. Метою досліджень було вивчення впливу передпосівної обробки насіння пшениці озимої такими препаратами, як Фульвогумін (хелатне добриво), 1-нафтил-оцтова кислота (ауксин) і бурштинова кислота (біогенний стимулятор росту, адаптоген) на проростання і біометричні параметри сходів. У процесі досліджень використовували лабораторні і статистичні методи. Встановлено, що за передпосівного намочування у 1% розчині Фульвогуміну підвищувалася енергія проростання насіння, сходи містили більшу частку сухої речовини, порівняно обробкою насіння 0,025% розчином бурштинової кислоти або 1-нафтил-оцтовою кислотою. За показниками енергії проростання і сирої маси коренів 7-денних сходів ефективною була комбінація препаратів Фульвогумін і 1-нафтил-оцтова кислота. Найбільші загальна довжина і сира маса 7-денних сходів були після сумісної обробки насіння Фульвогуміном і бурштиновою кислотою. За сукупністю даних формування сходів, рекомендується комплексна передпосівна обробка насіння пшениці озимої Фульвогуміном сумісно з бурштиновою кислотою і 1-нафтил-оцтовою кислотою. Неефективною була обробка лише 1-нафтил-оцтовою кислотою. Дані дослідження перспективні для програмування польової схожості насіння і математичного моделювання росту озимих культур на початкових фазах онтогенезу у польових умовах

Ключові слова: Фульвогумін, бурштинова кислота, 1-нафтил-оцтова кислота, проростання насіння, сходи пшениці

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Spread and Harmfulness of Infectious Diseases of the Main Forest-Forming Species in Zhytomyr Polissia of Ukraine

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Abstract. Given the multifunctional role of forests, there is an urgent need in forming biologically stable and productive tree stands to obtain the maximum ecological and economic effect for Ukraine in present-day conditions. Therefore, studies of the species composition, spread, pathogenic action, and harmful effects of pathogens of infectious diseases of the main forest-forming species in Zhytomyr Polissia of Ukraine allow designing a real phytosanitary situation that will take place in the forests in the coming years and developing timely measures to limit the spread of pathological phenomena, which is the relevant research vector. The purpose of this study is to assess the current phytosanitary state of the forests of the surveyed region and identify abiotic and biotic factors that most contribute to weakening and degradation of forest tree species. This paper uses classical forest inventory and phytopathological methods to establish the general phytosanitary condition of the surveyed forests. Special mycological and microbiological methods were also involved to investigate the aetiology and pathogenesis of infectious diseases pathogens. Currently, the comprehensive sanitary condition of the forests of Zhytomyr Oblast can be described as satisfactory. However, every year there is a slow but steady increase in the dieback of forest areas, specifically the main forest-forming species – pine, oak, ash, and birch. During the examinations, the authors noted typical symptoms of bacteriosis (bacterial dropsy), necrosis (dieback), vascular (graphiosis, tracheomycosis) and other diseases of contradictory aetiology (transverse cancer, dieback), as well as fruit bodies of aphilophoroid macromycetes, which are the causes of stem (pine polypore, birch polypore, false oak polypore, etc.) and root (heterobasidion perennial, honey mushroom) rot were noted. Monitoring of the phytosanitary condition with the allocation of predictors of degradation of forest stands will further allow forming a "global map of plant resilience and sensitivity" for analysis of phytosanitary risk and rapid and rational decision-making on forest protection measures

Keywords: aetiology, pathogenesis, wood-destroying fungi, bacteriosis, common pine, common oak, phytosanitary condition

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INTRODUCTION

The general digression of weakened woodlands is an irreversible natural process that has recently been reinforced by the impact of human civilization on forests and dramatic global climate change. The main priority task for forestry workers is to form biologically stable tree stands while preserving healthy trees and simultaneously strengthening their natural immunity.

Various pests and pathogens of infectious pathologies are constantly recorded in the forests of many states. There is no doubt that the forest is a powerful "food resource" for pathogens (Andreieva *et al*., 2018). Thus, it is known that forests around the world are critical habitats in terms of the biological diversity they contain and in terms of the ecological functions they perform. Environmental services of forests are also numerous. Forests regulate the local and global climate, mitigate weather events, regulate the hydrological cycle, protect watersheds and their vegetation, water flows and soils. The need to understand the values found in forests stems from the estimated rate of loss of forest area and, consequently, biological diversity. Currently, it is assumed that the rate of loss is thousands of species per year (Pearce & Pearce, 2001).

Presently, almost the entire Northern Hemisphere is affected by global warming, which results in the drainage of water bodies and subsequently the death of arboreal plants. On the territory of Ukraine (as of 01.01.2020), the total area of forest dieback exceeds 420 thousand hectares. Of them, stands of *Pinus sylvestris* L. – 222 thousand ha, *Quercus robur* L. – over 100 thousand ha, *Picea abies* (L.) Karst. – about 27 thousand hectares, other types of arboreal plants – 64 thousand hectares. There are alarming reports of new foci of forest dieback involving *P. sylvestris* on the territory of Ukraine by secondary pests, namely *Ips acuminatus* and *Ips sexdentatus* (Meshkova *et al*., 2018).

Over the past decades, there have been waves of mass drying of most species of arboreal plants – common pine, common oak, European silver fir, European spruce, common ash, silver birch, etc. Certain assumptions have been made about the causes of this phenomenon, this specifically refers to climate change (Zhang *et al*., 2018) and hydrothermal stress (Christopoulou *et al*., 2022; Rodriguez-Vallejo *et al*., 2021), invasive infectious agents and pests (Goychuk *et al*., 2020), natural biotope changes (Kobal *et al*., 2015), forestry activities (Meshkova *et al*., 2018) and polyfactorial causes (Elling *et al*., 2009).

Researchers name the following infectious agents as the main causes of oak dieback: *Phytophthora ramorum* (Grünwald *et al*., 2008), *Ceratocystis fagacearum* (Bretz.) Hunt. (Juzwik *et al*., 2008), *Ophiostoma roboris* Georgescu et Teodoru (Selochnik *et al*., 2000), *Lelliottia nimipressuralis* (Carter 1945) Brady *et al.* 2013 (Kulbanska *et al*., 2021).

The dieback of common pine trees in the world is mainly associated with diseases of infectious aetiology (Wyka *et al*., 2018), usually caused by *Heterobasidion annosum* ((Fries) Bref.) (Zhezhkun & Porohnyach, 2020), *Fusarium circinatum* (Elvira-Recuenco *et al*., 2020) and *Dothistroma needle* (Bulman *et al*., 2008), as well as xylotrophic insects – *Ips acuminatus* Gyll. (Porohnyach, 2018) and phytopathogenic nematodes – *Bursaphelenchus xylophilus* (Vicente *et al*., 2012; Yusuf *et al*., 2021; Zhen *et al*., 2010; Roques *et al*., 2015).

Degradation of European silver fir is associated with harmful activity of phytopathogenic fungi – *Heterobasidion annosum* (Fr.) Bref. s.l. and plants of the genus *Viscum* L. (Oliva & Colinas, 2010), *Phytophthora citricola* Sawada (Orlikowski *et al*., 2004); phytopathogenic bacteria – *Lelliottia nimipressuralis* (Carter 1945) Brady *et al.* 2013) (Kulbanska *et al*., 2022); insects – *Polygraphus proximus* Blandford (Kharuk, 2017) and high sensitivity to SO_2 emissions (Elling *et al*., 2009).

The weakening and death of silver birch is caused by the mass spread within the range of a dangerous disease of bacterial origin – bacterial dropsy (causing agent – *Lelliottia nimipressuralis* (Carter 1945) Brenner *et al*., 1988) (Goychuk *et al*., 2020). There is also information about mushroom (Pasonen *et al*., 2004; Nguyen *et al*., 2017) and viral (Rumbou *et al*., 2018) diseases that considerably affect the phytosanitary condition of birch.

Pathology of common ash is closely related to various causes of parasitic and non-parasitic origin. The main infectious agents that lead to ash dieback are fungi (Chandelier *et al*., 2016; Lygis *et al*., 2005; Langer, 2017) (*Hymenoscyphus fraxineus*, which is present in Ukraine) (Davydenko *et al*., 2013), bacteria (*Pseudomonas syringae* pv. *savastanoi*, which causes ash tuberculosis and is registered in the forests of Ukraine) (Goychuk *et al*., 2020), nematodes (Ryss & Polyanina, 2018), mycoplasmosis (Bricker & Stutz, 2004), representatives of harmful entomofauna (Davydenko & Meshkova, 2017; Korda *et al*., 2019), as well as the influence of climatic and soil-hydrological factors (Goberville *et al*., 2016).

Currently, there is a great need for a global map of arboreal plant resistance for phytosanitary risk analysis (Magarey *et al*., 2008). The creation of a dieback forecast based on the FORKOME simulation computer model allowed estimating the bioclimatic effects on the growth, stability, survival, and death of spruce and other arboreal plant species (Kozak & Parpan, 2019).

Thus, given the catastrophic extent of degradation that occurs with almost all types of arboreal plants, the disappearance of forests is a complex socio-economic, cultural, and political event. It is a mistake to attribute forest decline to a simple causal relationship, or to assume that the relationship will stay unchanged over time (Contreras-Hermosilla, 2000).

The purpose of this study is to investigate the aetiology and features of symptoms of infectious diseases of the main forest-forming species in the forest coenoses of Zhytomyr Polessia of Ukraine, which will allow for high-quality early diagnosis of pathologies and prompt and rational implementation of forest protection measures.

MATERIALS AND METHODS

The spread and harmfulness of infectious diseases of the main forest-forming species of arboreal plants was investigated in the forests of Zhytomyr Regional Department of Forestry and Hunting (Zhytomyr RDFH). Statistical materials of the State Specialized Forest Protection Enterprise "Vinnytsialisozahyst" (SSFPE "Vinnytsialisozakhyst") and the Department of Forestry, Forest Security and Protection of Zhytomyr RDFH were used to figure out the volume of dieback in tree stands. Reconnaissance and detailed surveys were performed in forest stands with signs of weakening and drying of arboreal plants. In the surveyed forest areas, forest pathological routes were laid, covering tree stands involving common pine, common oak, silver birch, and common ash of different age classes. Detailed studies were performed on permanent and temporary trial areas in SE "Yemilchynske Forestry", SE "Korostenske Forest Hunting Range", SE "Horodnytske Forestry", SE "Zhytomyrske Forestry", SE "Korostyshyvske Forestry", SE "Malynske Forestry", SE "Ovrutske Forestry" and SE "Slovechanske Forestry" according to generally accepted methods and requirements of SOU 02.02-37-476:2006 "Experimental plots of forest management. Method of laying" SOU 02.02.-37-476, 2006 (Forest inventory, 2007). 9 model trees were cut down for research. More than 123 samples (individual tissues and organs) of arboreal plants with visual signs of infectious diseases (trees of the II and III categories of sanitary condition) were selected for mycological and microbiological studies, which were performed per standard protocols and according to Patyka *et al.* (2017). The category of sanitary condition of trees was figured out according to the current requirements (Sanitary rules in the forests of Ukraine, 2020). Based on the results of accounting, the average category (index) of the sanitary condition of the tree stand was calculated. The sanitary condition of coenoses was estimated using the weighted average index of the sanitary condition of the tree stand (I.s.), calculated according to the following the formula:

$$I_s = \frac{\sum k_i n_i}{N}; \quad (1)$$

where: I_s is the phytosanitary index; $k_1 - k_6$ is the category of phytosanitary condition of woody plants; n_i is the total number of arboreal plants in the context of a certain category of tree stand; N is the total number of all trees. Special macroscopic, microscopic, and mycological research methods were used to figure out the species composition of infectious disease pathogens. The collected phytopathological material was identified directly using light microscopy methods in the laboratory. To identify phytopathogenic macromycetes, measures were taken to promote abundant sporulation. For this purpose, the affected parts of arboreal plants were placed in wet chambers of Petri dishes and cultivated for 7-10 days at 25°C.

Specimens of tree destroyers were identified and examined in laboratories using an MBS-9 binocular magnifier and an MBI-3 microscope. Macroscopic structures were studied at microscope magnifications from ×8 to ×100, while microstructure studies were performed on temporary micro-preparations (microscope eyepiece ×15, lenses ×8, ×20, ×40).

Latin names of higher plant species are given according to generally accepted methods (The Plant List, 2022; Index Fungorum, 2022; List of Prokaryotic names..., 2022). Calculation and statistical calculations of the obtained results were performed using *Microsoft Excel* software.

RESULTS AND DISCUSSION

According to the results obtained upon monitoring the sanitary condition of the region under study, it was found that the tree stands of Zhytomyr RDFH involving the main forest-forming species are weakened in phytosanitary condition and medium-damaged in the degree of degradation.

The total area of spread of infectious pathologies in the stands of Zhytomyr RDFH in 2021 covered 4961.7 ha and practically stayed unchanged compared to 2020 (4760.3 ha). The main reason for the mass weakening of the surveyed tree stands are pathogens of stem rot, namely pine polypore (affected area 658.7 ha in 2021 and 312.4 ha in 2020) and birch polypore (affected area 445.6 ha in 2021 and 226.0 ha in 2020), as well as pathogens of root rot, mainly Heterobasidion annosum (affected area 2233.5 ha in 2021 and 2146.6 ha in 2020) and honey fungus (affected area 672.6 ha in 2021 and 811.5 ha in 2020).

For this reason, the largest area of damage (for the last 5 years of observations) of the surveyed forests was recorded in 2019 and amounts to 5548.3 ha, which significantly exceeds the indicators of 2018 and 2017, when the foci of the spread of infectious diseases covered an area of 3913.9 ha and 2301.0 ha, respectively (Fig. 1).

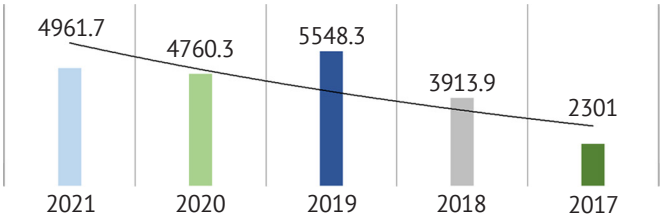

***Figure 1**. Area of distribution of infectious pathologies in the stands of Zhytomyr RDFH, ha*
***Source:** Statistical data of SSFPE "Vinnytsialisozakhyst" (2017-2021)*

Presently, the leading infectious agent that weakens and leads to the death of the surveyed forests is Heterobasidion annosum, the affected area of which stays almost unchanged from year to year (despite constant measures to limit its spread). Thus, the focus of *H. annosum* in 2019 was 2312.9 ha, in 2018 – 2094.1 ha, and in 2017 – 1338.5 ha. Other infectious agents (bacterial dropsy and dieback) that have now spread to smaller areas of the surveyed forests should also be identified, but by their nature, namely virulence and aggressiveness, as well as the ability to form mass epiphytotics, may soon become the main cause of forest degradation.

The species composition of infectious pathologies in the stands of Zhytomyr RDFH and types of woody plants with symptoms of damage are presented in Table 1. In the composition of the mycobiota of the forest coenoses under study, a significant quantitative and qualitative composition is the fraction of aphilophoroid macromycetes (wood destroyers), namely basidiomycetes-xylotrophs. Therewith, the role of the latter in forest ecosystems is ambiguous. On the one hand, as pathogens of rot (root and stem), aphilophoroid macromycetes can have a harmful effect on the sanitary condition. On the other hand, saprotrophic species of basidiomycetes-xylotrophs are destructors of tree mortality; fruit bodies and mycelium of aphilophoroid representatives are an essential component in the trophic chains of many insect species, and certain types of such fungi can be used as indicators of forests unchanged by anthropogenic activity (Methodology, 2018).

***Table 1**. Species composition of infectious pathologies in tree stands of Zhytomyr RDFH*

Latin name (According to Index Fungorum and List of Prokaryotic names with Standing in Nomenclature)	**Type of arboreal plant on which the pathogen is detected (According to The Plant List)**
Pathogens of stem rot	
Phellinus pini (Brot.) Pilát 1941	*Pinus sylvestris L.*
Fomitopsis betulina (Bull.) B.K. Cui, M.L. Han & Y.C. Dai 2016	*Betula pendula Roth.*
Phellinus robustus (P. Karst.) Bourdot & Galzin 1928	*Quercus robur L.*
Phellinus igniarius (L.) Quél. 1886	*B. pendula*
Laetiporus sulphureus (Bull.) Murrill 1920	*Q. robur, Fraxinus excelsior L.*
Fomes fomentarius (L.) Fr. 1849	*Q. robur, B. pendula, F. excelsior*
Fomitopsis pinicola (Sw.) P. Karst. 1881	*P. sylvestris, Q. robur, B. pendula, F. excelsior*
Trichaptum fuscoviolaceum (Ehrenb.) Ryvarden 1972	*P. sylvestris*
Botryobasidium subcoronatum (Höhn. & Litsch.) Donk 1931	*P. sylvestris*
Stereum hirsutum (Willd.) Pers. 1800	*Q. robur*
Chondrostereum purpureum (Pers.) Pouzar 1959	*B. pendula*
Exidia nigricans (With.) P. Roberts 2009	*B. pendula*
Pathogens of root rot	
Heterobasidion annosum (Fr.) Bref. 1888	*P. sylvestris, F. excelsior*
Armillaria mellea (Vahl) P. Kumm. 1871	*Q. robur, P. sylvestris*
Ganoderma applanatum (Pers.) Pat. 1887	*F. excelsior*
Bacterioses	
Lelliottia nimipressuralis (Carter 1945) Brady et al. 2013	*B. pendula*, *Q. robur*
Necroses	
Hymenoscyphus pseudoalbidus Queloz, Grünig, Berndt, T. Kowalski, T.N. Sieber & Holdenr. 2011	*F. excelsior*
Vascular diseases	
Ophiostoma roboris Georgescu & Teodoru 1948	*Q. robur*
Ophiostoma ulmi (Buisman) Nannf. 1934	*Ulmus glabra* Huds.
Diseases of unknown aetiology	
No pathogen detected	*P. sylvestris*
No pathogen detected	*Q. robur*

***Source:** Statistical data of SSFPE "Vinnytsialisozakhyst" (2017-2021)*

The total area of damage to forest stands of Zhytomyr RDFH by pathogens of stem and root rot in 2021 was 4961.7 ha, in 2020 – 4760.3 ha, in 2019 – 5548.3 ha (the highest for the surveyed period), in 2018 – 3913.9 ha, in 2017 – 2301.0 ha (the lowest for the surveyed period). Detailed information on the spread of particular types of pathogens is presented in Table 2.

Table 2. *Areas of distribution of infectious pathologies in the tree stands of Zhytomyr RDFH*

Phytopathogen	Year and distribution area, ha				
	2021	**2020**	**2019**	**2018**	**2017**
Of which, pathogens of stem rot:	*1881.4*	*942.6*	*1499.3*	*924.5*	*355.4*
P. pini	658.7	312.4	488.3	185.3	118.5
F. betulina	445.6	226.0	309.6	162.6	44.8
P. robustus	200.1	315.5	196.1	309.3	0.0
P. tremulae	12.5	0.0	60.9	5.7	10.0
L. sulphureus	165.8	0.0	150.3	74.2	123.9
F. fomentarius	398.7	88.7	294.1	187.4	58.2
Of which, pathogens of root rot:	*2906.1*	*2981.2*	*3725.2*	*2427.0*	*1647.2*
H. annosum	2233.5	2146.6	2312.9	2094.1	1338.5
A. mellea	672.6	811.5	1412.3	332.9	299.0
G. applanatum	0.0	23.1	0.0	0.0	9.7
Bacterial dropsy	148.0	394.3	106.5	261.8	0.0
Transverse cancer	10.0	125.2	89.5	174.4	190.2
H. pseudoalbidus	16.2	0.0	0.0	0.0	0.0
Vascular mycosis (tracheomycosis)	0.0	262.7	62.9	0.6	108.2
Dutch elm disease	0.0	0.0	2.0	0.0	0.0
Dieback of unknown aetiology	0.0	54.3	62.9	125.6	0.0
Total	**4961.7**	**4760.3**	**5548.3**	**3913.9**	**2301.0**

Source: *Statistical data of SSFPE "Vinnytsialisozakhyst" (2017–2021)*

The structure of the species composition of pathologies in the areas under study in 2021 is presented in Figure 2. During the conducted studies, it was found that a larger number of species of aphilophoroid macromycetes, in relation to tree species, have an unequal degree of trophic specialization. Most of the identified aphilophoroid macromycetes belong to xylosaprotrophs that develop on dry and fallen wood. The largest number of species is registered on common pine, common oak, silver birch, and common ash. Thus, aphilophoroid macromycetes are characterized by specialization in substrate-forming tree species (so-called ecosystem engineers).

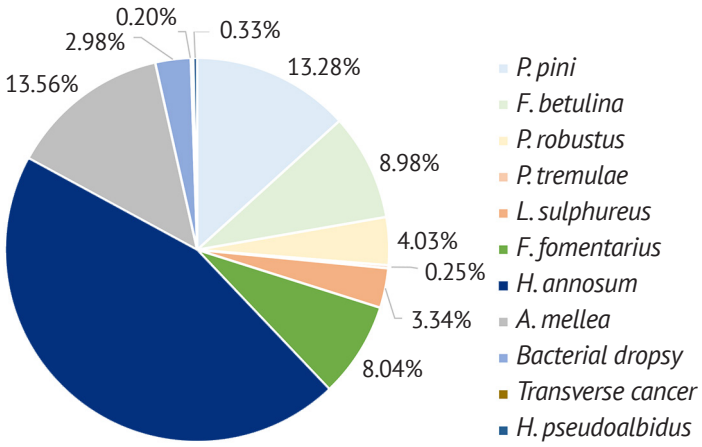

Figure 2. *Area of distribution of infectious pathologies in the stands of Zhytomyr RDFH, ha*
Source: *Statistical data of SSFPE "Vinnytsialisozakhyst" (2017-2021)*

6 species of aphilophoroid macromycetes were detected and identified on common pine. Thus, on the roots, on the lower parts of trunks, on the stumps and trunks of living trees, *Heterobasidion annosum* develops, which causes variegated central rot of wood and roots and is one of the most dangerous pathogens of mycotic diseases and mass dieback of coniferous forests (Fig. 3). H. annosum massively affects pine stands and leads to the loss of a considerable amount of wood, significantly weakens the protective properties of forests. It is established that this pathogen causes the gradual death of arboreal plants, where over time dense stands turn into woodlands. In weakened plants, resin pressure becomes less because it is a mechanical and physiological barrier against the harmful effects of stem pests. This phenomenon is usually typical for weakened trees that grow in a state of physiological stress.

***Figure 3**. Destruction of the root system and common pine fallout from the tree stand due to the influence of wood-destroying fungi*

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

On the growing trunks of common pine trees, the spread of *Phellinus pini* was recorded. On weakened, dead and fallen trunks, fruit bodies of *Fomitopsis pinicola*, *Trichaptum fuscoviolaceum* and *Botryobasidium subcoronatum* are common.

5 species of aphilophoroid macromycetes were detected and identified on common oak: on the trunks and branches of living trees – *Phellinus robustus*, *Laetiporus sulphureus* and *Fomes fomentarius* (Fig. 4). A group of fruiting bodies of *Stereum hirsutum* and single fruiting bodies of *Fomitopsis pinicola* were also noted on dying and dead trunks.

***Figure 4**. Basidiomas of Fomes fomentarius and Phellinus robustus*

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

The most common was P. robustus (affected area in 2021 was 200.1 ha, in 2020 – 315.5 ha, in 2019 – 196.1 ha, in 2018 – 309.3 ha, in 2017 – not registered), which causes the destruction of the core part of the wood, subsequently penetrates into sapwood, cambium, and bast. As a result, dents form on the trunk, and later – cancer wounds.

The overall health of common oak is also substantially affected by the spread of transverse oak cancer (a disease of unknown aetiology). Pathology can affect trunks with thick branches, where it leads to the formation of cancerous growths. At the beginning of the disease, small tumourlike thickenings are formed, which later grow. An important sign is the presence of a transverse crack, which often exposes the wood. The trunk becomes noticeably deformed and the growth of the tree is delayed. It is customary to distinguish between 3 forms of transverse cancer: open, transient, and closed. This disease is moderate in severity, since it does not kill trees (i.e., it does not cause environmental damage), but only reduces the yield of commercial varieties (economic damage to the farm). In 2021, a significant reduction in the area affected by transverse cancer was found

(10.0 hectares were affected). Meanwhile, in 2020 this area reached 125.2 hectares, in 2019 – 89.5 hectares, in 2018 – 174.4 hectares, in 2017 – 190.2 hectares. It was found that the intensity of oak damage by transverse cancer mainly depends on several environmental and forestry factors. Thus, as a rule, in pure oak stands, the intensity of damage is higher than in mixed ones, which is confirmed during reconnaissance and detailed surveys. Various mechanical damages and excessive overgrowth of young stands contribute to the progression of pathology. During the examinations, typical symptoms of bacterial dropsy of oak were noted (Fig. 5). The primary symptoms of the lesion are formed on oak trunks and have the appearance of small (1-2 mm) purple, deep brown spots located under the secondary integumentary tissues. Under favourable conditions for the development of bacteria, the disease progresses, the tree bark becomes deep brown, and a depression is formed.

***Figure 5**. Typical symptoms of the pathogenesis of transverse cancer (left) and bacterial dropsy (right) recorded on common oak*

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

On young oak plants (up to 10 years old), black convex spots first appear. Often in early spring or late autumn, a brown exudate that turns black in the air appears. Under the experimental conditions, the release of bacterial fluid is most often observed in June and lasts for a short time. Diseased oaks have dry tops, fall behind in growth, bush profusely, many buds appear on them, and subsequently water shoots. If the disease develops for an extended period, then the resulting necrosis looks like a step cancer of deciduous plants. With the development of bacterial dropsy of oak, the growth of the focus of infection is not as intense as in cancer pathologies. Internal signs of bacteriosis are described by the presence of deep brown wet wood on the trunk and the presence of a sharp sour smell. On the cross-section, the wet wood area has a regular rounded shape, impregnated with a liquid of a viscous consistency that has an alkaline reaction. Within the surveyed forest stands, bacterial dropsy most intensively affects oaks of ripening and ripe age in various mixing schemes, as well as oaks in clean plantings. The highest percentage of damage is noted on low landforms.

According to the conducted observations in all age categories of stands (involving oak), the greatest danger is vascular mycosis, the spread of which was periodically noted within the surveyed forest stands (affected area: 2021 – 0 ha, 2020 – 262.7 ha, 2019 – 62.9 ha, 2018 – 0.6 ha, 2017 – 108.2 ha). Thus, in the conditions of forestry enterprises of Zhytomyr RDFH, the chronic form of the disease prevails. External signs of oak vascular mycosis damage on mature trees appeared in mid or late summer, while yellowing and leaf fall on individual branches of diseased trees were observed (as a result of the active development of the disease in the spring and summer period). The bark on such branches lost turgor, and the wood was dehydrated. In spring, partial or complete death of individual branches was noticed on the affected trees, buds on them bloomed late or did not develop at all. The leaves that formed on weakly affected branches were smaller. Subsequent stages of the disease were characterized by openness of crown, dryness, formation of water shoots on the trunk and gradual dieback of the tree. An internal sign of oak disease with vascular mycosis is browning of wood elements under the influence of a pathogen (branches, trunk, root system, growth). On the longitudinal section of the branches and the trunk, the water supply elements (large vessels) affected by the pathogen acquire a brown colour with different shades and have the appearance of intermittent lines-strands. Dark solid or fragmentary rings or semi-rings were recorded on a cross-section of the infected tree. This is the result of damage by fungal toxins that cause the death of living parenchymal cells, the formation of humic-like substances that clog the lumen of blood vessels. Often affected trees (to a weak and medium degree with drying branches from 10 to 50%) in subsequent years gradually restore the crown due to water shoots and can remain viable for many years.

Vascular mycosis was detected in all examined middle-aged, maturing, mature, and over-mature tree stands of Zhytomyr RDFH. It was found that with increasing age, the area of oak stands with signs of vascular mycosis and the intensity of its development gradually increases. In mature forests, it makes up an average of 18.0% of the surveyed area. Most of the examined tree stands involving oak are affected by vascular mycosis in mild to moderate degrees. One of the reasons for the high incidence of oak vascular mycosis is stem pests, which are the main carriers of fungal infection.

Aphilophoroid macromycetes of silver burch consortia are represented by three species diagnosed on live trunks: *Fomitopsis betulina*, *Fomes fomentarius* and *Phellinus igniarius*. On stumps and fallen trunks, the spread of *Chondrostereum purpureum* and *Exidia nigricanswas* noted (Fig.6).

Figure 6. *Fruit bodies of C. purpureum (left) and E. nigricans (right)*
Source: *photographed by the authors*

However, the main cause of phytosanitary deterioration and death of trees in the tree stands of Zhytomyr RDFH is the spread of bacterial dropsy (Fig. 7). At an early stage of the disease, its indirect signs are broken canopy, the presence of dry branches and watery shoots, and visual – small oval spots on the bark of a light rusty colour, followed by the leakage of liquid that has an unpleasant smell. The final stage of the disease is characterized by the appearance of large rusty-brown, almost black spots, which subsequently turn into one large wound up to several meters long. The bast part of the wood is destroyed, and the wood is infected with various types of bacteria and wood-destroying fungi.

Figure 6. *Symptoms of birch bacterial dropsy*
Source: *photographed by the authors*

Characteristic signs of ash weakening in the surveyed tree stands are the presence of dry branches and wet shoots (11.0%). In some stands, crown weakening, mechanical damage (3.0%) and frost damage (1.0%) are observed. Among the factors of infectious origin of ash tree damage, pathogens of Hymenoscyphus pseudoalbidus (5.7%) and stem and root rot (20.6%) are most often found.

Characteristic symptoms of ash damage by *Hymenoscyphus pseudoalbidus* are the dieback of individual branches in the crown and the formation of bushy "water" shoots. The infection spreads through the central vein to the leaf petiole and penetrates the shoots of the current year, which subsequently die off and turn brown. In place of the diseased shoot, two shoots are formed next year, which in turn can also become infected. This phenomenon then leads to the formation of bundles of shoots of different drying ages (such as those inherent in growth from stumps). Chronic late blight causes the death of individual large branches and reduces the overall resistance of arboreal plants. This

disease is characterized by a chronic pathogenesis, i.e., it does not "kill" the tree, but makes it more vulnerable (e.g., to secondary pathogens – several types of stump disease). Weather anomalies increase the stress of trees and reduce their resistance to root pathogens. The wood of the root systems is rapidly destroyed, which leads to intense windbreaks. Fruit bodies of wood-destroying fungi *Trichoptera flatworm* and *Heterobasidium annosum* were diagnosed on weakened and dying ash trees.

In summary, the key factor in the forest dieback in Zhytomyr Oblast is the complex effect of ecological and climatic processes with the harmful effect of infectious pathogens. The research results are limited and have not been described by scientists for a thorough scientific comparison.

CONCLUSIONS

Due to the influence of persistent ecological and climatic factors, especially sharp changes in the hydrological regime with a significant lack of moisture in the forests of Zhytomyr Oblast, we observe a significant decrease in the biological stability of the main forest-forming species. On all the surveyed test areas, there is an accumulation of dead wood inhabited by stem pests.

The results of the study of the current phytosanitary condition of forest stands subordinate to Zhytomyr RDFH suggest that the process of deterioration of the phytosanitary condition of arboreal plants of common oak, common ash, common pine, and silver birch closely correlates with the influence of infectious pathogens. The most dangerous diseases are *L. nimipressuralis* and *H. pseudoalbidus* which have an acute nature of the pathological process, the ability to cause large areas of tree mortality, and lead to dieback of affected trees in several years. The total area of damage to forest stands of Zhytomyr RDFH by pathogens of bacteriosis in 2021 was 164.2 hectares. More common, but not so aggressive are pathogens of fungal diseases. Analysing the species composition of the phytopathogenic mycobiota of the forest coenoses under study, the quantitative and qualitative representation is dominated by the fraction of aphilophoroid macromycetes, namely xylotrophic basidiomycetes, which cause root and stem rot. The most dangerous and widespread wood-destroying fungus that causes root rot on common pine and common ash trees is *Heterobasidion annosum*. The largest number of species is registered on common pine (*Phellinus pini*, *Fomitopsis pinicola*, *Trichaptum fuscoviolaceum*, *Botryobasidium subcoronatum*, *Heterobasidion annosum*, *Armillaria mellea*) and common oak (*Phellinus robustus*, *Laetiporus sulphureus*, *Fomes fomentarius*, *Fomitopsis pinicola*, *Stereum hirsutum*, *Armillaria mellea*). Apart from the group of wood-destroying fungi, typical symptoms of tree damage by vascular mycosis (tracheomycosis) (*Ophiostoma roboris*) and graphiosis (*Ophiostoma ulmi*) are also registered, which considerably affect the sanitary condition of common oak and Scots elm. In addition, the general sanitary condition of common oak trees is significantly affected by the spread of a disease of unknown aetiology – transverse oak cancer, which does not cause drying of woody plants, but substantially reduces the marketability of wood.

The total area of distribution of foci of infectious diseases in the forests of Zhytomyr RDFH in 2021 was 4961.7 hectares. Therewith, the indicator of the weighted average phytosanitary index of the surveyed massifs, obtained from the results of dividing arboreal plants into six categories, is satisfactory. In general, the detected and identified diseases are mainly chronic and develop on trees over many years, gradually weakening them.

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Поширення та шкодочинність інфекційних хвороб основних лісотвірних видів у Житомирському Поліссі України

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Анотація. Зважаючи на багатофункціональну роль лісів, є нагальна необхідність у процесі формування біологічно стійких і продуктивних деревостанів для отримання максимального еколого-економічного ефекту для нашої країни в сучасних умовах. Тому дослідження видового складу, поширення, патогенної дії та шкодочинного впливу збудників інфекційних хвороб основних лісотвірних видів у Житомирському Поліссі України дозволяють спроєктувати реальну фітосанітарну ситуацію, що буде в лісах у найближчі роки і вчасно розробити заходи щодо обмеження поширення патологічних явищ, що наразі і є актуальним напрямком дослідження. Мета роботи полягає в оцінці сучасного фітосанітарного стану лісів обстежуваного регіону та виокремленні абіотичних і біотичних чинників, що найвагоміше впливають на ослаблення та деградацію лісових деревних видів. В роботі використано класичні лісівничо-таксаційні та фітопатологічні методи для встановлення загального фітосанітарного стану обстежуваних лісів. А також застосовано спеціальні мікологічні та мікробіологічні методи в частині дослідження етіології та особливостей патогенезу збудників інфекційних хвороб. На даний час комплексний санітарний стан лісових масивів Житомирщини можна охарактеризувати як задовільний. Проте щорічно фіксуєтьсся повільне, але стійке збільшення площ всихання лісових масивів, зокрема основних лісотвірних видів – сосни, дуба, ясена і берези. У ході обстежень відмічено типову симптоматику бактеріозів (бактеріальну водянку), некрозів (халаровий некроз), судинних (графіоз, трахеомікоз) та інших хвороб суперечливої етіології (поперечний рак, всихання), а також плодові тіла афілофороїдних макроміцетів, які є причинами стовбурових (соснова губка, березова губка, несправжній дубовий трутовик та ін.) та кореневих (гереробазидіон багаторічний, опеньок осінній) гнилей. Моніторинг фітосанітарного стану з виокремленням предикторів деградації лісових деревостанів у подальшому дозволить сформувати «глобальну карту стійкості та чутливості рослин» для аналізів фітосанітарного ризику та швидкого і раціонально прийняття рішень щодо проведення лісозахисних заходів

Ключові слова: етіологія, патогенез, дереворуйнівні гриби, бактеріози, сосна звичайна, дуб звичайний, фітосанітарний стан

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Efficiency of Using Plant Antioxidants in the Meat Processing Industry

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Abstract. A decrease in the nutritional value and a reduction in the shelf life of meat and meat products occurs primarily due to lipid peroxidation and microbial contamination of the product during the cold chain. The intensive development of organic animal husbandry requires market operators to ban the use of synthetic preservatives in the food industry and strictly control their use at production facilities. However, although plant extracts look promising as an alternative to antibiotics or chemical preservatives, there is extraordinarily little available information about the effective dose that can be used without the risk of toxic effects in consumers, which determined the relevance of this study. The purpose of this study was to analyse the effectiveness of using plant extracts as natural antioxidants for the meat processing industry. To fulfil the purpose of this study, an analytical method was used. The main factors that reduce the quality and safety of food products during storage were analysed. The paper analyses the effectiveness of using natural, biologically safe antioxidants for the meat processing industry. It was found that the use of bioantioxidants allows minimising oxidative changes and microbial contamination, which can adversely affect the quality of meat and meat-based products. Based on the literature data, it was found that extracts of spicy plants (rosemary, green tea, sage, cloves, mustard, nutmeg, licorice root, ginger, garlic), berries (black currant, cranberry, strawberry, pomegranate, Maki and goji berries, grapes, barberry, plum) and their compositions are effective natural antioxidants that ensure long-term food storage. The intensive development of organic animal husbandry on the territory of Ukraine requires market operators to ban the use of synthetic preservatives in the food industry and strictly control their use at the production facilities of market operators

Keywords: plant extracts, natural antioxidants, meat products, quality and safety, meat storage

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INTRODUCTION

Creating high-quality products and storage stability is one of the key tasks of the meat processing industry. The main factors that reduce food quality and safety indicators are lipid peroxidation and microbial contamination, which lead to changes in sensory properties (colour, texture, smell, and taste), reduced nutritional value and reduced warranty period (Bal-Prylypko *et al*., 2016). That is why the search for effective biopreservants for the food industry and their testing in production conditions is the first urgent task of scientists around the world, which will contribute to the development of organic production and ensure the effective preservation of quality and safety indicators of the finished product during storage with prolonged action.

Lipid peroxidation products can also cause significant harm to the consumer's health. They can directly affect cellular and genomic stability by disrupting gene expression and cellular signalling pathways. The oxidation products of n-3 and n-6 polyunsaturated fatty acids exhibit atherogenic, mutagenic, and carcinogenic effects. Carbonyl and peroxide compounds, formed because of the reaction of oxidised lipids with proteins, can cause atypical cell proliferation and contribute to the development of inflammation and fibrosis (Soletska, 2017).

In their scientific papers, scientists (Qian-Qian *et al*., 2019) note that essential oils of spices are effective natural food preservatives. Most essential oils have a considerable antimicrobial effect against food pathogens that cause food spoilage. It is their use in food production technology that allows the food industry to extend the shelf life of food products, primarily meat and meat-based products, provided that quality and safety indicators are maintained. Thus, delaying lipid peroxidation and preventing cross-bacterial contamination are critical tasks for the food industry.

The purpose of this study was to analyse the effectiveness of using plant extracts as natural antioxidants for the meat processing industry.

LITERATURE REVIEW

One of the ways to improve the quality and safety of perishable products is to use natural, biologically safe preservatives and antioxidants (Ukrainets *et al.,* 2016; Velázquez *et al*., 2021).

With the development of organic production technology and extreme demand in the food industry, namely meat processing, natural plant antioxidants have been acquired (Zheludenko *et al*., 2014; Liebiedieva *et al*., 2016).

The most popular plant antioxidants and preservatives are spicy plants. Presently, there are more than one and a half thousand spices in the world with a unique taste and aroma profile. The most valuable of them grow in tropical countries, where the warm climate contributes to the production of high-quality products due to the rapid accumulation of essential oils. Manual harvesting technology allows preserving the integrity of plant tissues, and therefore the maximum content of active ingredients (Stasyuk, 2018; Atanasova *et al*., 2022).

The world's leading exporters of spices are India, Vietnam, China, Indonesia, Egypt, Brazil, and other countries of southern latitudes. Transportation of natural spices, which ensures the preservation of their quality and safety throughout the transport chain, is a challenging task for logistics companies. Violation of the cold chain, sanitary requirements, hygiene of working personnel, fluctuations in moisture and air speed, integrity of transport containers – all this reduces the quality of spices and leads to secondary contamination by microorganisms. During transportation, they gradually lose their flavour due to evaporation or oxidation of essential oils and other valuable organic compounds that determine their properties (Kovinko, 2016; Khalajji, 2017; Stasyuk, 2018).

Preserving the quality of natural spices and ensuring their stability during storage has become one of the key tasks of modern technologists and engineers. Among the currently developed technologies for processing plant raw materials, the extraction method deserves special attention. Modern production technologies allow obtaining two versions of extracts: dry (powder, complex, granular, encapsulated) and liquid (water-soluble, emulsion, and functional mixtures). Such technologies open wide opportunities for their application (Feng *et al*., 2022).

The use of natural antioxidants of plant origin in the meat processing industry allows regulating the protein, lipid, amino acid, fatty acid, carbohydrate, trace element, and vitamin composition of the final product – meat and meat-based products. An essential factor is that natural antioxidants, unlike synthetic ones, not only do not have a toxic effect on the human body, but also due to the content of several biologically active substances, they can positively affect the health of the consumer (Bilous *et al*., 2019).

RESULTS AND DISCUSSION

Extracts of plant origin (spices, berries, fruits, vegetables) have antimicrobial, antiviral, and anti-inflammatory activity. The antiviral effect is associated with the content of biologically active compounds in the composition of plant components: polyphenols, tocopherols, flavonoids, ubiquinones, vitamins, etc. The antimicrobial activity of plant extracts is determined by the content of phenolic compounds (phytoncides): tannins, flavonoids, simple phenols and their glycosides, phenolic acids, phenol alcohols, anthocyanins (Pasichnyi & Zheludenko, 2014; Valiukh *et al*., 2016).

Polyphenols of plant extracts inhibit the growth of microorganisms (including pathogenic ones) and mould fungi. The mechanism of their action is understudied,

but there is evidence that polyphenols can cause morphological changes in the bacterial cell of microorganisms by damaging its walls and affect the formation of biofilms. They also affect protein biosynthesis, alter metabolic processes in bacterial cells, and inhibit the synthesis of ATP and DNA (by inhibiting DNA gyrase). Thanks to this antibacterial activity of phenolic compounds, plant extracts are an alternative to the use of chemical preservatives in the meat industry, especially sodium nitrite (Efenberger-Szmechtyk *et al*., 2021).

Phenolic compounds have extremely high activity to capture and repair free radicals. Due to this property, natural antioxidants rich in polyphenols can affect lipid oxidation in meat, providing its inhibition. In the meat processing industry, to slow down the oxidation of lipids, plant extracts (berries, fruits, spicy and medicinal plants) with a high content of phenolic compounds are added to meat raw materials: grape seed extract, black currant extract, plum juice concentrate, etc. (Zheludenko *et al*., 2014; Liebiedieva *et al*., 2016).

Recent studies show that due to the high content of polyphenols, certain spices, berries, and their extracts exhibit antimicrobial and antioxidant properties, and therefore they provide a viable alternative to conventional synthetic antioxidants (Bozhko *et al*., 2017). The use of natural additives of plant origin can not only positively affect the shelf life of meat products, but also increase their biological and nutritional value. Sumy's scientists have proved that the antioxidant properties of black currant extract are conditioned upon the high content of anthocyanides in the berry (Bozhko *et al*., 2017; Bozhko *et al*., 2021).

Their research proves (Patent for the invention No. 119078 UA, 2019) that the use of black currant extract (0.01%, 0.02%, 0.03% per 100 kg of minced meat) in the technology of meat-containing boiled sausage based on poultry meat (duck and turkey) can slow down the course of oxidative spoilage of lipids of boiled sausages. Experimentally, the effectiveness of using black currant extract at a concentration of 0.01% was proved: the peroxide number on day 5 was 0.02% J2, while in the control this indicator was 0.13% J2, which is 6.5 times higher.

Furthermore, it was found (Bozhko *et al*., 2021) that the addition of black currant extract doses of 0.1…0.2% to minced meat-containing semifinished products prevents hydrolytic oxidation of fat during long-term storage for 100 days at negative temperatures and, as a result, reduces fat hydrolysis and reduces the peroxidation of free fatty acids.

The use of strawberry extract in the meat processing industry is also effective. Strawberries are a source of antioxidants and hold vitamins, anthocyanins, flavonoids, and phenolic acids (Lorenzo *et al*., 2018). Scientists have proven that adding chopped strawberry extract to minced chicken cutlets in concentrations of 5% and 10% helps to slow down lipid oxidation and ensures their stability during storage at subzero temperatures for 24 days. Furthermore, strawberry extract is thermally stable: during heat treatment, flavonoids are reduced by only 2% and provitamin A, which gives colour to berries (Manessis *et al*., 2020).

Armenteros *et al*. (2013) and Ganhão *et al*. (2013) found that due to the polyphenol content in strawberries (4.3 mg per 1 g of fruit), lipid oxidation in meat and meat-based products slows down. They agreed that the addition of strawberry extract as a natural antioxidant to meat-based products inhibits protein oxidation, as evidenced by the formation of carbon atoms during heat treatment. This ensures the preservation of the quality indicators of Frankfurter sausages for 20 days, provided that they are stored at a temperature of 4°C (Armenteros *et al*., 2013) and pork cutlets for hamburgers (frozen and heat-treated) for 12 days, provided that they are stored in vacuum packaging at 2°C (Ganhão *et al*., 2013).

The use of cranberry extract is also considered promising in the meat processing industry. Cranberry berries hold natural preservatives – organic acids (malic, lemon, chlorogenic, quinic, benzoic) and flavanoids (oxycoric acids, flavones, and anthocyanins). They are characterised by a high content of dietary fibre, sugars (sucrose, glucose, and fructose), essential amino acids (tryptophan, threonine, isoleucine, leucine, lysine, methionine, phenylalanine, valine) and polyunsaturated fatty acids (linoleic and linolenic) (Bozhko *et al*., 2017). Experiments on the use of cranberry extract (concentration of 0.1…0.2% by weight of raw materials) in the recipe of cooked-smoked meat-rich sausage confirmed the property of the extract to slow down the oxidation of lipids and extend the shelf life to 35 days (Bozhko *et al*., 2020).

The method of production of meat-containing boiled sausage "Kachyna" with the addition of cranberry extract to the minced meat for 0.01…0.03% of the mass of the main raw material has been developed. It was found that this amount is best for providing significant inhibition of oxidative processes of the lipid fraction of minced meat, stabilisation of microbiological parameters and high organoleptic parameters of the product (Patent for utility model No. 119891 UA, 2017; Patent for the invention No. 119079 UA, 2019).

Plum fruits also hold phenolic compounds, namely coumarins, flavonoids, and phenolic acid derivatives, as well as substantial amounts of chlorogenic, cryptochlorogenic, and non-chlorogenic acids, which have antioxidant properties (Ahmad *et al*., 2015). Thanks to these compounds, the ingredients obtained from plums exhibit antioxidant and antimicrobial properties. It is proved that when 5% fresh plum juice concentrate, 2.5% or 5% prune juice concentrate are injected into roast beef, protein, and lipid oxidation is inhibited during chilled storage. At the same time, the added ingredients minimally affect the tasting characteristics of meat (Nucez de Gonzalez *et al*., 2008).

Upon studying the antioxidant properties of 10% water infusion of pomegranate (10 ml of infusion was

added to 1 kg of minced meat), Zazharska *et al*. noted the impracticality of its use in the technology of making blood sausage. It was noted that the standard sample of blood sausage according to DSTU 4334:2004 and prototypes with the addition of water infusion of pomegranate (from the membranes and crust) have the same sensory and microbiological indicators during the shelf life regulated by the standard (Zazharska *et al*., 2018).

One of the exotic berries that recently has attracted the attention of scientists as a natural nutraceutical in the food industry has become maqui berry. This is a new Chilean superfruit, the fruit of an evergreen shrub, with high nutraceutical value. Maqui berry (*Aristotelia chilensis*) is also called Chilean blackberries, which are grown in central/southern Chile and southwestern Argentina. It has antioxidant activity due to its high content of biologically active compounds, such as phenolic acids, anthocyanins, and flavonoids (Genskowsky *et al*., 2015; Quispe-Fuentes et al, 2018; Bastías-Montes *et al*., 2020). Furthermore, Maqui berry is a natural antitumour and anti-inflammatory agent that can absorb oxygen radicals, inhibit xanthine oxidase, oxidise lipids, and reduce intracellular oxidative stress (Chen *et al*., 2020).

It is proved that among the proven drying methods (freesing, convective, solar, infrared, and vacuum drying) of Maqui berries, the most effective is the vacuum drying method. It is vacuum-dried fruits that retain the highest content of free flavanols and antioxidant capacity compared to other processing methods (Bastías-Montes, *et al*., 2020). Goji berries hold many nutrients and bioactive compounds, which allowed classifying them as superfruits. They are a source of many biologically active compounds characterised by high antioxidant potential (Kulczyński & Gramza-Michałowska, 2016). Unlike other fruits and fruit extracts, Maqui berries have been tested for use in the meat processing industry as an additive in packaging films (Baek *et al*., 2019).

Scientists from many countries have developed a technology for creating and investigating the effectiveness of using active edible films holding biopolymers (polysaccharides and/or proteins) and active compounds (plant extracts, essential oils, nanofilters, etc.) to reduce losses and extend the shelf life of food products, namely meat-based products. They noted that the inclusion of organic acids, enzymes, antimicrobial proteins, phenolic compounds, or other functional ingredients, such as plant-based nutraceuticals, not only improves the quality of food products, but also extends their shelf life (Benbettaïeb *et al*., 2019).

Two basic statements should be outlined in favour of using active biopolymer films compared to direct administration of antioxidant and/or antimicrobial components in meat products: (1) it is possible to control the diffusion of active compounds to the surface of the finished product and (2) the amount of preservatives added (Jamróz & Kopel, 2020; Smaoui *et al*., 2022).

The authors found that the edible packaging film made of oat starch with the addition of 20% Maqui berry extract for packaging salmon exhibits antioxidant properties by suppressing lipid oxidation under the condition of storage of experimental samples at 4°C (Baek *et al*., 2019).

Analysing the antibacterial ability of edible chitosan films, which include 0.5% or 1% *Maqui berry* extract, it was found that they exhibit a bacteriostatic effect against S. *marcescens*, S. *putrefaciens*, P. *fluorescens*, A.*denitrificans*, A. *hydrophila*, A. *faecalis* and C. *freundii*, L. *innocua*. Furthermore, they exhibit high antioxidant activity (Jamróz & Kopel, 2020; Smaoui *et al*., 2022).

Ginger (Singiber officinale Roscoe) has won the favour of food technologists due to its powerful antibacterial effect and its ability to hydrolyse native collagen (Mao *et al*., 2019; Beristain-Bauza *et al*., 2019). The group of scientists (Hamad *et al*., 2016) proved that ginger essential oil at a concentration of 1250 μg/ml can be used as a natural preservative of fresh chicken meat for 12 days and can extend the shelf life to 6 days at 3-7°C.

Sage extracts (*Salvia officinalis* and *Salvia fruticosa*) hold a wide range of antioxidant compounds (carnosol, carnosic acid, rosmanol, apigenin, luteolin methylcarnosate, rozmadial, Rosmanol-9-ethyl ether, epirosmanol, isorosmanol, and galdozol) that provide its antioxidant, antibacterial, and antifungal effects (Agric, 2013). The literature provides data on the use of sage extract in the technological formulation of meat products (liver pates, meatballs, and sausages) that provides protection against lipid and protein oxidation.

The content of phenols also provides antioxidant properties in barberry leaves, which creates potential for their use in the meat industry. Scientists found that the use of gelatin film with freeze-dried barberry leaves (1 g/kg) for packing meat cutlets considerably inhibits lipid oxidation during 20 days of their storage (Mohd Azman *et al*., 2016).

As a natural antioxidant, green tea extract is used – a source of polyphenols. The use of active packaging made of antioxidant polyamide impregnated with green tea extract for packaging fresh minced meat allows extending its storage period on an industrial scale up to 23 days (Borzi *et al*., 2019).

A source of phytocomponents (gallic acid, catechin, and epicatechin) and phenolic compounds with a high antioxidant effect is grape seed extract (Zheludenko *et al*., 2014). The antioxidant effect of phenolic substances contained in grape seeds is mainly due to two-, three-, oligo-, or polymer proanthocyanidins. Application of a natural preparation made from red grape seeds (in a concentration of 0.5…1.5% by weight of minced meat) in the manufacture of boiled and smoked meat-containing sausages with freshwater aquaculture meat in a natural shell effectively inhibits fat oxidation, which allows preserving the quality of the finished product during storage for 35 days (Bozhko & Tyshchenko, 2020).

I.O. Litvinova (2016) developed a method for obtaining extract from grape seeds and a technology for obtaining an antioxidant additive "Maltovin" on its basis, which contains a phenolic complex and maltodextrin. The use of Maltovin additives in the technological formulation of meat products (meat pate, fried sausage, quick-frozen minced meat semifinished products) (by replacing 2% of the main raw materials) improves rheological parameters, the structure of minced meat, slows down oxidative processes, shows bacteriostatic and fungicidal action, which helps extend their shelf life (Litvinova, 2016).

Among the flavonoid-rich additives, plant-based antioxidants, a special place belongs to rosemary, whose leaves and stems contain more than 12 types of antioxidants, namely rosemarinic, caffeic, carnosic acids, as well as those that can inhibit the oxidation of ascorbic acid and carotenoid fats (Cherednichenko *et al*., 2021).

The most active components of rosemary extracts are carnosic acid and carnosol. They are powerful inhibitors of lipid peroxidation in the microsomal and liposomal systems and absorbers of peroxyl radicals and superoxide anion, thus providing 90% of the antioxidant properties of rosemary. Rosemary extract has a cascading ability to renew vitamin E and is also involved in the carnosic acid cascade. As soon as the antioxidant molecule of carnosic acid "captures" the free radical, it changes its structure turning into carnosine. In the future, carnosol also "captures" the free radical and changes again, transforming into rosmanol. Rosmanol again "captures" radicals and turns into galdozol. Thus, a continuous cascade process is implemented.

Riabovol & Bal-Prylypko (2021) proved that adding 0.15% rosemary extract to minced meat reduces the level of fat peroxide during storage of the finished product for 10 days and slows down the growth rate of fatty acids present in minced meat.

Bozhko *et al.* (2017) developed recipes for meat-rich sausages (Patent for utility model No. 130387 UA, 2018) and meat-rich boiled and smoked sausage (Patent for the invention No. 121263 UA, 2020) made from waterfowl meat, which include the antioxidant of natural origin rosemary extract is injected in a concentration (0.03…0.05% by weight of the raw material). The results obtained by scientists indicate that rosemary extract at a concentration of 0.05% most effectively inhibits the hydrolytic breakdown of acylglycerides (Bozhko *et al*., 2017).

Valiukh *et al.* (2016) found that the use of a 0.02% solution of rosemary extract in minced meat from waterfowl (Peking duck) ensures its microbiological stability for 90 days of storage at -18°C. The amount of mesophilic aerobic and facultative anaerobic microorganisms in prototypes of minced meat at the end of the shelf life is 5.5 times less compared to the control.

Some scientists believe that the use of compositions from plant extracts is more effective. It is the joint mixtures that polygranically increase the biological value of products by introducing essential oils contained in extracts, bioflavonoids (catechins and phenolic acids), and also provide a preventive and restorative effect on the health of the consumer. Furthermore, each of the components of the mixture enhances the effect of the other.

Studies of N.V. Bozhko and L.U. Voytsehivska and others are dedicated to justifying the feasibility of using rosemary and green tea plant extracts and analysing their influence on the physico-chemical parameters of hydrolytic and oxidising processes in minced meat mixtures for meat semifinished products (Bozhko *et al*., 2017)

Scientists of the Institute of Food Resources of the National Academy of Agrarian Sciences (Voitsekhivska *et al*., 2020) found the optimal amounts of addition of biologically active substances to poultry meat of mechanical deboning, which provide the most effective manifestation of their antioxidant properties: dihydroquercetin – 0.025%; fat-soluble and water-soluble green tea extract – 0.05%; water-soluble rosemary extract – 0.07%; fat-soluble rosemary extract – 0.1%. The authors proved the effectiveness of the mentioned extracts in inhibiting oxidation processes during storage for 10…15 days, 3.7…5.2 times compared to the control sample. In addition, Voytsechivska L.U. *et al.* found (Voitsekhivska *et al*., 2020) the effectiveness of the use of these natural antioxidants in stabilising the colour of the meat of mechanically deboned poultry during grinding due to the delay in the oxidation of unsaturated fatty acids, which are catalysed by divalent iron ions present in the pigments of muscle tissue.

The use of natural bioactive substances, such as extracts of rosemary, green tea, and dihydroquercetin, apart from effectively protecting meat lipids from oxidative spoilage, performs the stabilising function of organoleptic parameters – colour and smell (Voitsekhivska *et al*., 2020).

The use of a composition of rosemary and grape seed extracts also reduces the rate of lipid oxidation. The study (Bozhko & Pasichnyi, 2018) investigated the effectiveness of introducing such a composition into minced meat from Peking duck. It was found that the addition of rosemary extract (0.01-0.03%) and grape seeds (0.05-0.15%) helps slow down the hydrolytic lipid oxidation of minced meat by 35.63-57.09%. The results of research prove the prospects of using the composition in the technology of minced meat products with a high fat content.

Zahorui *et al.* (2020) investigated the antioxidant properties of a mixture of spice extracts – cloves, mustard, nutmeg, and garlic (0.5%), when storing melted pork fat under conditions of accelerated kinetic oxidation (102°C). It was found that the use of the mixture extends the duration of fat storage up to 56 days. This mixture of extracts is a promising highly effective bioantioxidant.

CONCLUSIONS

Thus, with the development of organic production in Ukraine, meat industry researchers are focusing on creating new recipes for meat products using natural

and safe ingredients. In this sense, plant extracts are an excellent alternative to synthetic preservatives. Extraction allows protecting the resulting phyto-raw materials from microbiological and mechanical impurities, reducing the volume of industrial storage facilities and, most importantly, extending their shelf life.

Furthermore, this method of processing allows transporting plant extracts over long distances while keeping their quality and safety. With the development of agricultural technologies over the past century, progress has also been made in acclimating spices in temperate countries and matching them with local native plants, which also eases transport logistics. Plant products hold compounds such as flavonoids, vitamins, organic, and phenolic acids, are effective against oxidative processes and have a bacteriostatic effect against bacteria. Extracts of spicy plants (rosemary, green tea, sage, cloves, mustard, nutmeg, licorice root, ginger, garlic), berries (black currant, cranberry, strawberry, pomegranate, Maqui and goji berries, grapes, barberry, plum) and their compositions are promising bioantioxidants with high efficiency. They not only extend the shelf life of meat and meat products, but also positively affect the health of consumers.

The prospects of further studies lie in the development of an effective biopreservative for the meat processing industry based on plant extract of Maqui and goji berries, which will ensure long-term storage of boiled sausages and chilled beef and pork meat.

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Ефективність використання рослинних антиоксидантів у м'ясопереробній промисловості

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Анотація. Зниженння харчової цінності та скорочення терміну придатності м'яса та м'ясопродуктів відбувається, перш за все, внаслідок перекисного окислення ліпідів та мікробної контамінації продукту під час холодового ланцюга. Інтенсивний розвиток органічного тваринництва вимагає від операторів ринку заборону використання синтетичних консервантів у харчовій промисловості та суворий контроль за їх використанням на виробничих потужностях. Однак, незважаючи на те, що рослинні екскракти виглядають багатообіцяючими як альтернатива антибіотикам або хімічним консервантам, доступної інформації про ефективну дозу, яку можна використовувати без ризику токсичних ефектів у споживачів дуже мало, що зумовило актульність даного дослідження. Метою роботи було проаналізувати ефективність використання рослинних екстрактів, як натуральних антиоксидантів для м'ясопереробної промисловості. Для досягнення мети дослідження використано аналітичний метод. Проаналізовано основні фактори, які знижують показники якості та безпечності харчових продуктів під час зберігання. Надано аналіз ефективності використання натуральних біологічно безпечних антиоксидантів для м'ясопереробної промисловості. Встановлено, що використання біоантиоксидантів дає можливість мінімізувати окислювальні зміни та мікробну контамінацію, які можуть негативно впливати на якість м'яса та м'ясопродуктів. На основі літературних даних визначено, шо екстракти пряних рослин (розмарин, зелений чай, шавлія, гвоздика, гірчиця, мускатний горіх, корінь солодки, імбир, часник), ягід (чорна смородина, журавлина, полуниця, гранат, ягода макі та годжи, виноград, барбарис, слива) та їх композиції є ефективними натуральними антиоксидантами, які забезпечують довготривале зберігання харчових продуктів. Інтенсивний розвиток органічного тваринництва на території України вимагає від операторів ринку заборону використання синтетичних консервантів у харчовій промисловості та суворий контроль на виробничих потужностях операторів ринку за їх використанням

Ключові слова: рослинні екстракти, натуральні антиоксиданти, м'ясопродукти, якість та безпечність, зберігання м'яса

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Evaluating the Effectiveness of Catch Crops and Tillage Systems for Carbon Farming

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Abstract. In modern agriculture, it is necessary to identify strategic steps that will reduce greenhouse gas emissions: on the one hand, reducing emissions by cutting down fuel consumption, reducing soil interference, limiting nitrogen losses when using fertilisers, and on the other hand – increasing the efficiency of carbon extraction from the atmosphere through plant photosynthesis and sequestration as organic matter of the soil. The purpose of this study is to figure out the influence on the carbon balance of such elements of the agricultural system as the system of tillage and the use of intermediate cover crops in a model 4-field crop rotation in the Steppe zone of Ukraine. This work was performed using the method of empirical calculations based on the online calculator of greenhouse gas emissions Cool Farm Tool. The influence of intermediate crops in two fields of crop rotation (after the early grain predecessors – wheat and winter barley) and tillage systems (traditional, reduced, and no-till) on the balance of carbon emissions and sequestration in the model 4-field crop rotation was analysed. According to the results, it was found that during the model 4-field crop rotation under the conditions of the classical system of tillage for sunflower and maize without intermediate crops and reduced processing for wheat and barley, the total greenhouse gas emissions amount to 4015 kg/ha of CO_2-eq. in 4 years. Switching to a reduced tillage system has been shown to reduce emissions by 30.1%. The addition of two intermediate crops in two crop rotation fields before spring crops allows obtaining a negative balance of greenhouse gas emissions of -377 kg/ha of CO_2-eq. during this period, and when switching to no-till for all crops -1221 kg/ha of CO_2-eq. for a 4-year rotation period. This study will help identify strategic steps and their potential contribution to the development and implementation of agricultural systems with minimal greenhouse gas emissions

Keywords: carbon farming, soil health, greenhouse gas emissions, intermediate crops, comprehensive quality assessment of intermediate crops, carbon balance

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INTRODUCTION

This paper is the first to consider the principal elements of the agricultural system in terms of reducing greenhouse gas emissions, and to estimate the potential impact of tillage systems and the use of intermediate crops on greenhouse gas emissions in the Southern Steppe of Ukraine.

Natural and anthropogenic factors in global terms are a constant source of emissions of greenhouse gases into the atmosphere, the main of which are CO_2 (carbon dioxide), CH_4 (methane) and N_2O (nitrogen oxide). Historically, for a prolonged period of time, these substances were in a safe ratio and humanity almost did not pay attention to the possibility of a violation of the balance and the emergence of threatening situations. But with the growing anthropogenic impact on the environment, already in the mid-20th century, there was a substantial increase in greenhouse gas emissions associated with the burning of fossil fuels, which over time led to the creation of a greenhouse effect and the threat of global warming (Sixth Assessment Report, 2021; Balyuk & Kucher, 2019; Bedernicek, 2017). Over the past 50 years, the concentration of CO2 increased from 0.03% to 0.042% (Carbon dioxide peaks…, 2021). The result was a noticeable increase in global air temperature by 1.5°C (Sixth Assessment Report, 2021). The average annual air temperature in Ukraine has increased by 1.4°C over 100 years (the average annual air…, 2020). This phenomenon has a global spread on all continents of the planet.

It is important to understand that agriculture also contributes substantially to greenhouse gas emissions. For instance, in 2019, the total emissions of greenhouse gases in Ukraine amounted to 332.1 million tonnes of CO_2-eq., of which the share of agriculture was 13% (Ukraine's greenhouse…, 2021). The main sources in the industries are animal husbandry and crop production. In the field of crop production, the main reasons for a substantial amount of greenhouse gas emissions are related to such reasons as considerable ploughing of land and conventional approaches in agriculture, which involve the desire to get rid of plant residues as soon as possible and substantial intervention in the soil upon its mechanical processing. Special attention should be paid to the increase in the use of nitrogen fertilisers and the associated increase in N_2O emissions, which has a 298-fold higher greenhouse effect (Boychenko, 2002).

Instead, implementing the principles of carbon farming can move it from emissions to carbon sequestration and will not become a source of emissions, but a powerful tool for extracting carbon from the atmosphere.

Research by scientists (Fiorini, 2020; Sauvadet, 2018, Tkachuk & Trofimenko, 2020) in different countries of the world has found that cover crops are an effective tool for carbon sequestration. All these data are summarised and used in various tools for calculating carbon balance, including the Cool Farm Tool (2022).

According to the studies by Poeplauab & Don (2015), the time since the introduction of cover crops into crop rotation was linearly correlated with the change in organic carbon reserves in the soil (R2=0.19) with an annual rate of change at 0.32±0.08 t/ha per year at an average soil depth of 22 cm, the observation period is 54 years.

A study by Tribouillois *et al.* (2018) showed that cover crops can improve the average direct GHG balance by 315 kg/ha CO_2-eq. per year in the long term compared to no cover crops, which could cause a reduction of 4.5-9% of annual greenhouse gas emissions in French agriculture and forestry.

In research by Brazilian scientists, Velosoa *et al.* (2018), the combination that provided the greatest increase in soil organic carbon was a no-till combination with two legume cover crops without nitrogen fertilisers (1.15 t/ha per year) compared to a conventional tillage system. Cover crops of the legume family were twice as efficient at storing organic carbon as nitrogen fertilisers, with 1 kg of applied residues converted to 0.15 kg of soil organic carbon. Changes in soil organic carbon reserves were mainly attributed to plant carbon intake (R2=80%).

The results of studies by Fiorini *et al.* (2020) showed that N_2O emissions under a no-till system were 40-55% lower than under a conventional tillage system. No-till technology also increased the organic carbon content of the soil (by 28%; 0-5 cm) and the number of earthworms (by 5 times) compared to the conventional tillage system. In no-till systems, N_2O emissions were 20-36% lower with rye cover crop than with vetch cover crop ($P<0.05$), which was a consequence of lower availability of mineral nitrogen in the soil under rye than under vetch due to high C/N ratio of rye residues. The combination of no-till and cover rye resulted in the lowest N_2O emissions and the highest yields and should be recommended in the Po Valley region of Italy.

The authors Ruis & Blanco-Canqui (2017) figured out the effect of cover crops and the removal of plant residues from the field of major crops when used for certain purposes. Thus, the removal of more than half of plant residues reduces soil organic carbon reserves by 0.87 t/ha per year, and less than half – by 0.31 t/ha per year. Cover crops increase the organic carbon content of the soil by 0.49 t/ha per year, which indicates that cover crops can compensate for at least some of the organic matter lost with the removal of residues.

The results of the research of Sauvadet *et al.* (2018) showed that the enzymatic efficiency of microorganisms in the soil under a reduced tillage system increased by 49% and 61% in the presence of residues of ripe and flowering wheat, respectively. These results showed that the soil with reduced cultivation benefited from both an increase in the number of residues included in microbial biomass and a decrease in soil carbon loss due to the priming effect, regardless of the degree of decomposition of residues.

In Ukraine, most studies on the effect of intermediate crops on improving soil health have been investigated on green manure. The results of Razanov's (2021) research show that the vegetative mass of green manure of winter wheat, spring barley, winter rapeseed, and peas, incorporated in the soil in post-harvest crops, contributes to an increase in humus content by 0.11-0.14%, alkaline hydrolysed nitrogen – by 1.7-7.1%, exchange potassium – by 27.4-32.2%. The larger the vegetative mass of green manure, the more the content of humus and essential nutrients in the soil.

Egorov (2021) notes that in sod-podzolic soils of Polissia, along with the introduction of manure, the use of straw, green manure, and the use of legumes in crop rotations (namely lupine for green mass and green manure) contributes to the preservation and reproduction of humus content in the soil, improves the balance of nutrients and increases the productivity of arable land in crop rotations, and in its effectiveness approaches the introduction of 10 t/ha of manure in the crop rotation area.

According to Tkachuk & Trofimenko (2020), over a 36-year research period, humus losses on the background of fertiliser-free ploughing annually amounted to 0.13 t/ha, while on non-soil cultivation – 0.11 t/ha. At an average CO_2 emission intensity of 6.3 kg/ha/h from the soil, during the day the volume of emissions is about 167 kg per 1 ha, and for the entire growing season about 20.1 t/ha of carbon dioxide. During the growing season of crops, on sod-podzolic sandy loam soil, non-productive losses of CO_2 range within 2.1-4.2 kg/ha/h.

The purpose of this study is to figure out the influence of soil cultivation and the use of catch cover crops on the carbon balance.

MATERIALS AND METHODS

The paper uses the methodology of empirical calculations for crops of a typical 4-field arable crop rotation in dry land conditions of the Steppe of Ukraine based on the Cool Farm Tool (CFT) greenhouse gas emissions calculator (2022), which is based on IPCC methods (Hansen *et al.*, 2013) and is FAO-approved (Review of GHG calculators…, 2012).

The experimental plot is located near the village of Myrne in the Odesa District of the Odesa Oblast of Ukraine. The plot is located within the Dniester-Buh lowland region of the Black Sea region of the Middle-Steppe subzone of the Steppe zone (geographical coordinates: N 46.47444046488163, E30.40456107404692). According to agropedological zoning, the territory characterises the subzone of the southern Steppe, for which southern chernozems on forest rocks are typical. A strictly arid agroclimatic zone, where the hydrothermal coefficient (HTC) is about 0.7. The study was conducted based on data from 2021.

The crop rotation model and initial data for calculations based on information from standard technological maps of the farm and data on programmed yield for crop moisture availability are presented in Table 1.

***Table 1**. Initial data for calculating greenhouse gas emissions*

Crop rotation culture	Planned yield, t/ha	Fertiliser system*	Plant protection system**	Diesel fuel consumption (excluding crop export), l/ha		
				CT	RT	NT***
Winter barley	4.3	$N_{76}P_{36}K_{18}$, CAM32 – 181 kg/ha, Superagro 12: 24:12 – 150 kg/ha	P – 0.04 l/ha (23.5%), H – 0.07 l/ha (17.5%), F – 0.75 l/ha (30%), I – 0.18 l/ha (24.7%)	–	41.1	26.6
Maize	5.4	$N_{78}P_{34}K_{17}$, Carbamide – 132 kg/ha, Supeagro 12:24:12 – 142 kg/ha	P – 0.05 l/ha (50%), H1 – 2 l/ha (48%), H2 – 2 l/ha (10.5%), I – 0.2 l/ha (15%)	61.0	50.6	33.7
Winter wheat	4.2	$N_{74}P_{33}K_{17}$, CAM32 – 180 kg/ha, DAP – 138 kg/ha	P – 0.04 l/ha (23.5%), H – 0.07 l/ha (17.5%), F – 0.75 l/ha (30%), I – 0.18 l/ha (24.7%)	–	41.1	26.6
Sunflower	2.4	$N_{71}P_{23}K_{12}$, Carbamide – 128 kg/ha, DAP – 96 kg/ha	P – 0.04 l/ha (50%), H1 – 2 l/ha (48%), H2 – 0.05 kg/ha (75%), F – 0.75 l/ha (25%), I – 0.18 l/ha (24.7%)	62.3	51.9	34.5

***Note:** *the rate of nitrogen is calculated for removal by the main part of the crop considering the nitrogen use efficiency approach (NUE) (Oenema, 2015), the rates of phosphorus and potassium fertilisers are calculated based on the law of returning – only for the removed part of the crop. **(P – protectant, H – herbicide, F – fungicide, I – insecticide), rate, l/ha, % a.s.). ***CT – classic tillage is prescribed for maize and sunflower, the main tillage is ploughing and added operations, RT – reduced tillage for wheat and barley – disk ploughing, cultivation, for sunflower and maize – deep tiller (chiselling), NT – no-till*

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

Factors under study:

Factor A. Tillage systems: Variant 1. CT – classic tillage: for maize and sunflower, stubble scouring, ploughing, and cultivation. Variant 2. RT – reduced tillage: for maize and sunflower – deep tillage (chiselling), cultivation; for winter wheat and barley – disk ploughing, cultivation. Variant 3. NT – direct no-till sowing for all crops.

Factor B. Use of catch crops (Table 2):

***Table 2**. Scheme of field employment with main and catch crops in the model 4-field arable crop rotation of the farm*

Option 1 – no catch cover crops:

Crop rotation field	Months of the year											
	1	2	3	4	5	6	7	8	9	10	11	12
1	Winter barley											
2				Grain maize						Winter wheat		
3	Winter wheat											
4				Sunflower						Winter barley		

Option 2 – with catch cover crops:

Crop rotation field	Months of the year											
	1	2	3	4	5	6	7	8	9	10	11	12
1	Winter barley						Catch culture					
2	Catch culture			Grain maize						Winter wheat		
3	Winter barley						Catch culture					
4	Catch culture			Sunflower						Winter barley		

– no crops
– main culture
– catch culture

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

In this study, only the absence or presence of a catch crop in crop rotation is important, regardless of its type, duration of the growing season and biological features, as per the CFT methods. The catch crop is provided here only to "fill in the pauses" between the main crops of crop rotation, continue to sequester carbon from the atmosphere and maintain soil health in the periods between the main crops. In Steppe conditions, one of the most common intermediate crops for this can be mustard, phacelia, spring vetch, millet, etc. These crops, sown in July after grain harvesting, overwinter and their remains stay until the next crop is sown, or are ploughed as green manure in case of a classic tillage system – in both cases, Cool Farm Tool standards make provision for a positive impact from their use.

RESULTS

One of the most principal issues is the correct definition of terms. According to DSTU 4691:2006 (2006), repeated (intermediate) crops are those grown in a crop rotation field when it is free from the main crop. In world standards, the concept of repeated crops intended specifically for preserving soil health is defined by the term "*cover crops*", green manure – "*manure crops*". According to the "Conservation practice standard 340" (2020) – this corresponds to the domestic term "intermediate crops", but with an important clarification that these crops are left in the field without harvesting any biomass of these crops and without burning this biomass. According to the EU definition, "cover crops" are crops sown on arable land specifically to reduce the loss of soil, nutrients, and plant protection products during winter or during other periods when the land would otherwise be exposed and prone to loss. They are usually ploughed in the spring before sowing the next main crop, not harvested, or used for grazing (Cover crop, 2018). In other words, the European policy is not categorical about banning the harvesting of catch crops.

According to DSTU 4691:2006 (2006), underplant, or inter row crop, is a crop sown in a crop rotation field under the cover of the main crop. Such approaches are well known when growing alfalfa or sainfoin under the cover of barley, millet, etc.

Green fertilisers (*green manure*) are plants that are temporarily grown on vacant plots of land to improve soil fertility (DSTU 4691:2006). The international definition of the term "*green manure*" is crops that are ploughed into the soil (using conventional or disc plough) while they are green (Adrian, 1927). Thus, these concepts are identical in Ukraine and the world.

Therefore, using the term "*cover crop*" in modern search engines and international scientific literature, one can find catch crops in the understanding of Ukrainian science as an essential element of carbon farming and restoring soil health. And it is this understanding of the term "catch crops" that is discussed in this paper.

Along with the concept of "soil fertility", a new one has emerged – "soil health". It is known that soil fertility is its ability to provide plants with a complex of conditions for harvest formation (DSTU 4362:2004, 2004). In contrast to fertility, the term "soil health" refers to its compliance with the spectral functions of an ecosystem according to its environment (Soil Health, 2022). This is the harmonious action of living and non-living components of the soil: microbiota, plants, and animals. Unfortunately, Ukrainian agrarian science does not identify or consider this important concept at all, which in its complex meaning defines that the soil is part of nature. Organic matter of the soil is the main factor of its health and fertility, and the primary source of organic matter in the soil is plants.

According to many studies (Fiorini *et al*., 2020; Sauvadet *et al*., 2018, Tkachuk & Trofimenko, 2020), perhaps the greatest contribution to greenhouse gas emissions in crop production is made by tillage through the activation of the processes of mineralisation of plant residues and organic matter. Previously, this was almost the key purpose of cultivation. But currently the views have changed. Therewith, the scientific community has determined that minimising tillage and switching to a no-till farming system substantially reduces emissions and switching to reduced tillage technologies, as defined in the international carbon farming standards (IPCC Assessment Report 6).

In the experiments of Reicosky (1997), in 19 days, as a result of mineralisation, emissions of carbon (C) were as follows: 249 g/m^2 after ploughing, 106.6 g/m^2 after disk ploughing, 99.8 g/m^2 after chiselling and 49.9 g/m^2 after no-till. Therewith, 185 g/m^2 of carbon was accumulated with the remains of the crop – spring wheat, which was harvested before processing. That is, after ploughing for 19 days, the amount of carbon lost was substantially higher than the amount accumulated by the harvested crop, which means the loss of organic carbon of the soil accumulated by previous crops in previous years. The highest amount of greenhouse gas emissions in agriculture occurs precisely because the fields are without plants in the off-season and conventional approaches to tillage.

The increase in the concentration of CO_2 from the standpoint of agronomic science also has positive consequences. The growth of plant biomass on the planet is noted due to the increase in the efficiency of photosynthesis caused by the increase in CO_2 concentration. By 2100, the yield of the main products is expected to increase by 10%, and the biomass of plants – by 12% (Terrer *et al*., 2019). This very phenomenon became the basis for the emergence of a new field in agriculture – carbon agriculture, which involves the effective extraction of CO_2 from the atmosphere due to plant photosynthesis and its preservation in the soil in the form of organic matter (sequestration). The potential for removing CO_2 from the air and sequestering it in the soil with the implementation of carbon farming approaches on the entire arable land of the world is estimated at 10% of current annual emissions, or 8-10 Gt/year (Hansen *et al*., 2013).

Therewith, carbon technology also makes provision for preserving the health of the soil. To some extent, it destroys conventional ideas about "scientifically sound" measures, which ultimately lead to the destruction of both fertility and soil health indicators. Presently, in the EU (Carbon farming, n.d.) identified the following components of carbon farming:

– plants (both the main and catch crops of crop rotation), as one of the main factors of soil formation, which should occupy the field for as long as possible during the year. This means that "rest" in the form of the absence of plants is harmful to soil health;

– tillage minimisation by introducing reduced tillage technologies: minimal, strip, vertical (mini-till, strip-till, verti-till, respectively) and no-till, which are energy-saving at the same time;

– accumulation and preservation of plant residues on the soil surface, which prevents their rapid mineralisation and risks of soil erosion;

– complete elimination of clean vapours as an element of technology that considerably accelerates the mineralisation of organic matter in the soil and increases greenhouse gas emissions;

– measures aimed at reducing N_2O emissions when applying nitrogen fertilisers: methods of applying nitrogen fertilisers with soil wrapping use of nitrification inhibitors to prevent nitrogen loss;

– harmonious management of fertiliser and plant protection systems.

Each of these links in carbon farming is a complex and multi-vector task that needs to be solved comprehensively. This paper analyses and highlights the role of such elements of the agricultural system as soil cultivation systems and the effectiveness of intermediate crops to reduce the time spent in the field without plants, as the main factor of soil formation.

If one analyses modern crop rotations by the periods of time when the field is occupied by the main crop, and when there are potential periods – intervals for catch crops, then often these intervals are longer than the time occupied by the main crops (Table 3).

***Table 2**. Scheme of field employment with main and catch crops in the model 4-field arable crop rotation of the farm*

Crop	Date		Duration of field occupancy, days	Duration of time suitable for growing repeated crops, days
	Sowing	Harvesting		
Winter wheat	25.09	05.07	283	82
Winter rapeseed	05.09	10.07	317	72
Winter barley	10.10	25.06	260	92
Maize	20.04	15.09	148	60 (autumn) 35 (spring)
Sunflower	15.04	05.09	143	70 (autumn) 25 (spring)
Peas	20.03	25.06	96	131
Buckwheat	20.04	20.08	107	103
Silage maize	20.04	20.08	120	85
Alfalfa (2 mowings)	–	20.06	176	136
Pea-oatmeal mixture	20.03	15.06	55	124

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

Therefore, only winter crops occupy the field for 70-85% of the time. Other crops occupy the field 20-37% of the entire year. And if there is black steam in the crop rotation, this figure is only 13%. This disrupts healthy soil processes, so it is recommended to abandon the specified precursor.

Any crop can be either basic or catch. For instance, if buckwheat is sown in the spring, and it is included in the crop alternation scheme, then this is the main crop, and if the same buckwheat is sown in the summer after winter barley, which was in the alternation scheme, then this buckwheat should be considered a repeated crop.

If one calculates the duration of all periods when the field is occupied by the main crop, the winter period when the conditions do not meet the requirements of crops, as well as the period when the field is free, but it is not used for growing repeated crops, then these three periods are 32-35% (Fig. 1).

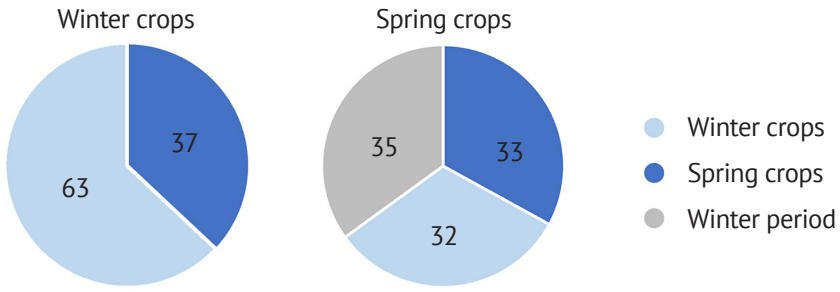


***Figure 1**. Ratio of field occupancy periods with winter and spring crops, %*
***Source:** compiled by the authors*

The above figure convinces of the extreme harmfulness of keeping a field without growing cultivated plants. And here attention is drawn not to the economic component (shortage of products), but to the negative environmental consequences discussed above. Depending on the place allocated for growing in crop rotation, intermediate crops are divided into the following groups (Fig. 2).

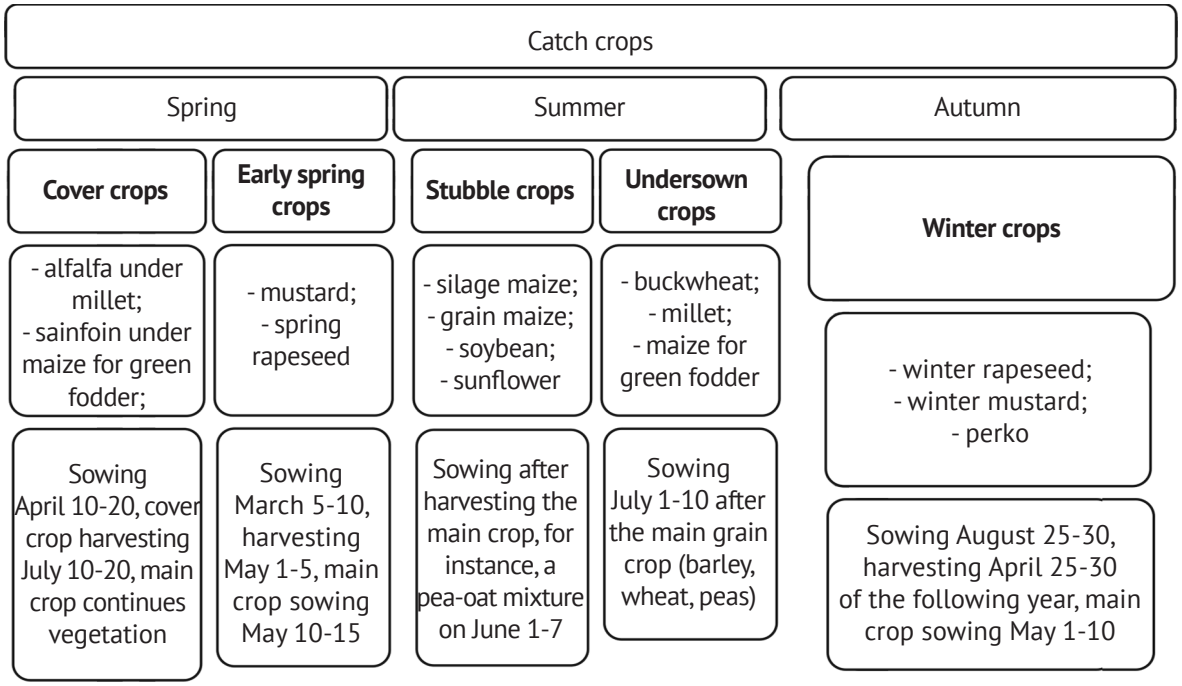


***Figure 2**. Classification of catch crops*

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

From an ecological standpoint, growing catch crops is always a positive measure. But for producers of agricultural products, it is also important to consider the economic feasibility and the possibility of including a catch crop without its adverse impact and the technology of the main crops of crop rotation (sowing dates, contamination, soil water regime, etc.).

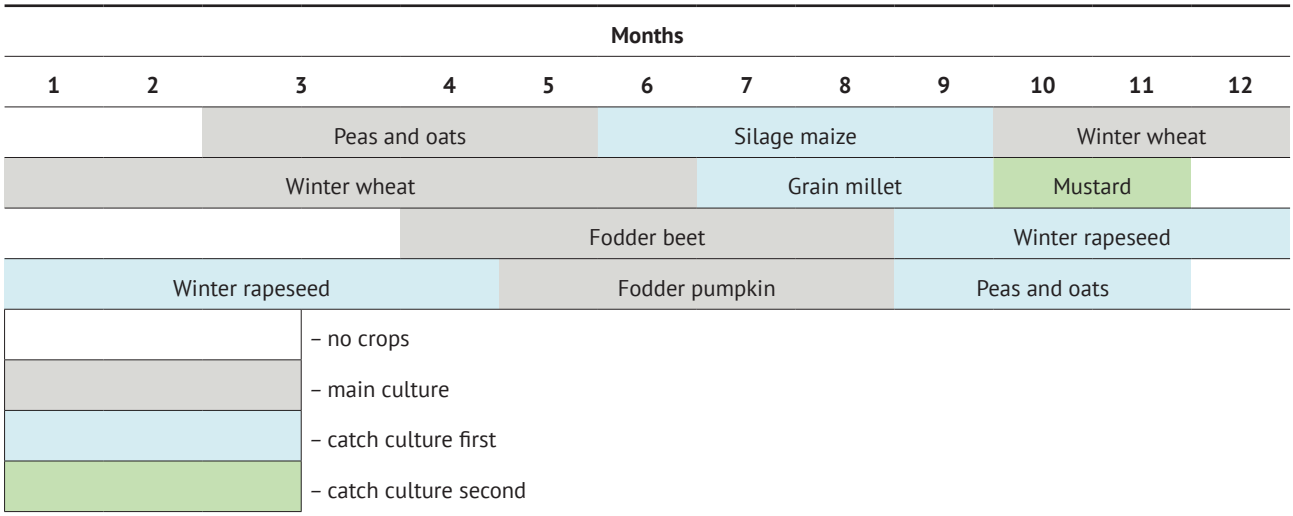
Therefore, for an objective comprehensive assessment, it is necessary to evaluate each intermediate crop by as many indicators as possible, and then figure out the best ones by the sum of places that a particular crop will occupy. It is more appropriate to give an assessment not for the entire set of catch crops, but within their classification groups. For a comprehensive assessment, it is advisable to choose the widest possible range of indicators, but the following are crucial:

1. Duration of vegetation of the catch crop (days).
2. Crude biomass yield, t/ha, as the main indicator from the standpoint of economic activity.
3. Influence of the catch crop on the best parameters of the main crop. In most cases, when growing catch crops, the sowing time of the main crop may shift for a certain time (number of days).
4. Competitiveness of the catch crop in relation to weeds. The contamination is assessed on a 10-point scale. The higher the contamination – the higher the score.
5. Total greenhouse gas emissions during the growing season of the catch crop. The higher this indicator, the worse the quality of the catch crop (measured in t/ha of CO_2-eq.).
6. Direct production costs for growing catch crops, UAH/ha. The lower the cost, the better.

It is also important to consider the potential negative allelopathic effect of the catch crop as a precursor to the next main crop. Such influence should be excluded. The most widespread in the conditions of Ukrainian Steppe are catch crops of the post-harvest group. From the time of harvesting winter cereals (wheat, barley) to the transition of the average daily temperature through +5°C (November 5-16), there are about 90-97 days with the sum of temperatures of 2100°C. This resource ensures the cultivation of most field crops, but the limiting factor in this case is moisture, which is especially scarce in the second half of summer. Therefore, the possibilities of growing catch crops are significantly limited, and these calculations clearly prove this. Evidently, according to the comprehensive indicators, mustard has a substantial advantage, which has a reserve of vegetation and can be sown when the appropriate conditions for moisture supply appear. To increase grain production, millet and buckwheat are quite satisfactory, which as catch crops are not inferior to the main crops in terms of productivity, and often exceed them.

In the presence of intermediate crops with an ultra-short growing season, it is possible to obtain not only two, but three or more crops per year. This possibility is available in fodder and vegetable crop rotations. For instance, in a 4-field fodder crop rotation with alternating crops: 1. pea-oat mixture; 2. winter wheat; 3. fodder beet; 4. fodder pumpkin, it is allowed to grow several intermediate crops between the main ones (Table 4).

***Table 4**. Scheme of field occupancy by main and catch crops in a 4-field fodder crop rotation*



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

If one accurately calculates the duration of vegetation of the main and catch crops, as well as the time when the field was left without crops, then in total for the crop rotation, the results are as follows (Table 5).

***Table 5**. The specific weight of the occupation of the fields by main and catch crops for the 4-field forage crop rotation (1460 days)*

Field occupancy	No catch crops		With catch crops	
	Days	%	Days	%
With main crop	565	38.7	565	38.7
With catch crops	–	–	635	43.5
Duration of the no-sowing period	895	61.3	260	17.8

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

Without catch crops, the field is not covered with plants for 61.3% (almost 2/3 of the time). If one intensifies production, this figure is reduced to 17.8%, with all the resulting environmental consequences discussed above.

Vegetable crop rotations have even greater opportunities for multi-yielding fields, where certain crops have an ultra-short growing season and are grown under irrigation conditions. These crops include radishes, leafy vegetables, early cucumbers, early cabbage, early potatoes, vegetable peas, and many others. There is a lot of room for imagination based on yielding 3-5 crops a year. For instance:

1. radish – 35 days (15.03-25.04),
2. early ripe tomatoes – 75 days (05.05-20.07),
3. cucumbers – 50 days (25.07-15.09),
4. dill + parsley – 40 days (20.09-30.10)

In just 200 days, one can harvest 5 crops, which not only has a positive economic effect, but also radically optimises the carbon balance. Since the CFT tool has extensive capabilities for analysis, there is enough data to figure out the structure of greenhouse gas emissions and sequestration, as well as the influence of the factors under study in the authors' model (Figs. 3; 4).

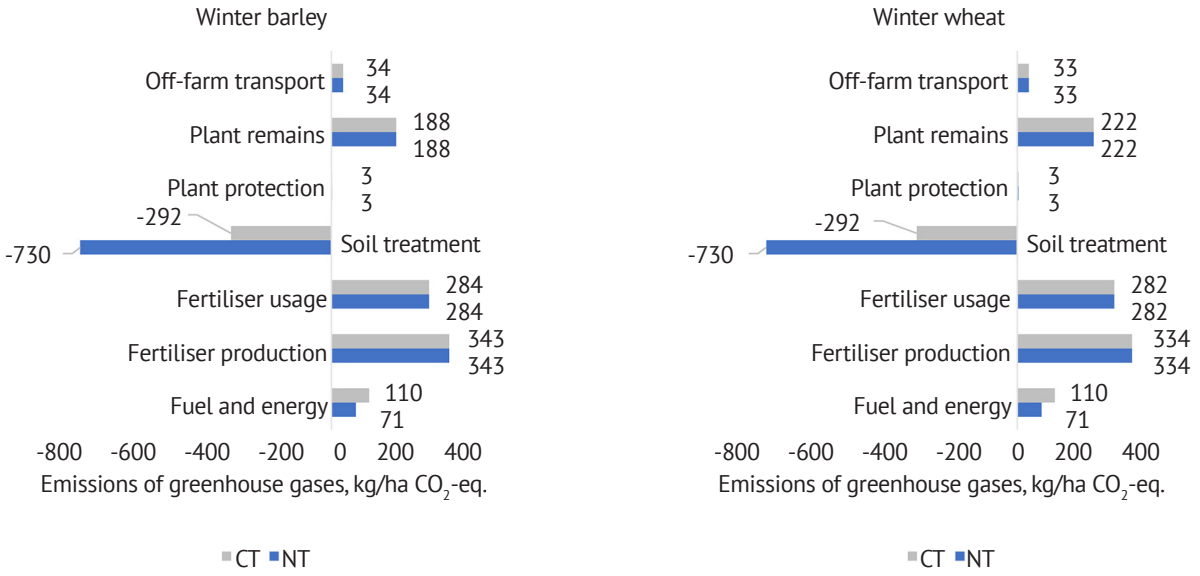


***Figure 3**. Balance of greenhouse gases during the cultivation of barley and winter wheat depending on soil cultivation systems, kg/ha CO_2-eq*

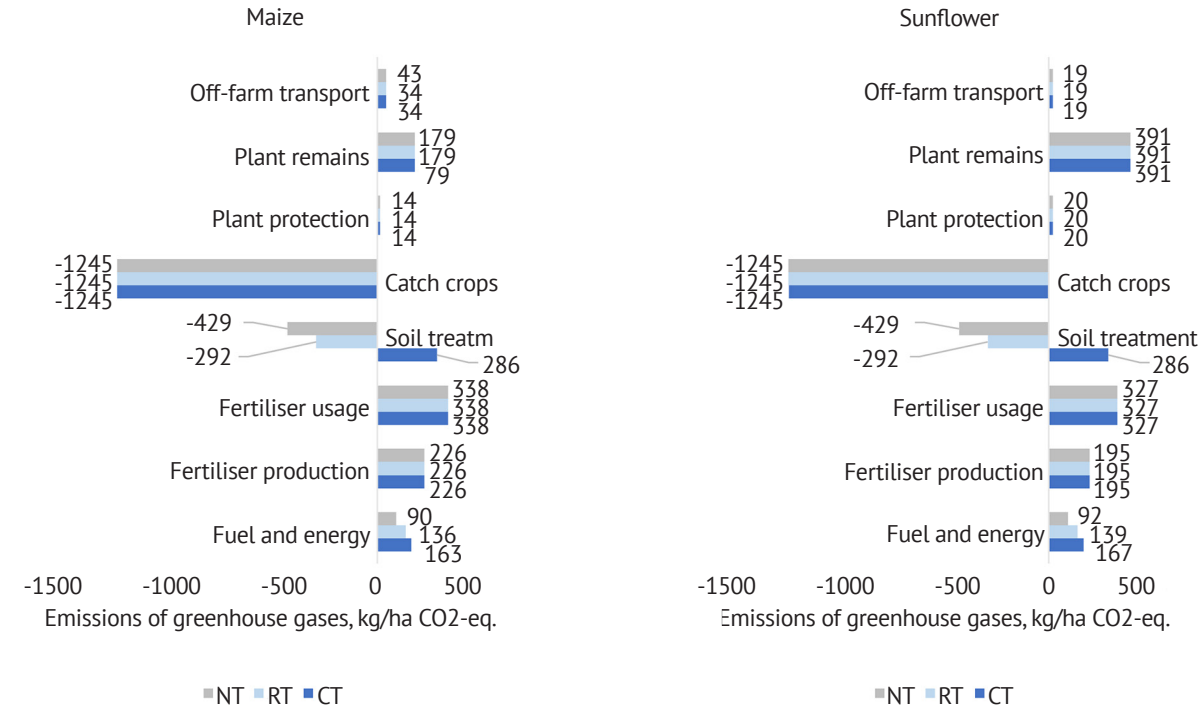


***Figure 4**. Balance of greenhouse gases during the cultivation of maize and sunflower depending on the systems of soil cultivation and catch crops, kg/ha CO_2-eq*

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

As the graphs show, in the structure of greenhouse gas emissions during the cultivation of winter wheat and barley, the largest share is occupied by emissions associated with the use of mineral fertilisers – about 29% and the management of plant residues –22% – in both cases, the tool assumes that in the options under study they stay on the surface. Emissions associated with fertiliser production are transmitted from the producer (plant) to the farmer as indirect (Scope 3). Fuel use accounts for a small share of emissions – 11.2% with reduced tillage and decreases to 7.5% with no-till, while transport emissions account for about 3.5%. Emissions from plant

protection products are only 0.3%. The greatest impact of tillage systems is on the level of sequestered carbon, which is clearly visible in the negative part of the graph related to tillage practices. It is clearly visible that upon reduced tillage, the sequestration reaches -292 kg/ha, with no-till it is almost twice as much – -730 kg/ha CO_2-eq., which compensates for greenhouse gas emissions from the entire technology. Thus, the transition from reduced tillage (mini-till) to no-till on winter grain crops can reduce greenhouse gas emissions by 69-71%, including by reducing fuel consumption – 5.6-5.8%.

Thus, catch crops, due to efficient carbon sequestration, allow obtaining a negative balance of greenhouse gas emissions with any tillage technology. For a 4-field crop rotation under the classical tillage system for sunflower and maize and without catch crops and reduced tillage for wheat and barley, the total emissions of greenhouse gases are 4015 kg/ha of CO_2-eq., under reduced tillage and without catch crops the total greenhouse gas emissions amount to 2805 kg/ha of CO_2-eq., when adding 2 catch crops between the winter grain predecessor before sowing late spring crops, it allows obtaining a negative balance of greenhouse gas emissions during this period – -377 kg/ha of CO_2-eq., and when switching to no-till for all crops – -1221 kg/ha of CO_2-eq. over 4 years (Table 6).

***Table 6**. Comparative characteristics of greenhouse gas emissions depending on the use of catch crops under different tillage systems, kg/ha per year*

Crop rotation culture	Catch culture	Greenhouse gas emissions, kg/ha of CO_2-eq.		
		CT	RT	NT
Winter barley	–	-*	669	192
Maize	No catch crops	1249	644	461
	With catch crops	7	-601	-784
Winter wheat	–	–*	692	215
Sunflower	No catch crops	1405	800	616
	With catch crops	163	-445	-629
Total per rotation	No catch crops	4015*	2805	1484
	With catch crops	839*	-377	-1221

***Note:** *to calculate the amount of emissions per rotation according to CT (classical tillage) for barley and winter wheat, data from RT (reduced tillage) were taken as the recommended and most common*
***Source:** compiled by the authors*

The use of catch crops allows reducing greenhouse gas emissions by 1245 kg/ha per year in the field where they are grown, and when using them twice in a 4-field crop rotation, by 794 kg/ha CO_2-eq. (79%) per year using classical tillage technology, by 795 kg/ha of CO_2-eq. (113%) per year with reduced tillage and by 676 kg/ha (181%) CO_2-eq. per year on the no-till farming system (Table 7).

***Table 7**. Greenhouse gas balance depending on the tillage system and the use of catch crops in the crop rotation under study, kg/ha CO_2-eq. for the year*

Catch crops	Tillage system		
	CT	RT	NT
No catch crops	1004	701	371
With catch crops	210	-94	-305

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

Reducing the intervention in the soil through tillage when growing maize and sunflower allows reducing greenhouse gas emissions when growing these crops upon switching from traditional ploughing with rotation to chiselling by 650 kg/ha of CO_2-eq. per year (43-48%), and when switching to no-till – by 788 kg/ha of CO_2-eq. (56-63%) compared to traditional ploughing, including as a result of reducing emissions from fuel – by 2-6%. This reduction in emissions is mainly explained by curbing the rate of mineralisation of organic matter and its more efficient sequestration in the soil. Cultivation of catch crops effectively improves the sequestration process – up to 1245 kg/ha of CO_2-eq. annually.

In calculations based on the Cool Farm Tool, the level of carbon sequestration from the use of catch crops is 1245 kg/ha of CO_2-eq. turned out to be substantially higher than in the studies of Poeplauab & Don (2015) – 0.32±0.08 t/ha and Tribouillois *et al.* (2018) – 315 kg/ha CO_2-eq. in a year. However, the indicator of this study is close to the results obtained by

Velosoa *et al.* (2018), where the combination of no-till with two cover legume crops showed a sequestration level of 1.15 t/ha of CO_2-eq. for a year.

Thus, such elements of the agricultural system as the system of tillage and the use of intermediate crops can substantially improve the carbon balance towards sequestration, which reduces greenhouse gas emissions into the atmosphere and increases the content of organic carbon in the soil.

CONCLUSIONS

Improving the carbon balance in agriculture is achieved by implementing a set of measures in two areas: reducing direct CO_2 emissions and improving its removal from the atmosphere through plant photosynthesis and sequestration in the form of organic matter in the soil. Reducing emissions involves measures to save fuel, reduce nitrogen losses from fertilisers, reduce soil interference through cultivation, and slow down the mineralisation of plant residues. The improvement of CO_2 extraction from the atmosphere is achieved by maximising the time the field is occupied by plants through the optimisation of crop rotation.

To improve the carbon balance in agrocenoses, it is important to reduce the field time in the state without plants by growing catch crops without harvesting them to avoid "pauses" in extracting carbon from the atmosphere and sequestering it in the soil as organic matter.

The use of catch crops allows reducing greenhouse gas emissions by 1245 kg/ha per year in the fields between winter grain predecessors before the summer crops in the year of cultivation, and when using them twice per a 4-field crop rotation – by an average of 794 kg/ha of CO_2-eq. (79%) per year using classical tillage technology, by 795 kg/ha of CO_2-eq. (113%) per year with reduced tillage and by 676 kg/ha (181%) CO_2-eq. per year on the no-till system.

Based on the results of calculations, it was found that the most substantial measures to improve the carbon balance are the transition to reduced tillage and no-till systems and the use of catch crops. Involvement of catch crops in a 4-field arable crop rotation in 2 seasons out of 4 allows reducing greenhouse gas emissions from 1004 kg/ha to 210 kg/ha CO_2-eq. a year. Switching from ploughing to a reduced tillage system for maize and sunflower allows reducing emissions by 303 kg/ha of CO_2-eq. a year. Switching to a no-till farming system for all crop rotations will reduce greenhouse gas emissions by an average of 633 kg/ha of CO_2-eq. a year. Switching to a no-till system for all crops and including catch crops in two crop rotation fields allows for a positive carbon balance – sequestration of an average of 305 kg/ha of CO_2-eq. a year.

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Оцінка ефективності проміжних культур і систем обробітку ґрунту для вуглецевого землеробства

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Анотація.В сучасному землеробстві необхідно визначити стратегічні кроки, що дозволять скоротити викиди парникових газів: з одного боку, скорочення викидів через зменшення витрат палива, зменшення втручання в ґрунт, обмеження втрат азоту при використанні добрив, а з іншого – підвищення ефективності вилучення вуглецю з атмосфери через фотосинтез рослин і секвестрацію його у вигляді органічної речовини ґрунту. Метою дослідження є визначення рівня впливу на баланс вуглецю таких елементів системи землеробства як система обробітку ґрунту і використання проміжних покривних культур у модельній 4-пільній польовій сівозміні в степовій зоні України. Дана робота була виконана за методикою емпіричних розрахунків на основі онлайн калькулятора викидів парникових газів Cool Farm Tool. Було проаналізовано вплив проміжних культур у двох полях сівозміни (після ранніх зернових попередників – пшениці і ячменю озимих) і систем обробітку ґрунту (традиційний, скорочений і no-till) на баланс викидів і секвестрації вуглецю в модельній 4-пільній польовій сівозміні. За результатами досліджень було встановлено, що за ротацію модельної 4-пільної сівозміни за умов класичної системи обробітку ґрунту для соняшнику і кукурудзи без проміжних культур і скороченої обробки для пшениці і ячменю, сумарні викиди парникових газів становлять 4015 кг/га CO_2-екв. за 4 роки. Було доведено, що перехід на систему скороченої обробки ґрунту зменшує викиди на 30.1%. Додавання двох проміжних культур у двох полях сівозміни перед ярими культурами дозволяє отримати за цей період від'ємний баланс викидів парникових газів -377 кг/га CO_2-екв., а при переході на no-till для всіх культур -1221 кг/га CO_2-екв. за 4 річній період ротації. Ця робота допоможе визначити стратегічні кроки та їхній потенційний внесок при розробці і впровадженні систем землеробства з мінімальними викидами парникових газів

Ключові слова: вуглецеве землеробство, здоров'я ґрунту, викиди парникових газів, проміжні культури, комплексна оцінка якості проміжних культур, вуглецевий баланс

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Cracking of Heavy Hydrocarbons on the Shankanai Zeolite of Modified HPA for the Synthesis of Long-Chain α-Olefins

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Abstract. The relevance of the subject of this scientific research is determined by the importance of the problem of using zeolites as catalysts of process of the heavy hydrocarbons' cracking and studying the prospects for modifying heteropolyacids for the synthesis of long-chain α-olefins. The purpose of this scientific research is to study the prospects for the implementation of process of the heavy hydrocarbon cracking on Shankanai zeolite modified with heteropolyacids for the synthesis of long-chain olefins. The basis of the methodological approach in this research work is a combination of methods for system analysis of heavy hydrocarbon cracking processes using natural zeolites as catalysts for this process, with an analytical study of the prospects for implementing this process when modifying natural zeolites with heteropolyacids in order to synthesize α-olefins. In order to analyze the products of α-olefins, the method of gas-liquid chromatography and nuclear magnetic resonance spectrometry was used. The study is carried out under the condition of using the heavy hydrocarbon cracking process of natural zeolites of the Shankanai deposit as catalyst. The obtained results testify to the high efficiency of the practical application of the Shankanai deposit zeolites, upgraded with heteropolyacids when used as catalysts for the cracking of heavy hydrocarbons. The results obtained in the course of this scientific study, as well as conclusions formulated on their basis, are of significant practical importance for developers of chemical processes for oil cracking using natural zeolites modified with heteropolyacids as effective catalysts of this process, as well as for employees of oil refineries, whose professional duties include the quality control of the implementation of this process

Keywords: heteropolyacids, catalyst, gasoline fraction, feedstock, heavy oil

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INTRODUCTION

Heteropolyacids (HPA) are strong acids, much stronger than sulfuric acid, as well as all known other inorganic polyacids (Maity *et al*., 2021). Heteropolyacids, together with some of their salts, are highly soluble in water and other acid-containing organic solvents, such as ethers, alcohols, ketones. They are relatively stable in neutral and acidic aqueous solutions, while rapidly decomposing in an alkaline environment. They are widely used as catalysts in the alkylation and dealkation of phenol and benzenes with alkenes; they have the ability to catalyze the acetone condensation into mesityl oxide, as well as the formation of ethers: simple and complex (Sels & Kustov, 2016).

To date, the industrial processing of various types of hydrocarbon raw materials, as well as wet gases of oil refining, provides a number of important substances for the economy of any state (Kandiyoti *et al*., 2016). The subsequent involvement of heavy hydrocarbons in oil refining processes requires a qualitative modernization of already existing schemes for processing oil products and the development of new and more efficient technological solutions that allow processing oil products on an industrial scale with obtaining a large amount of light oil fractions of high quality (Maity *et al*., 2021). At the same time, today there are practically no effective methods for processing heavy oil, with the production of a sufficient amount of light fractions. There are only individual technological solutions that allow efficient processing of oil residues that are formed during the primary processing of light and medium grade oils. Industrial processing of heavy oil with the use of currently existing technologies implies the need for significantly higher costs per one ton of processed raw materials (McKeon *et al*., 2016). It should also be taken into account that in order to improve the quality parameters of fuel fractions, it is necessary to introduce expensive secondary catalytic processes, in particular, such as hydrotreating and hydroforming (Sels & Kustov, 2016).

Issues related to various aspects of heavy oil refining are not new, but they remain relevant. The main negative impact of oil production and processing enterprises is on atmospheric air and water bodies. S. Yusup and N.A. Rashidi (2021) believe that the main sources of pollutants are the processes of sulfur extraction, regeneration of cracking catalysts, heaters, rectification columns, boilers, vessels for storing raw materials and finished products, water and oil separators, flares for burning a mixture of accompanying gases and air. According to J. Speight (2019), the industry annually emits up to 1,650,000 tons of harmful substances (the main share of emissions is liquid and gaseous substances). G.P. Da Ponte Jr (2021) is convinced that the negative impact on the environment is manifested in the following aspects: extraction of land resources for the construction of wells; land pollution; emissions of gaseous substances into the atmosphere; extraction of highly mineralized associated waters with oil; emergency oil spills with subsequent evaporation.

According to R. Kandiyoti *et al*. (2016), for now, the bulk of the oil refining companies of the Republic of Kazakhstan have not implemented high-quality technologies for the industrial processing of heavy oil, which leads to their preliminary mixing with light and medium oils during the implementation of this process. At the same time, the processing of oil with a high degree of viscosity causes significant difficulties, since the lack of a clear technology for implementing this process stipulates low profitability and difficulties in its implementation. This explains the need to find effective methods for implementing oil cracking processes using the catalytic elements, one of which is natural zeolites modified with heteropolyacids (HPA) for the synthesis of long-chain α-olefins. The technologies developed to date in different countries of the world for processing heavy high-viscosity oils into "synthetic" oil are mainly based on a combination of classical methods for processing oil residues. At the same time, T. McKeon *et al.* (2016) agree that the specific characteristics and complex composition of heavy hydrocarbon raw materials indicate that the classical recycling methods of light oils do not show proper efficiency. The problem can be effectively and timely solved by introducing the heavy hydrocarbon cracking technique using the modified zeolites as a catalyst.

To date, a large number of scientific studies (Yusup & Rashidi, 2021; Speight, 2019; Da Ponte Jr, 2021) have been devoted to the search for non-standard methods for solving the problem of ensuring the high quality of oil distillation processes using catalysts, which only emphasizes the seriousness and relevance of the problem under consideration. The main disadvantage of the conducted scientific research devoted to the comprehensive study of issues related to various aspects of the heavy hydrocarbons' cracking on natural zeolites is the insufficient knowledge of the problem of the implementation of the zeolites preliminary modification with heteropolyacids in the context of synthesis of the long-chain α-olefins. The task of this scientific research is to study the prospects for implementation of heavy hydrocarbon cracking processes on the Shankanai zeolite of modified HPA for the synthesis of long-chain olefins, as well as to obtain practical results that are important from the point of view of optimizing heavy oil cracking processes using natural zeolites as catalysts for this chemical process.

The aim of the article is to study the prospects for implementation of heavy hydrocarbon cracking processes on the Shankanai zeolite of modified HPA for the synthesis of long-chain olefins.

MATERIALS AND METHODS

The study was carried out under the condition of using the heavy hydrocarbon cracking process of natural zeolites of the Shankanai deposit as catalyst. In this scientific

study, nuclear magnetic resonance (NMR) and gas chromatography (GC) analyzes of al-olefins were implemented in practice, as well as certification of modified zeolite catalysts for cracking processes was carried out. TEM analysis of zeolites modified with heteropolyacids was made. The theoretical basis of this research work consisits of a number of studies of authors dedicated to the issues of cracking of heavy hydrocarbons on natural zeolites used as catalysts for this process, as well as the problems of modifying natural zeolites with heteropolyacids and the synthesis of long-chain α-olefins.

This scientific study was carried out in three main stages. At the first stage of this research work, a theoretical study of available publications dedicated to various problematic aspects of the cracking of heavy hydrocarbons on zeolite modified with heteropolyacids for the synthesis of long-chain α-olefins was made. On the basis of the formed theoretical background of scientific research, at this stage of scientific work, a systematic analysis of the processes of cracking of heavy hydrocarbons was performed using natural zeolites as catalysts for this process.

At the next stage, an analytical study of the prospects for the implementation of this process was carried out when modifying natural zeolites with heteropolyacids in order to synthesize α-olefins. The study proceeded under the condition that natural zeolites of the Shankanai deposit are used as a catalyst for the cracking of heavy hydrocarbons. Factual information was used regarding the characteristics of this kind of zeolites and the current state of their development processes, directly in the conditions of the Shankanai deposit of the Republic of Kazakhstan. To study the hydrocarbon composition of the cracking reaction products, as well as the target fractions, 6890N chromatograph with a plasma ionization type detector was used. Separation was carried out on HP-5 capillary column with the following characteristics: length – 30 m, inner diameter – 0.25 mm, film thickness in a nitrogen flow – 0.25 μm. The increase in temperature of the thermostat was increased sequentially from 50 to 300°C, at a heating intensity of 3° per minute.

To determine the composition of cracking gases, the gas-liquid chromatography method was used using Chromium-4 and Chromium-5 chromatographs (Czech Republic). On the Chromium-4 chromatograph, chromaton was used as a solid carrier, and squalane was used as an immobile liquid reagent, in a volume of 15% of the total mass of the solid carrier. The column of chromatographs separates saturated hydrocarbons, which are the components of source gas. For NMR analysis of α-olefin products, it was used NMR spectrometer Bruker AVANCE IIITM 400 MHz (Germany) with a superconductivity magnet, optimized for experiments on solid objects.

Also, at this stage of the research work, an analytical comparison of the main results obtained during its conduction with those obtained by other scientists during parallel studies of the stated problem was made. All this in combination provides an objective and high-quality picture of the performed scientific research. At the final stage of this research work, based on the results obtained during it, the final conclusions were formulated, acting as a logical presentation of these results and summing up the entire range of scientific studies that were carried out within the framework of the stated theme. In general, the results obtained in this research work and the conclusions formulated on their basis can subsequently serve as a qualitative methodological basis for further research in studying the processes of catalytic cracking of heavy hydrocarbons on natural zeolite that has passed the stage of modification using HPA.

RESULTS

Natural zeolites of the Shankanai deposit are pure aluminosilicate raw materials of multipurpose use. According to the mineralogical classification, zeolites can be referred to the highest class of zeolites of natural origin (Speight, 2019). Modification of natural zeolites with heteropolyacids improves their adsorption properties, which is essential for their subsequent use in hydrocarbon cracking processes as catalysts. The main chemical formula of zeolites (1):

$$M_2/N_0 \times Al_2O_3 \times xSiO_2 \times yH_2O \quad (1)$$

where: Al_2O_3 – alkali or alkaline earth metal; yH_2O – the degree of its oxidation.

Table 1 presents data regarding the content of oxides and auxiliary natural elements in the zeolites of the Shankanai deposit. Natural zeolites of the Shankanai deposit, modified with heteropolyacids, are used as an active component of the oil cracking process. The catalytic effect of such zeolites is determined by many components, among which it should be single out their acidic properties that can be regulated, with a change in the nature and concentration of exchange cations, the molar ratio, SiO_2/Al_2O_3, and also the main conditions of heat treatment. Heteropolyacids are extremely strong protonic acids, which are much stronger than all known inorganic acids. The specific acid characteristics of heteropolyacids are of significant interest for acid catalysis and the theory of acids and bases, since HPAs have a fairly high oxidizing potential. These and a number of other properties make it possible to use HPA in practice as selective catalysts for oxidation and reduction processes. Heteropolyacids have a wide variability in chemical composition and physico-chemical characteristics, by having a stable molecular structure (Da Ponte Jr, 2021).

In the course of experiment, the efficiency of the action of zeolite catalysts modified with heteropolyacids was estimated by the amount of light fractions formed in the process of oil cracking at t=350°C. Figure 1 shows gas chromatographic analysis of al-olefin products.

***Table 1**. The content of oxides and auxiliary natural elements in the zeolites of the Shankanai*

Element	Concentration. %	Content. atom. %	Oxides. %	Chemical formula
O	52.38	66.34	–	–
Si	39.57	28.85	85.28	SiO_2
Al	4.45	6.65	9.58	Al_2O_3
Ca	1.18	0.56	1.62	CaO
Fe	0.96	0.44	1.16	FeO
K	0.66	0.38	0.90	K_2O
Na	0.45	0.33	0.54	Na_2O
Mg	0.35	0.28	0.62	MgO
Total	100	–	–	–

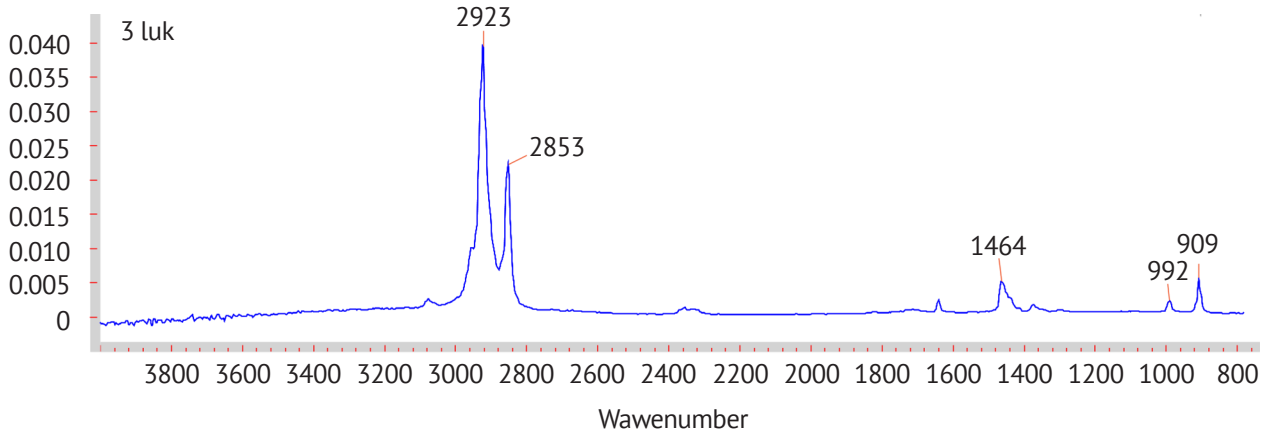

***Figure 1**. GC analysis data for α-olefin products*

Table 2 presents the results of GC analysis of the composition of gaseous products of cracking of heavy hydrocarbons on the natural zeolites of the Shankanai deposit that have not undergone the HPA modification.

***Table 2**. Results of GC analysis of the composition of gaseous products of cracking of heavy hydrocarbons using unmodified zeolite*

Element	1	2	3
Butadiene	0.09	0.02	0.04
Hydrogen	0.28	0.24	0.27
n-Butane	3.95	4.68	3.98
Methane	0.75	0.88	1.05
Propan	1.17	1.42	1.85
Propylene	5.86	6.65	7.62
Isobutane	0.87	1.01	1.16
Olefins C_4	6.56	6.82	6.97
Ethan	0.55	0.58	0.66
Ethylene	0.79	0.95	1.12

As follows from the data presented in Table 2, in the process of catalytic cracking, it is possible the formation of a significant amount of C1-C17 hydrocarbons, in particular C17 olefins and propylene, as well as hydrogen. These elements are of significant interest for the processes of alkylation and petrochemical synthesis of α-olefins. Table 3 presents the results of GC analysis of the composition of gaseous products of cracking of heavy hydrocarbons on natural zeolites of the Shankanai deposit modified with HPA.

***Table 3**. Results of GC analysis of the composition of gaseous products of cracking of heavy hydrocarbons using a zeolite modified with HPA*

Element	1	2	3
Butadiene	0.03	0.02	0.02
Hydrogen	0.12	0.11	0.1
n-Butane	3.81	4.55	3.62
Methane	0.73	0.82	1.06
Propan	1.06	1.22	1.45
Propylene	5.25	6.33	7.54
Isobutane	0.93	1.02	1.18
Olefins C4	6.57	6.99	6.94
Ethan	0.56	0.59	0.69
Ethylene	0.72	0.84	1.09

Figure 2 shows the main results of the validation of modified zeolite catalysts of cracking, expressed as the dependence of the conversion of heavy hydrocarbons into light gasoline fractions on the type of cracking catalyst used.

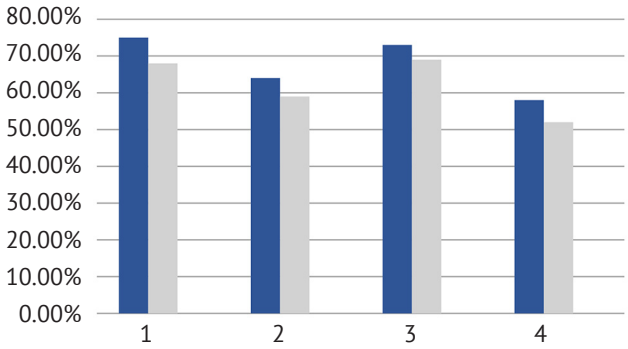

***Figure 2**. Dependence of the conversion value obtained in the process of heavy hydrocarbons' cracking of the type of catalyst used*

Experimental data confirm the higher efficiency of using HPA-modified zeolites as catalysts for the cracking of heavy hydrocarbons, which is reflected in a greater amount obtained as a result of C_{17} long-chain alpha-olefins. Thus, the cracking of heavy hydrocarbons on the zeolite of the Shankanai deposit, modified with HPA, ensures obtaining the target product – long-chain C_{17} alpha-olefins. In the course of this scientific study, it has been proven the high efficiency of using natural zeolite when modified with heteropolyacids to convert alkanes into long-chain olefins.

This means a proof of the practical feasibility of modifying the natural zeolites of the Shankanai deposit with heteropolyacids in order to enhance their catalytic properties, as well as to reduce the total volume of gaseous cracking products. The results of comparative tests of modified and unmodified natural zeolites as catalysts for cracking processes have shown that the adsorption properties of zeolites modified with heteropolyacids increase significantly, which leads to a significant increase in the efficiency of their use as catalysts for the cracking of heavy hydrocarbons (Mantas, 2019). Modification of natural zeolites with heteropolyacids plays an important role in their practical application as catalysts for oil cracking processes at modern oil refineries. The practical use of heteropolyacids as modifying elements increases the adsorption properties of natural zeolites, and it has a positive effect on the efficiency of their use as cracking catalysts.

This explains the possibility of obtaining long-chain C_{17} alpha-olefins, which are the target product of the process at the output. In addition, the use of heteropolyacids as modifying elements for the conversion of alkanes into long-chain olefins has a positive effect on the nature of the process under consideration, since it minimizes the likelihood of sharp jumps and problems with the subsequent use of secondary products of oil refining. Thus, the cracking of heavy hydrocarbons on the Shankanai zeolite modified with HPA for the synthesis of long-chain α-olefins contributes to the production of a significant amount of long-chain C_{17} alpha-olefins, which generally indicates the high efficiency of this process. It should be noted that in this context, the purity of the catalytic substance and

the high level of preparation of petroleum products for the process of their processing are of great importance, since the contamination of the modified natural catalyst used significantly complicates the process and negatively affects its final results. To date, there is a significant potential of the Shankanai deposit as a proven source of natural zeolites, which can be successfully used as effective catalysts for the cracking of heavy hydrocarbons, subject to the implementation of their high-quality modification with HPA. This is of great importance from the point of view of increasing the efficiency of the development of the Kazakhstan oil refining industry as a whole.

DISCUSSION

According to G. Mantas (2019), one of the critical problems of the oil refining industry is the irreversible loss of hydrocarbons during oil processing. These emissions enter the atmospheric air, water basin, or soil, polluting them. That is why the problem of emissions is not only a technical and economic one but also a global ecological one. Research by J. Jarvis *et al.* (2018) showed that among the causes of hydrocarbons entering reservoirs, the discharge of oil-containing effluents and the entry of oil products into groundwater are the most significant. R. Wang *et al.* (2020) believe that heavy oil products destroy microorganisms that participate in the process of self-purification of water. As a result of the decay of sediments contaminated with oil products, harmful substances have released that poison the water.

J. Jarvis *et al.* (2018) assert that today, in the context of a large-scale decrease in reserves, as well as in the volume of oil production, including the so-called "light" oil, the need to use new, non-standard methods for refining hydrocarbon sources is becoming more acute, primarily for bitumen and heavy oils. First of all, it concerns the bitumen and heavy oils. The subsequent increase in the production of heavy oils necessitates an increase in the efficiency of options for their industrial processing to obtain the maximum possible volume of light oil products with qualitatively better characteristics. According to R. Wang *et al.* (2020), the steadily growing consumption of motor fuels in the economically developed countries of the world, encourages leading oil companies to start developing new methods of oil production, as well as developing new oil and bitumen deposits amid the general decline in the share of light oil in the overall balance of oil production. In recent years, the issues of improving the processing of oil and bitumen have become increasingly important, since the natural reserves of raw materials of this kind are several times higher than the natural reserves of medium and light oils. According to numerous expert estimates, it is expected a steady increase in their share in the total volume of hydrocarbon production, taking into account the current balance of production and processing of hydrocarbons.

The task of deepening the oil refining is one of the most urgent tasks of chemical technology for building oil refining processes using catalysts. The processes of catalytic cracking of oil, along with thermal, hydrocracking and visbreaking, are the main methods for processing heavy oil residues. The high energy cost of these processes and their material and capital intensity do not allow timely and full implementation of these processes at most enterprises of the modern oil refining industry. In particular, according to A. Hameed *et al.* (2021), technologies for the industrial processing of vacuum gas oil through catalytic cracking include two additional stages (in addition to the main one) such as hydraulic purification from sulfur compounds of the initial vacuum gas oil, as well as the final products of its cracking. While the hydrocracking process implies the need of practical application of complex technology, it is combined with a high hydrogen pressure. Catalytic cracking ensures the production of high-quality products, and this technique is distinguished by the possibility of processing oil and oil fractions in order to obtain high-quality final products. At the same time, there is a real possibility of combining this process with technological operations of alkylation, adsorption purification. The main disadvantage of this technique is its relative high cost, which predetermines the need to stop the spread of catalytic cracking in many modern enterprises of the oil refining industry. For medium power plants, this process may not always justify itself and fully pay off.

An analysis of the currently existing technologies for cracking of heavy oil residues indicates that catalytic cracking requires significant financial costs and, in fact, is a very expensive operation. S. Chattaraj *et al.* (2016) think that the possibility of obtaining light oil products in sufficient quantities should be considered an undoubted advantage of such technologies. The possibility of practical use in the process of natural zeolites' cracking as catalysts for this process contributes to a significant reduction in its cost and increase in overall efficiency per unit time. Actual high rates of activity of catalysts' cracking when starting these processes in industrial plants can be obtained through the purposefully reducing its specific surface area and thorough deterioration of the active part. According to K.M. Zohdy *et al.* (2021), catalytic cracking should be considered the leader among all processes for improving the efficiency of oil distillation processes in the modern oil refining industry, since one fourth of the world's gasoline is produced through the use of this technology. In addition to gasoline, cracking should also be considered a key producer of diesel fuel and propane-propylene, butane-butylene fraction – the main feedstock for petrochemical processes. The role of catalytic cracking in the development of the economy, especially for countries that are large exporters and producers of oil, is very significant, since it has a significant impact on the depth and thoroughness of oil refining. It also determines the final

volume of oil and oil products consumption, including per capita and for specific types of fuel, by exerting a key influence on the growth rate of the actual gross domestic product of the state (2021).

Zeolites of type X, Y and ZSM-5 (Zeolite Socony Mobil-5) are widely used in the production of cracking catalysts in the fluidized bed. Aluminum oxide and amorphous aluminosilicates are most often used as the catalyst matrix. A. Atmayudha *et al.* (2021) consider that the active matrix makes the main contribution to the overall performance of the catalyst, because the zeolite pores can have a small size for the cracking of especially large molecules of heavy hydrocarbons with a boiling point of more than 480°C. In addition, with an increase in the level of activity of the catalytic substance, various components are added to it that can activate the combustion process (CO) and reduce the concentration of SOx and NOx. Various distillate fractions can be used as feedstock components of the catalytic cracking process, and during their conversion, it is observed a catalyst coking and contamination with heavy metals (V and Ni) and sulfur. For now, there is a continuing trend towards an increase in the total weight of the fractional composition of the feedstock that is involved in the catalytic cracking process, which inevitably becomes one of the reasons for the increase in the concentration in the feedstock of polynuclear aromatics, as well as sulfur, nitrogen and heavy metals. All this, in turn, leads to an increase in the coking capacity of raw materials. Today, the most urgent tasks should be considered the tasks of gradually increasing the depth of the catalytic cracking process and increasing its selectivity, as well as maintaining the activity level of the selected catalyst for a long time of operation (2018).

Nowadays, many countries of the world have almost completely exhausted the possibilities for improving oil refining processes in order to meet the real needs for fuels of various types, which can only be realized by increasing the volume of industrial oil production. Various options for solving this problem are proposed, one of which is a gradual transition to the use of catalysts for cracking processes of natural origin. Zeolites are one of these variants (2021). The practical use of natural zeolites as cracking catalysts makes it possible to achieve a high intensity and efficiency of this process, with a significant reduction in energy and material costs. The implementation of secondary catalytic processes requires preliminary upgrading of the initial feedstock. Dementalization and denasalizing should be attributed to the category of refining processes, while the thermal adsorption variety of these processes can also be attributed to the indicated type. According to A. Al-Qasim *et al.* (2020), in all these processes, partial thermodestructive transformations can act as methods for refining the hydrocarbon residues, at the same time there is a parallel and simultaneous adsorption of the resins formed, asphaltenes and carboids, as well as organometallic, organosulfur and organonitrogen compounds on the surface of the resulting adsorbing substances.

The processing of heavy hydrocarbon residues is carried out in various ways and methods, which should be grouped for subsequent selection when developing the proposed schemes for oil refineries. In this context, the use of heavy hydrocarbon residues as the main components of fuels significantly reduces the depth of processing of hydrocarbon raw materials at oil refineries. This necessitates a high-quality organization of the processes of industrial processing of hydrocarbon residues at oil refineries in order to increase the efficiency of these enterprises and obtain high-quality oil refining products. A. Al-Qasim *et al.* (2020) believe that such processes are characterized by significant technological complexity and require a qualitatively new approach to their organization in order to obtain optimal results, which can be expressed in the high quality of the oil distillation products obtained as a result of oil refining processes. Adding a certain amount of zeolite to the processed oil helps to increase the volume of output of light fractions, as well as coke and gas. When conducting studies of the composition of gaseous products released during the implementation of oil cracking processes, it is found that catalytic cracking products contain a significantly larger number of lower olefins, including propylene, butenes and pentenes, as well as a smaller number of alkanes compared to the gas phase that occurs in thermal cracking.

In addition, J.A. Curiale & J.B. Curtis (2016) are convinced that a significantly higher content of isostructural alkanes (15.2%) and a significant amount of hydrogen (1.7%) can be detected in the gas formation products that are released during oil cracking in the presence of catalyst, which is clear evidence of the development of dehydrogenation reactions and further redistribution of hydrogen. At the same time, the oil straight-run gasoline fraction immediately before the start of the catalytic cracking process and the products' gasoline fractions of its thermal and catalytic cracking differ significantly in the group concentration of hydrocarbons. The gasoline fractions obtained contain a significantly larger amount of n-alkanes and a significantly lower concentration of nevtenes and alkenes. At the same time, the octane numbers of gasolines produced through the implementation of catalytic and thermal cracking processes are significantly higher than similar indicators of the straight-run gasoline component of the original oil. The composition of the resulting gasolines also determines significant differences in the technology of thermal and catalytic cracking of oil. In this context, it should be noted that gasoline obtained through the implementation of thermal cracking processes contains more alkanes of normal structure and less naphthenes and alkenes than gasoline obtained as a result of catalytic cracking. When catalytic cracking is implemented on natural

zeolites as catalysts for the process, the cracking itself with dehydrogenation, isomerization and aromatization is the key reactions for the conversion of hydrocarbons. However, in the thermal cracking, disconnection of C-C connection in combination with polycondensation is observed.

CONCLUSIONS

To date, science offers various options for improving the processes of industrial oil refining, one of which is the use of zeolites upgraded with heteropolyacids as catalysts for the cracking of heavy hydrocarbons. In almost every case of launching an oil refining process through catalytic cracking, the best option for upgrading zeolites in order to obtain a larger amount of light oil fractions amid the synthesis of long-chain α-olefins can be selected. It should also be noted that the gasoline fractions of catalytic cracking processes using the Shankanai zeolites (Kazakhstan) modified with heteropolyacid include a large number of components with a high-octane number, which significantly exceeds the octane number of their analogues that were obtained by thermal cracking.

The preparedness of the feedstock should be considered the most important aspect that directly affects the results of the catalytic cracking process on modified natural zeolites in order to avoid contamination of the natural catalyst used for this process. Before starting the cracking process, it is necessary to prepare the feedstock in order to prevent poisoning of the natural zeolite acting as a catalyst, which will adversely affect the results of the synthesis of long-chain α-olefins. In addition, the cracking of heavy hydrocarbons on the Shankanai zeolite, which has undergone the HPA modernization, implies the need for a careful selection of zeolites, taking into account their actual characteristics, which can be significant in terms of the actual results of this process, expressed in obtaining a significant amount of light gasoline fractions. In general, the results obtained in the course of this research work showed the high practical efficiency of modifying the natural zeolites of the Shankanai deposit with heteropolyacids when they are used as catalysts for the cracking of heavy hydrocarbons. This indicates the significant prospects for the subsequent mass use of zeolites modified with HPA in processes of catalytic cracking of heavy hydrocarbons for the synthesis of long-chain α-olefins.

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Крекінг важких вуглеводнів на шанканайському цеоліті модифікованого ГПА для синтезу довголанцюгових α-олефінів

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Анотація. Актуальність теми наукового дослідження визначається важливістю проблеми використання цеолітів як каталізаторів процесу крекінгу важких вуглеводнів та вивченням перспектив модифікування гетерополікислот для синтезу довголанцюгових α-олефінів. Метою наукової роботи є вивчення перспектив реалізації процесу крекінгу важких вуглеводнів на шанканайському цеоліті, модифікованому гетерополікислотами, для синтезу довголанцюгових олефінів. Основою методологічного підходу в цій науково-дослідній роботі є поєднання методів системного аналізу процесів крекінгу важких вуглеводнів з використанням природних цеолітів як каталізаторів цього процесу, з аналітичним дослідженням перспектив реалізації цього процесу при модифікуванні природних цеолітів гетерополікислотами з метою синтезу α-олефінів. Для аналізу продуктів синтезу α-олефінів використано метод газорідинної хроматографії та ядерно-магнітно-резонансної спектрометрії. Дослідження проведено за умови використання в якості каталізатора процесу крекінгу важких вуглеводнів природних цеолітів Шанканайського родовища. Отримані результати свідчать про високу ефективність практичного застосування цеолітів Шанканайського родовища, модернізованих гетерополікислотами, при використанні в якості каталізаторів крекінгу важких вуглеводнів. Отримані в ході наукового дослідження результати, а також сформульовані на їх основі висновки мають практичне значення для розробників хімічних процесів крекінгу нафти з використанням природних цеолітів, модифікованих гетерополікислотами, як ефективних каталізаторів цього процесу, а також для працівників нафтопереробних заводів, до професійних обов'язків яких входить контроль якості здійснення даного процесу

Ключові слова: гетерополікислоти, каталізатор, бензинова фракція, сировина, важка нафта

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The Main Areas of Development of Organic Agriculture in the Republic of Kazakhstan

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Abstract. The Republic of Kazakhstan has good potential for developing environmentally friendly agricultural production: extensive agricultural land, a large number of farms, and interest in producing environmentally-friendly products. These factors determined the relevance of the study, the main purpose of which was to investigate the main areas of the development of organic agriculture in Kazakhstan. The statistical data and reports of Research Institute of Organic Agriculture, International Federation of Organic Agriculture Movement, and Food and Agriculture Organization of the United Nations were analyzed; logical and empirical methods were used; a survey of consumers and producers of environmentally-friendly products in Kazakhstan was conducted. This paper shows the main trends in the development of the organic sector in Kazakhstan and in the world in general. Studies have shown a growing tendency of organic production in the world, however, many countries are still in their infancy regarding this trend. At the moment, the world's land under organic agriculture is 74.9 million hectares, but in Kazakhstan, since 2016, there has been a reduction in such areas. In 2020, they amounted to 114 thousand hectares or 0.1% of all farmland in the country, which is associated with the coronavirus pandemic and the global economic crisis. According to the international organisations Research Institute of Organic Agriculture and International Federation of Organic Agriculture Movement, in 2020, out of 190 countries of the world, Kazakhstan entered the lists of 50 producing countries and 20 exporting countries of organic agricultural products, and the export of these products amounted to about EUR 9 million. It is concluded that the necessary conditions for the introduction of ecological agriculture are gradually being created in the Republic of Kazakhstan, which would improve the economic situation of the country. The practical significance of the study was to conduct a sociological survey of consumers and producers of ecological agricultural products in four regions of Kazakhstan to identify the state and main areas of development of the domestic market of organic agriculture

Keywords: environmentally-friendly product, export, standardisation, certification, green economy

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INTRODUCTION

Every year, there is a positive growth trend in the organic agriculture market worldwide and an increasing demand for organic products. According to S. Das, A. Chatterjee, T.K. Pal (2020), the expansion of this sector of the economy is conditioned by the receipt of higher profits from the sale of organic products and the transition of small farms to organic farming. American researchers (Reganold & Wachter, 2016) predict that population growth will reach 9-10 billion people by 2050, while enormous attention will be paid to environmental protection. They claim that the large-scale introduction of organic and resource-saving agriculture will ensure the future of humanity with safe food and improve the ecosystem. Similar information has been published in a paper by Indian researchers (Prusty *et al*., 2021; Thakur *et al*., 2022) and in the Food and Agriculture Organization of the United Nations (FAO) (2022) report, where it is noted that among the most effective strategies for improving the quality of food, strengthening the sustainability of ecological and agrifood systems, organic agriculture, soil protection and resource-saving agriculture are singled out. Thus, according to FAO, Belgium, Croatia, Estonia, Finland, Germany, Hungary, Ireland, Spain, the Netherlands, Norway, Poland, Slovakia, Slovenia, Sweden, and the United Kingdom have achieved positive results in maintaining biodiversity in the production of agricultural products with the help of organic agriculture.

According to the Research Institute of Organic Agriculture (FiBL) (2022) and the International Federation of Organic Agriculture Movement (IFOAM) (2022), Oceania countries are considered the largest among organic producers, in which 35.9 million hectares of land are occupied by ecological agriculture, of which 27.2 million hectares are located in Australia (Paull and Hennig, 2018), followed by countries in Europe (17.1 million hectares), Latin America (9.9 million hectares), Asia (6.1 million hectares), North America (3.7 million hectares), and Africa (2.1 million hectares) (Willer *et al*., 2021). FiBL (2022) and IFOAM (2022) reports over the past 20 years indicate a constant increase in land areas for organic production (Grigoruk and Klimov, 2016). Both the number of countries and the number of organic producers within these states are growing (Proshchalykina *et al*., 2019). According to H. El Bilali (2020), in 2018, 2.8 million farmers were engaged in organic agriculture worldwide on an area of 71.5 million hectares.

A.Kh. Berdiev and H.K. Rasulov (2020) state that the USA (United States of America), EU (European Union), and China are the largest markets for organic products, the volume of which is 24.3, 22, and 2.4 billion euro, respectively, and the majority of consumed environmentally-friendly products account for Germany, France, and the USA (Anderberg, 2020). According to the calculations of O.O. Karamatov (2021), in the republics of Central Asia, by 2025, the land area under organic agriculture would increase to 762 thousand hectares. However, the growth of organic production in developing countries still depends heavily on foreign markets. According to many researchers in the Republic of Kazakhstan, the development of organic agriculture is in an active phase. Thus, more than 300 thousand hectares of farmland were allocated for the production of environmentally friendly products in 2016, while about 30 farms certified in accordance with international standards were registered (Uskenov et al, 2016). According to some researchers (Bulkhairova *et al*., 2020; Canwat & Onakuse, 2022), the largest producer of organic products in the republic is the Akmola company Edelweiss Invest LLP (limited liability partnership), which grows certified organic products on 27 thousand hectares. The annual export of eco-products from Kazakhstan to the EU reaches about USD 10 million. The main exporters are Akmola, Kostanay, Pavlodar, East Kazakhstan, Karaganda, and Almaty regions. Seeds, oilseeds, and legumes are in great demand, and there are about 20 types of products in total (Wiśniewski *et al*., 2021).

The growing interest of the world community in organic agriculture and the opportunity to improve the economic situation in Kazakhstan through the production of eco-friendly products determined the relevance of this study. *The purpose of the study* was to investigate trends in the development of organic agriculture in the Republic of Kazakhstan.

MATERIALS AND METHODS

The conceptual and theoretical basis and the methodology of "organic agriculture" were developed at the beginning of the 20th century. The countries of Europe and the USA took an active part in this process. However, this term has analogues. For example, in Germany and France it is "biological" (or "bio"), and in Poland, the Czech Republic, and the Netherlands – "ecological" (or "eco"). All these concepts imply that the production of organic products includes the minimisation or complete exclusion of synthetic chemical means of protection and plant growth stimulants, and the rejection of the use of genetic engineering and genetically modified organisms (GMOs), that is, the products safe for humans and the environment. The study of the main trends in the development of organic agriculture in the Republic of Kazakhstan was conducted based on the analysis of the dynamics of domestic and foreign markets, consideration of the problems of standardisation and certification of eco-products, and the social aspect.

The main objects of the study were: regulatory and legal documentation on organic agriculture in Kazakhstan, producers of ecological agricultural products, and consumers of the external and internal markets of organic goods. The paper uses data from reliable, open sources: reports of the FiBL (2022) and the IFOAM (2022), the FAO (2022). These data allowed identifying the main areas of the development of organic agriculture in the

world and Kazakhstan, and understanding the existing problems of this sector of the economy. To investigate the production and sale of organic products using questionnaires (Table 1), a survey of its consumers and producers was conducted. The survey was conducted in the North Kazakhstan, Akmola, Karaganda, and Kostanay regions of the Republic of Kazakhstan. The questionnaire was distributed to consumers of organic agricultural products and farm managers in the regions under study, as well as using an online service for creating feedback forms, online tests, and surveys – Google Forms. 322 respondents took part in the survey: 198 – consumers in the areas under study and 124 – through Google Forms.

***Table 1**. Survey of consumers and producers of organic products*

No.	Questions	Answers
1.	Are you familiar with organic agricultural products and the benefits of their consumption for your health?	a) yes b) no
2.	What are the sources of information from which you learned about organic products?	a) Internet b) specialised department in the store c) another source
3.	Which manufacturers' organic products did you purchase?	a) Kazakh producers b) imported manufacturers
4.	What types of products that have the status of organic have you bought most often?	a) bakery and pasta products b) meat products c) dairy products d) vegetables and fruits
5.	Your income is:	a) 50-100 thousand tenge b) 100-150 thousand tenge c) 150-200 thousand tenge d) more than 200 thousand tenge
6.	How much of your income are you willing to spend to buy organic agricultural products?	a) up to 10% b) 10-15% c) 15-20% d) more than 20%
7.	Arrange the options from 1 to 5 depending on the degree of importance for you when choosing organic agricultural products.	a) quality b) appearance c) price d) manufacturer e) environmental characteristics
8.	Choose the reasons why you like organic agricultural products?	a) high quality b) health benefits c) preservation of ecology d) fashion trend

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

In general, the study was conducted using abstract and logical, analytical, graphical, computational, and constructive research methods, and methods for comparing values and groupings, generalising statistical indicators.

RESULTS

An analysis of the state of organic agriculture in the world has shown positive growth dynamics on a global scale (Fig. 1), however, in a number of countries, including Kazakhstan, in recent years there has been a decline in both production and export of biological products (Willer *et al.*, 2021).

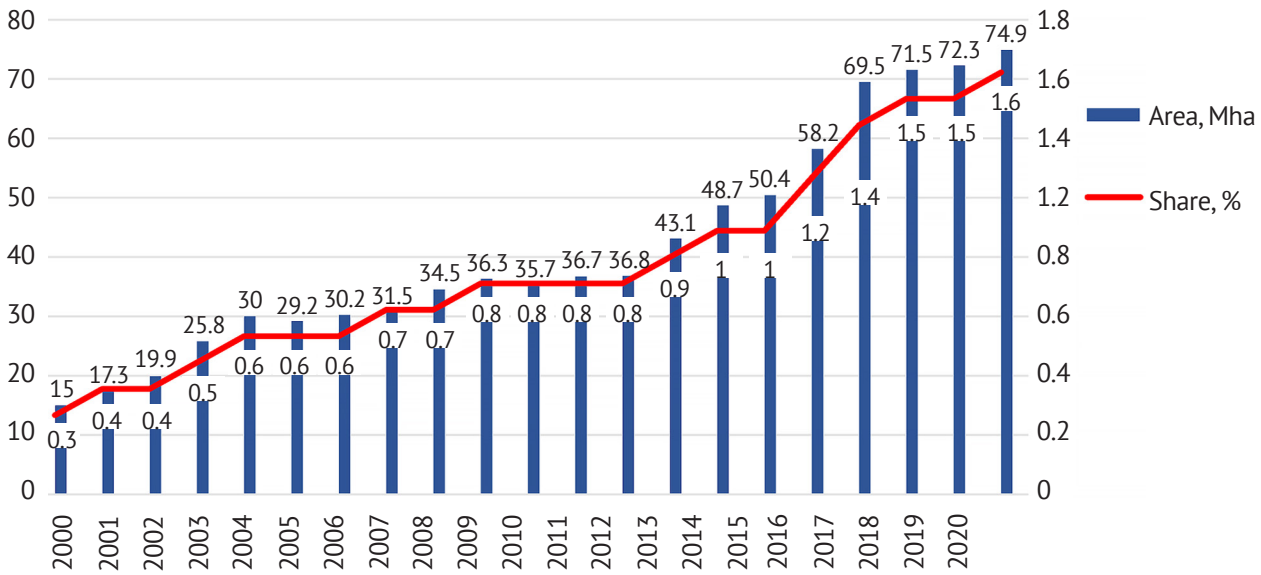

***Figure 1**. Growth of areas under organic agriculture in the world (2000-2020)*
***Source:** compiled and adapted based on H. Willer et al. (2020; 2021)*

In recent years, 1.6% of the world's agricultural lands have been certified organic according to international standards. For the period 2000-2020, there was a five-fold increase in the area under organic agriculture, their size by 2020 amounted to 74.9 million hectares. Notably, the spread of organic farming across continents and countries was uneven. According to the FiBL (2022) and the IFOAM (2022) in 2020, Kazakhstan entered 50 out of 190 countries producing environmentally safe products (Table 2).

***Table 2**. Ranking of 50 countries of the world, including Kazakhstan, by areas under organic agriculture and the dynamics of their growth over the past 10 years*

Rating, number	Country	Area in 2020		Growth in 10 years (2010-2020)	
		thousand hectares	%	thousand hectares	%
1	Australia	35687	9.9	24488	218.7
2	Argentina	4453	3.0	657	17.3
3	Uruguay	2742	19.6	1811	194.6
4	India	2657	1.5	1573	145.1
5	France	2548	8.8	1573	161.4
6	Spain	2437	10.0	815	50.3
7	China	2435	0.5	535	28.2
8	United States of America	2326	0.6	148	6.8
9	Italy	2095	16.0	998	91.0
10	Germany	1702	10.2	686	67.6
11	Canada	1417	2.4	576	68.5
12	Brazil	1319	0.6	632	92.0
13	Austria	679	26.5	117	20.9
14	russian federation	615	0.3	488	385.0
15	Sweden	613	20.4	133	27.9
16	Czech Republic	539	15.3	79	17.2
17	Greece	534	10.1	321	150.7
18	Poland	507	3.5	-101	-16.7
19	United Kingdom	473	2.7	-165	-25.8

Table 2, Continued

20	Romania	468	3.5	238	103.9
21	Ukraine	462	1.1	191	71.0
22	Turkey	382	1.0	-59	-13.5
23	Peru	342	1.5	156	84.3
24	Portugal	319	8.1	119	59.6
25	Finland	315	13.9	126	67.4
26	Hungary	301	6.0	177	142.3
27	Denmark	299	11.4	137	85.0
28	Tunisia	297	3.0	118	66.4
29	Latvia	291	14.8	107	58.2
30	Lithuania	235	8.0	83	54.6
31	Ethiopia	234	0.6	94	67.0
32	Slovakia	222	11.7	56	33.7
33	Estonia	220	22.4	87	65.0
34	Sierra Leone	219	5.6	83	54.6
35	Mexico	215	0.2	-151	-41.2
36	Tanzania	198	0.5	83	72.3
37	Philippines	191	1.5	95	99.1
38	Bolivia	179	0.5	33	23.0
39	Switzerland	177	17.0	61	52.6
40	Thailand	160	0.7	125	361.7
41	Chile	156	1.0	127	439.5
42	Togo	127	3.3	126	9,464.5
43	Kenya	123	0.4	118	2,390.3
44	Democratic Republic of Congo	118	0.4	77	188.2
45	Dominican Republic	117	4.8	-69	-37.2
46	Uganda	116	0.8	-111	-49.0
47	Bulgaria	116	2.3	91	364.6
48	Egypt	116	3.0	33	41.2
49	Kazakhstan	114	0.1	-81	-41.4
50	Croatia	108	7.2	76	239.0

Source: *compiled and adapted based on H. Willer et al. (2021)*

The leading position in the field of organic agriculture, since 2000, has been occupied by Australia, which accounts for more than half of the world's certified land area (54%). In 2020, its area under organic farming was 35687 thousand hectares, or 9.9% of all farmland (Willer *et al.*, 2021), which is 1.1% higher than in 2018 (Paull & Hennig, 2018). In second place, by a wide margin, was Argentina, which observed stable organic farming on an area of 4453 thousand hectares. In other countries, smaller areas have been allocated for ecological agriculture. In modern conditions of development of organic agriculture, Kazakhstan lags far behind and occupies only a small part of the total area of the world's land resources used in the production of environmentally safe products. Thus, as of 2020, 114 thousand hectares were occupied under organic farming, which accounted for 0.1% of all farmland in the country (Willer *et al.*, 2021).

According to FiBL (2022) and IFOAM (2022), since 2013, cereals (rice, wheat, and corn) have been considered the most important organic field crops; their area in 2020 was almost 1.3 million hectares. In second place are oilseeds (mainly soybeans), occupying almost 578 thousand hectares, and textile crops (mainly cotton) grown on an area of more than 355 thousand hectares. The largest areas of grain and oilseed organic crops in 2020 were in China (almost 1.9 million hectares), cotton and soybeans – in India (more than 415 thousand hectares), rice – in Thailand (more than 143 thousand hectares), grain, textile, and oilseed crops – in Kazakhstan (more than 107 thousand hectares). In terms of the number of areas occupied by organic wheat, Kazakhstan ranked second (more than 24 thousand hectares) after China (240 thousand hectares), China was also the leader in organic corn (230 thousand hectares).

In 2020, compared to 2019, there was an increase in the volume of imports of tropical fruits, nuts, spices, vegetables, coffee, mate tea, soybeans, olive oil, and rice. The remaining categories of goods in the top 10 decreased compared to last year: cake – by 22%, sugar – by 10%, oilseeds, except soybeans – by 12%, and especially cereals, except wheat and rice – by 41%. The volume of organic wheat, which in 2019 was among the top 10 imported organic products in the EU, also sharply decreased by 34% in 2020 (Willer *et al*., 2021). The decrease in the import of cake in 2020 was caused by a sharp reduction in imports from China (-47%), which is the main supplier. In addition, the import of corn and wheat decreased significantly due to a considerable reduction in the volume of products coming from Ukraine (-44%) and Kazakhstan (-96%).

Regarding the dynamics of the development of organic agriculture in the world over the past decade, among the countries with the largest growth in production areas, African countries stand out: Togo and Kenya. The positive dynamics of the increase in land for organic production are characterised by 44 of the 50 countries under study that are engaged in this area. A reduction of more than 40% of the area under ecological production was observed in Uganda, Kazakhstan, and Mexico, which is probably conditioned by the internal problems of these countries (Willer *et al*., 2021). In Kazakhstan, organic agriculture developed slowly due to the fact that the population was not sufficiently informed about the potential opportunities of this area (obtaining higher profits than from traditional production, maintaining the ecological balance in agrocenoses, improving living standards, etc.), and there were also no organic farming technologies developed and adapted to local extreme agro-climatic conditions in the country. The study of world market relations showed that the largest markets for organic agricultural products were located in the USA and the EU (Germany, Great Britain, the Netherlands, France, Italy, Poland, etc.) (Fig. 2), they accounted for 45 and 42% of world trade turnover, respectively. China and Canada were next in the ranking, by a large margin – 8% and 3%, respectively (Willer *et al*., 2020).

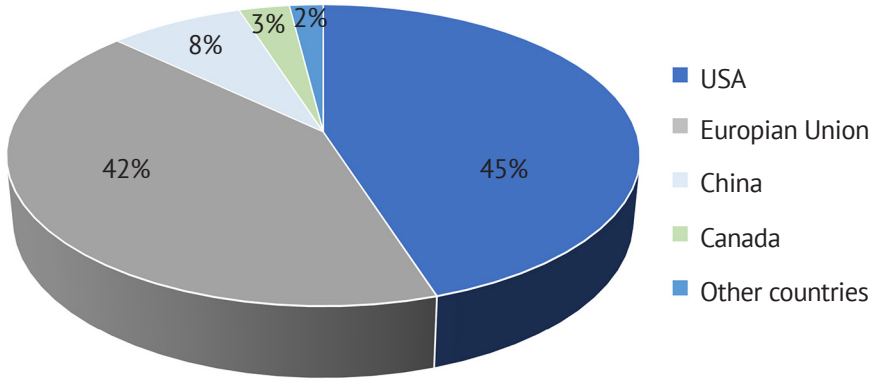

Figure 2. *World market of organic products consumption, 2018*
Source: *H. Willer et al. (2020)*

In 2020, the Republic of Kazakhstan was one of the 20 exporting countries of organic products (Table 3), however, due to the coronavirus pandemic and the deterioration of the country's economy, exports fell by 30.8% compared to 2019. A similar situation, albeit on a smaller scale, occurred with China, Ukraine, Turkey, and other countries (Willer *et al*., 2021).

Table 3. *The volume of imports of organic agricultural products by EU exporting countries, including Kazakhstan, in 2019 and 2020*

Rating, number	Exporter	Imports in 2019, million tonnes	Imports in 2020, million tonnes	Dynamics, %
1	Ecuador	299971	324071	8.0
2	Dominican Republic	229218	252293	10.1
3	China	359057	227669	-36.6
4	Ukraine	282427	217210	-23.1
5	Peru	207938	200860	-3.4
6	India	152678	174311	14.2
7	Turkey	173026	155741	-10.0
8	Colombia	79167	106766	34.9
9	Brazil	75676	67225	-11.2

Table 3, Continued

Rating, number	Exporter	Imports in 2019, million tonnes	Imports in 2020, million tonnes	Dynamics, %
10	Mexico	67427	66127	-1.9
11	Tunisia	42519	58516	37.6
12	Argentina	59456	56361	-5.2
13	Togo	44684	54017	20.9
14	Egypt	53233	51292	-3.6
15	Pakistan	33432	44942	34.4
16	Honduras	35961	41800	16.2
17	Kazakhstan	58785	40692	-30.8
18	Sri Lanka	29198	37166	27.3
19	Ivory Coast	23487	35475	51.0
20	Canada	28457	33350	17.2

Source: *H. Willer et al. (2021)*

According to the International Federation of Organic Agriculture Movements (2022) in 2020, the number of producers of environmentally-friendly products in the Republic of Kazakhstan was 279, and about EUR 9 million worth of products was exported. The main goods exported by Kazakhstan to EU countries were wheat, soybeans, seeds, and oilseed cake (Bulkhairova *et al.*, 2020). The legislative framework of the producing country is of great importance in promoting organic agriculture. According to S. Le Douarin (2020), for 2019, the relevant documents were developed and adopted in 103 countries, while in other states they were at the stage of preparation for ratification. A law on organic farming was adopted in Kazakhstan a few years ago, but its implementation has not yet been completed.

Despite a number of problems: the insufficiently developed infrastructure that ensures the efficient operation of the organic market, the high cost of certification of eco-products and dependence on international experts and certification bodies, the Kazakh government has made significant efforts to introduce the production of environmentally friendly agricultural products into the country's economy. Thus, adopted the Strategy "Kazakhstan-2050" (2012), the Concept for the transition of the Republic of Kazakhstan to a "green economy" (Decree of the…, 2013) and the Law of the Republic of Kazakhstan No. 423-V ZRK "On the production of organic products" (2015) laid the legal basis for the country's organic agriculture (Grigoruk & Klimov, 2016; Karamatov, 2021). Thus, the Republic of Kazakhstan is on the way to the effective management of organic agriculture, and the adopted regulatory documents and active cooperation with international organisations to promote environmentally-friendly products in the future would allow attracting additional funds into the country's economy. The most important factor in the expansion of the global and domestic markets for organic products is the growth of consumer demand for eco-products. The annual reports and reports of the FAO (2022), FiBL (2022), and IFOAM (2022) on the state of the organic products market testify to the international growth in demand for these products, and in Kazakhstan – the appearance of specialised stores and the sale of environmentally friendly goods via the Internet. As a result of the research, it was confirmed that Kazakh internal market of organic products was underdeveloped. According to V.V. Grigoruk and E.V. Klimov (2016) and O.O. Karamatov (2021) the main reason for this is the lack of demand for Kazakhstan's environmentally safe products in the domestic market.

As a result of the study, it was found that in the specialised departments of the republic's stores, eco-products were represented by goods of imported origin, the most common were: buckwheat, mash, lentils, red quinoa, dried cranberries, walnuts, flour (whole wheat, oatmeal, chickpeas, corn, rice, and buckwheat), semolina, coconut milk powder, organic coconut sugar, etc. Their cost exceeded similar traditional goods by 2-7 times, and local products that have not passed certification for compliance with organic standards – by 2-3 times. Most goods, except exotic ones, can be produced by Kazakh farmers, but this requires appropriate conditions. Organic products have a number of characteristics that are divided into individual and social (environmental). The group of individual characteristics of organic food includes taste, health value, freshness, usefulness, and social attributes include aspects of environmental protection (Grzybowska-Brzezińska *et al.*, 2017).

To investigate the state and growth prospects of the domestic organic production market, a survey was conducted using questionnaires in the North Kazakhstan, Akmola, Karaganda, and Kostanay regions of the Republic of Kazakhstan. Data on the retail market in these areas were obtained. The survey showed that the consumers of Kazakhstan made a choice in favour of organic products, focusing on such characteristics as the impact of an eco-product on health and the environment, and its taste qualities. Among the consumers of eco-products, families with high incomes or with children under 5-7 years old prevailed, as well as young people engaged in sports. The analysis of the survey showed that the main consumers of organic products were two categories of people aged 31 to 40 years and 26 to 30 years, which accounted for 37.6% and 32.3% of the total number of respondents, respectively (Table 4).

***Table 4**. Structure of Kazakh respondents – consumers of organic products*

Consumer group by age category			Gender of the consumer of eco-products, %	
Years	**Peoples**	**%**	**Male**	**Female**
18-25	47	14.6	38.3	61.7
26-30	104	32.3	27.9	72.1
31-40	121	37.6	43.8	56.2
41-50	21	6.5	33.3	66.7
51-59	18	5.6	44.4	55.6
over 60	11	3.4	18.2	81.8
Total	322	100	–	–
Average	–	–	34.3	65.7

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

According to the gender ratio, women predominated among the respondents, that is, 65.7% of women and 34.3% of men out of 322 respondents. The analysis of responses showed that the majority of consumers (68.3%) were familiar with organic products and used them frequently, the rest (41.7%) have heard something or did not know anything about it. Nevertheless, such awareness of the population and the consumption of eco-products are quite low. Most of the respondents learned about eco-products from advertising on the Internet (31.2%), the second-ranked source of information was familiarisation with products in organic departments of stores (38.6%), 19.7% of survey participants learned about organic products from other sources. It was found that the majority of consumers (59.4%) preferred the products of Kazakh manufacturers. They believe that if local products have a "BIO" or "ECO" mark, then they are organic. According to the analysis of the survey, the most frequent buyers purchased dairy products (41%), while meat products, pasta and bakery products ranked second in popularity and gained 28% each (Fig. 3).

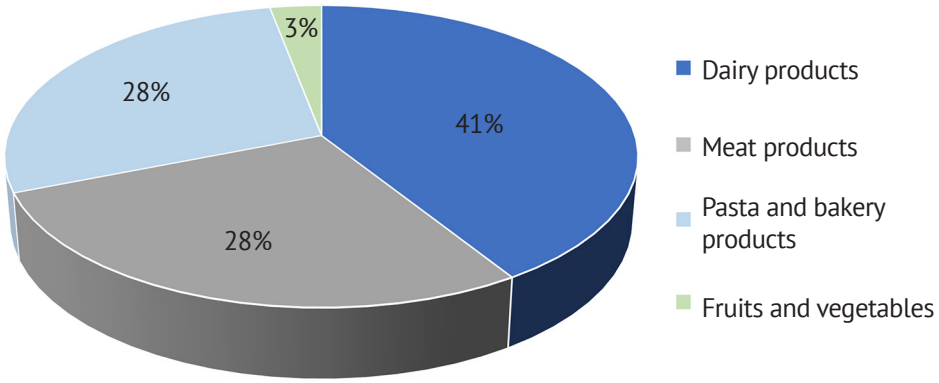

***Figure 3**. Diagram of consumption of organic products in the Republic of Kazakhstan*
***Source:** compiled by the authors*

The survey also showed that 34% of consumers of eco-products had an income of KZT 100-150 thousand, 29.5% – KZT 150-200 thousand, 19% – over KZT 200 thousand, and 17.5% – KZT 50-100 thousand. This indicated that the majority of respondents who spent enough money on organic products had an average income and could buy these products, but not on a permanent basis. At the same time, 42.5% of respondents were willing to spend 10-15% of their personal income on eco-products, 15-20% of their income – 38.2%, more than 20% of their income – 6.9%, and less than 10% of their income – 12.4% of respondents. When buying organic goods, most of the respondents focused on the quality of products (62.5%), environmental characteristics (52%), and price (50.5%). Among the reasons for buying organic agricultural products were health benefits (62.2%) and high quality of eco-products (45.8%). Thus, the survey showed an increase in interest in organic products and the establishment of a steady demand for the purchase of environmentally friendly products.

DISCUSSION

It is believed that organic agriculture is less productive in terms of yield than traditional agriculture, nevertheless, it is able to bring 3-5 times more profit due to the higher price of the final product, as well as eco-products

are safe for human health, animals, and the environment. Unfortunately, organic production is not able to feed the ever-growing population of the planet, therefore, to achieve food and environmental security of all countries in the future, joint management of traditional and organic agriculture will be promising. The transition to organic farming does not mean simplification and does not exclude an integrated approach to solving problems using modern methods and tools, but only introduces some restrictions, for example, on the use of mineral fertilisers, pesticides, GMOs (Bulkhairova *et al*., 2020). In addition, according to A. Proshchalykina *et al*. (2019), the market of ecological agricultural products is niche, that is, it is focused on some buyers who are willing to overpay for a better product. Despite the above, organic agriculture is gaining more and more momentum on a global scale. H. El Bilali (2020) suggests that it can contribute to climate change mitigation, biodiversity conservation, and environmental impact reduction. However, the comparative indicators of organic farming depend on the specific region of production, just as the difference in the yield of organic and traditional agricultural plants depends on the crops and methods of their cultivation.

T.C. Durham and T. Mizik (2021) have proved that organic farming is less capital-intensive in contrast to traditional. This may be of particular interest to small farmers, who usually do not have the financial means to purchase resources. Thus, organic farmers may be less exposed to financial risks associated with fluctuations in market prices for synthetic fertilisers and plant protection products. Studies by A. Proshchalykina *et al*. (2019) have shown that the market of organic agricultural products can reach a very high level only with balanced consumption and quality control of manufactured products. Examples are such Nordic countries as Denmark, Sweden, France, Switzerland, and Germany. The high level of consumer income also contributes to the development of this market. This can be seen in the example of the USA, Canada, China, and Australia. The prospects for the development of the organic products market in the country are closely related to the development of the following groups of factors: organisational and legal, financial and economic, technological, and socio-psychological. Such a systematisation of factors allows improving and developing effective measures for the introduction of an organic agricultural production system, considering the influence of these factors (Turner *et al*., 2015).

V.V. Grigoruk and E.V. Klimov (2016) report that since 2000, the agricultural sector of the Republic of Kazakhstan has shown a positive development trend, except for certain dry periods that reduce agricultural production. Over 15 years, grain production has increased by 1.6 times, potatoes – by 2.1 times, and vegetables by 2.2 times. In addition, the area for oilseed crops has expanded by 4.5 times. At the same time, the gross grain harvest fully meets the needs of the domestic market of the country and provides the potential to export up to 8 million tonnes of wheat grain. The Republic of Kazakhstan is one of the leading exporting countries of organic wheat, soybeans, cake, and sunflower seeds. These products are mainly imported by the countries of the European Union. According to J. Rustamov *et al*. (2020) and Baydildina *et al*. (2000) Kazakhstan is among the top 10 leading exporters of organic products in the EU. In 2019, the export of all environmentally-friendly products increased by 70.5% compared to the previous year, while the volume of exports of sunflower cakes during this period fell by 4.6 thousand tonnes. Organic wheat exports had good indicators, its volume increased by 31.8 thousand tonnes over the same period. In 2019, the republic also increased the supply of organic oilseeds to the EU market by 60.7%. A similar pattern was observed in the export of ecological soybeans, the volume of exports of which to the EU market increased by 3.4 thousand tonnes.

A brief analysis of the main areas of development of organic agriculture in Kazakhstan showed that the Concept for the transition of the Republic of Kazakhstan to a "green economy" (Decree of the…, 2013) and the Law of the Republic of Kazakhstan No. 423-V ZRK "On the Production of Organic Products" (2015) laid the legal basis for ecological agriculture. According to a number of researchers, to improve the state of organic farming in the republic at all stages of the production of environmentally-friendly products, it is necessary to introduce a system of total control, clear labelling of eco-products, carry out inspections and certifications of production (Uskenov *et al*., 2016; Rustamov *et al*., 2020). Currently, in Kazakhstan, only the regulations of importing countries are applied for the export of organic goods, which have some differences from the requirements of national legislation. This hinders the development of the organic industry. According to a number of researchers (Raihan & Tuspekova, 2022), only technical support from IFOAM (2022) can ensure the promotion of Kazakhstan's environmentally friendly products on the global organic market, and the implementation of the legislation of the republic into the IFOAM Family of Standards can give additional advantages, for example, the recognition of the Law of the Republic of Kazakhstan No. 423-V ZRK "On the Production of Organic Products" (2015) by the export markets of Saudi Arabia and Australia, and the private programmes, for example, the Global Organic Textile Standard.

O.O. Karamatov (2021) and Lazzat *et al*. (2014) argues that, despite the results achieved on the introduction of organic agriculture, there are a number of problems in the Republic of Kazakhstan: there are no organisational and economic mechanisms for the implementation of adopted legislative documents designed to stimulate organic production in the country; farmers incur significant costs when obtaining international certificates

of eco-products from international experts and auditors; there is no single database where all information on the structure and volumes of production and export of organic products would be collected; there is an acute shortage of specialists in this field; there is an insufficiently developed infrastructure for the distribution and marketing of environmentally safe products. To improve the situation, the authors of this study suggests the following solutions: to develop a regional programme for the development of environmentally-friendly production, which should include tools for optimising and specialising producers of organic goods, considering the specific conditions of the republic; to provide financial and technical assistance for the effective implementation of organic projects and programmes; it is necessary to create a common information database of demand and supply for eco-products in markets of all levels (from local to global); to create a national certification service; to improve the infrastructure for the transportation and storage of organic agricultural products.

CONCLUSIONS

The investigation of the main global trends has shown a positive trend in the development of organic agriculture on a global scale. However, at the regional level in a number of countries, this process is still in its infancy. Over the past 20 years, the global area under organic agriculture has increased fivefold and in 2020 amounted to 74.9 million hectares. Togo and Kenya had the largest increase in organic production areas, while Uganda, Kazakhstan, and Mexico had a significant decrease (more than 40%). The growth of farmland under organic production was observed in 44 of the 50 countries under study. In the Republic of Kazakhstan, after the active growth of organic agricultural areas since 2012, which reached more than 300 thousand hectares in 2016, their gradual reduction began and by 2020, the land under organic farming amounted to 114 thousand hectares or 0.1% of all farmland in the country. This negative phenomenon is associated with the deterioration of the economy as a result of the coronavirus pandemic and the global economic crisis. Internal problems also contributed to this: low awareness of the population about the prospects for the development of organic agriculture, underdeveloped infrastructure, lack of a domestic market, high prices for international eco-product certificates, and dependence on international experts.

The results of the reports of the FiBL and the IFOAM showed that in 2020 Kazakhstan entered the 50 countries-producers of eco-products in terms of occupied organic areas and took 49th place among 190 certified producers. It was also among the 20 exporting countries of environmentally friendly products, however, exports decreased by 30.8% compared to 2019. The decline in export volumes also occurred in China, Ukraine, Turkey and other countries. In 2020, 279 organic producers were registered in the republic. The main exports to the EU countries were wheat, soybeans, seeds and oilcake, which brought about EUR 9 million to the country's economy. Analysis of the regulatory framework of Kazakhstan has shown that the country is actively working to promote organic agriculture. This is evidenced by the adopted the Law of the Republic of Kazakhstan No. 423-V ZRK "On the Production of Organic Products", the Strategy "Kazakhstan-2050", etc. The survey conducted by the population of four regions of Kazakhstan showed an increase in interest in eco-products, and the establishment of a steady demand for this type of goods: 42.5% of respondents agreed to spend 10-15% of their income on organic goods. For a more detailed study of this issue, it is necessary to conduct regular surveys in all regions of the republic, and it is also necessary to create a common database on social, environmental and economic indicators of organic agriculture in Kazakhstan.

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Основні напрями розвитку органічного сільського господарства в Республіці Казахстан

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Анотація. Республіка Казахстан має хороший потенціал для розвитку екологічно чистого сільськогосподарського виробництва: великі сільськогосподарські угіддя, велика кількість фермерських господарств, зацікавленість у виробництві екологічно чистої продукції. Ці фактори зумовили актуальність дослідження, основною метою якого було дослідити основні напрямки розвитку органічного сільського господарства в Казахстані. Проаналізовано статистичні дані та звіти Науково-дослідного інституту органічного сільського господарства, Міжнародної федерації руху органічного сільського господарства, Продовольчої та сільськогосподарської організації ООН; використано логічні та емпіричні методи; проведено опитування споживачів і виробників екологічно чистої продукції в Казахстані. У даній роботі показані основні тенденції розвитку органічного сектора в Казахстані і в світі в цілому. Дослідження показали тенденцію зростання органічного виробництва в світі, проте багато країн все ще знаходяться в зародковому стані щодо цієї тенденції. На даний момент світова площа земель під органічним сільським господарством становить 74,9 млн. га, але в Казахстані, починаючи з 2016 року, спостерігається скорочення таких площ. У 2020 році вони склали 114 тис. га або 0,1% всіх сільгоспугідь країни, що пов'язано з пандемією коронавірусу та світовою економічною кризою. За даними міжнародних організацій Research Institute of Organic Agriculture та International Federation of Organic Agriculture Movement, в 2020 році з 190 країн світу Казахстан увійшов до списків 50 країн-виробників і 20 країн-експортерів органічної сільськогосподарської продукції, а експорт цієї продукції склав близько 9 млн. євро. Зроблено висновок, що в Республіці Казахстан поступово створюються необхідні умови для впровадження екологічного сільського господарства, що сприятиме поліпшенню економічного становища країни. Практична значимість дослідження полягала в проведенні соціологічного опитування споживачів і виробників екологічної сільськогосподарської продукції в чотирьох регіонах Казахстану з метою виявлення стану та основних напрямів розвитку вітчизняного ринку органічного сільського господарства

Ключові слова: екологічно чиста продукція, експорт, стандартизація, сертифікація, зелена економіка

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Restrictions on Grain Exports During COVID-19: Features and Solutions

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Abstract. The world export of agricultural and of food products has been a relevant topic at all times, because it regulated the issue of economic and social development of many countries. However, this issue became even more urgent after the start of the COVID-19 pandemic, when the situation in this sector got very complicated. The aim of the study is to look at the features of grain export restrictions during the COVID-19 pandemic and how they can be addressed. The socio-economic method was applied to compare the economy with the market and take into account the multiplicity of economic behaviour. Using the functional method, the theoretical foundations of functional economics were established, focusing on the evolution of terminology to denote certain combinations of restrictions on the export of grain products and services during the COVID-19 pandemic. The method of institutional analysis was used to assess the quality of the institutional basis of the economy and political structures. It was determined that changes in the trade sphere stimulated the development of production in deficit areas. It was defined that the removal of tariff barriers to trade could be useful in overcoming the crisis caused by the pandemic and also an efficient link to reducing the costs of international trade. Another conclusion is that it is worth preserving access to food, rather than restricting exports in countries where people may suffer from hunger. The practical relevance lies in identifying the features of appropriate restrictions on grain exports during a pandemic and highlighting the main ways in which this problem can be addressed

Keywords: sustainable development, pandemic, food security, macroeconomic environment

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INTRODUCTION

The end of 2019 was marked by the outbreak of the coronavirus pandemic (COVID-19). This disease began to spread rapidly around the world and had a significant impact on various spheres of life (Glauber *et al*., 2020). At the beginning of 2020, a large number of measures were introduced to limit the spread of the coronavirus disease. Those measures were relieved in the summer of the same year, but by the end of 2020, all countries of the world still maintained certain restrictions (Sulser *et al*., 2020). These restrictions have had a negative impact on the economy of many countries, as well as on international trade. Many countries have encouraged imports to promote food security, while some other countries have restricted imports to protect their citizens from the spread of COVID-19 (Glauber *et al*., 2020). These various containment measures affected the production, processing, trade and distribution of cereal foodstuffs in the region, with the greatest impact occurring early in the pandemic. It should be noted that world exports of agricultural and food commodities remained relatively stable and unaffected by mines compared to previous years, while world trade in other commodities fell by 9.2% (Rude *et al*., 2019; Liefert *et al*., 2021; Headey, 2019). Most of the disruption to agriculture and food trade occurred early in the pandemic, when several countries-imposed blockages and other containment measures.

According to A. Mullen (2020), while various countries reacted by imposing export restrictions, leading to increases in prices of staple foods on the world market, this led to the limitation of the sale of grain by many exporters. In this way, it was possible to protect consumers from rising prices. In return, the countries that bought the grain were forced to lower the customs tax on food to stimulate internal trade. Consequently, rather than curbing price rises, these policies only increased prices on the world market. These restrictions, even temporary ones, seem entirely unnecessary. W. Martin & K. Anderson (2020) think that export bans by key exporters have restricted world supplies and have certainly pushed up world prices of staple foods. In April and May 2020, the total value of agricultural and food exports fell by 5-11% compared with the overall averages for the same months of 2018 and 2019 (International Trade Centre, 2020). Exports of grain and food products began to recover to previous indicators in the summer of 2020, however, since then, prices for the mentioned goods started to rise, if compared with the prices of previous years (International Trade Centre, 2020).

T.W. Hertel & M.E. Tsigas (2019) believe that the value of imports of agricultural products and foodstuffs followed a somewhat similar trend, but with greater volatility. According to S. Mitra & T. Josling (2019), the aggregate value of imports started to decline in February 2020 and by May had fallen by 15%, compared to 2018 and 2019. In the summer of 2020, sharp changes in the dynamics of world trade in agricultural products were observed. Researchers explain this phenomenon by the change in the number of products, as a result of the harvest, as well as by the increasing of taxes in many countries (Schewe *et al*., 2017). It should also be noted that throughout 2020, prices for grain and agricultural products fluctuated. Thus, in the winter and spring of 2020, export flows worldwide decreased by a quarter, compared to previous years. However, already in autumn, export flows increased; therefore, it was possible to normalize the situation (Puma *et al*., 2020; Sustainable Development Goals, 2022; Torero Cullen, 2020). The export of agricultural products should be divided into two types: primary (grain) and secondary (cotton, tobacco, fish). Thus, the export of primary goods was stable during 2020; therefore, it was possible to avoid a humanitarian catastrophe related to famine.

The purpose of the study is analysing the characteristics of grain export restrictions during the COVID-19 pandemic and how they can be addressed.

MATERIALS AND METHODS

The methodological basis of the study was formed by the following methods to the study of this topic: social and economic, functional and institutional. The social and economic method, complemented by the conceptualisation of a solidarity and pluralistic economy, likens the economy to the market and takes into consideration the plurality of economic behaviour, which is part of the substantive approach to the economy. It refers to the exchange between an individual and their natural and social environment. This exchange provides one with the means to satisfy their material needs. Indeed, the realisation of the latter requires the elimination of irregularities, such as the involuntary disconnection and separation of the global dimension of grain productivity during the pandemic; the ineffectiveness of the communication coordination and consultation system. To meet this need, the social and economic approach enriches management practices that base increased export performance during a pandemic on a single combination of factors, in particular, products, markets, and technologies, that view human capacity as the foundation and aspect that gives clear competitive advantage, hence as an important lever for improved economic performance.

The functional approach provides a brief overview of the origins and theoretical foundations of functional economics, focusing on the evolution of terminology to refer to particular combinations of constraints on the export of cereal products and services during the COVID-19 pandemic. Drawing inspiration from the variety of categories of solution proposals, a typology can be identified that is intended to characterise the main categories of restriction models that companies may use given the current problem, based on a description

of their mechanisms and a selection of thematic aspects that offer advantages from an economic point of view with respect to grain exports. A functional methodology that optimises the use of constraints and services focuses on the management of existing wealth in the form of cereal products. From this perspective, products in a pandemic appear to be mere inputs, intermediaries in a process aimed at fulfilling a function rather than a central object. Their economic value no longer lies in their exchange value, but in their use value, where one doesn't have to focus only on the cost of production, but on the total value of services that include the stages of operation, maintenance or end-of-life processing.

Institutional analysis is built around three basic principles. Firstly, it develops a programme that integrates various social science approaches, but goes beyond the traditional aporias of these methods, which often tend to focus on the stability of institutions. Second, it analytically develops a theory of endogenous change in institutions and an analysis of temporal dependence, in this case the timing of the extension of restrictive measures in relation to the pandemic, where individual interactions are studied in the context of grain export. Third, it reflects the proper functioning of markets, emphasising that institutional foundations of these markets are strong enough and that the functioning of political structures plays a major role here. Indeed, good institutions also stimulate grain export production by generating savings, investment in human and physical capital, and the development and deployment of useful knowledge, particularly during the COVID-19 period. The quality of the institutional framework of the economy and political structures determines decisively the well-being of a society.

RESULTS AND DISCUSSION

Public health crisis associated with the COVID-19 pandemic threatens global food security in one way or another by slowing down grain logistics and transportation chains. This is enough to make grain-importing countries fear rising prices while their financial resources deteriorate. Although global food security has gradually recovered over the past five years, with more than 820 million people globally undernourished in 2018, the long-term trend has nevertheless shown a clear decline in global hunger over 25 years (Sustainable Development Goals However, 2022), the COVID-19 epidemic has now forced nearly half of humanity to stay in their homes, jeopardising the ability of populations that are already vulnerable or in the process of becoming so. The reversal of this crisis is a powerful indicator of the fragility of food supply chains. After the outbreak of the epidemic, agriculture and food seem to be the decisive sectors in the fight against the pandemic, although they have already been in particular demand since the beginning of the crisis.

All Eurasian zone countries have adopted common measures (introduction of export duty, limit volumes of grain export) to limit exports of raw and processed grain products (Headey, 2019). To limit inflation on processed grain products such as bread, Ukraine has restricted wheat exports to 20.2 million tonnes for the 2019/2020 campaign. Announcements of export restrictions by Black Sea countries have triggered a panic effect on the market and, consequently, significant purchases of wheat for storage, jeopardising food security in sub-Saharan Africa. More generally, the unbalanced structure of agrifood trade on the African continent, i.e., value-added exports compared to basic food imports, makes it unnecessary to divert export flows of products that are not staples for the basic food ration, unlike wheat or rice. All the more so as the value of export earnings from these commodities is likely to fall sharply in the coming years. This loss of income at the macroeconomic level for these countries can also be approached in terms of the micro-economy of households, where the isolation of population in these countries blocks the ability to work and earn wages. As can be seen, the global health crisis raises the urgent issue of food security. To alleviate supply constraints, the challenge is to re-establish national or even regional agricultural policies, where possible, and to strengthen a production system capable of meeting food needs of local populations.

Isolating grain exports during pandemic can severely limit the workforce that must work in the fields or food processing plants, directly affecting food production system. But the problem of self-isolation is much more global, since for the majority of population in developing countries, income from activities is daily and allows them buying food. However, by depriving or restricting labour market with containment measures, the most precarious workers risk, at best, facing a sharp drop-in activity and, hence, a loss of income, which would jeopardise their ability to feed themselves. The state of public finances in most of these cases does not allow for a social safety system to compensate for this labour market failure, especially as much of the activity is concentrated in the informal economy. Populations in urban areas with high population densities thus risk being particularly weakened by these disincentives, which will disrupt formal and informal labour markets. Loss of income caused by this reduction in activity will even worse if it is combined with an increase in the price of imported staple foods (Pugachov *et al.*, 2021; Kaletnik & Lutkovskaya, 2020).

Faced with the spread of the pandemic, countries naturally took measures to restrict exports to make the national food security of their fellow citizens a priority. According to the International Trade Centre (ITC) (International Trade Centre, 2020), 80 countries have adopted export-restrictive measures since the start of the crisis, while 57 countries, on the contrary, have liberalised their imports to boost supply. Of the 130 announced export restriction or liberalisation measures worldwide, about 10 concerns the agrifood sector and

are concentrated in countries of strategic importance in the global food balance (International Trade Centre, 2020). Food autonomy seems largely secured, but the global agrifood balance is severely disrupted by this health crisis and leads some transition and developing countries to depend on external food source in a new period of food insecurity. Since late March 2019, due to fears related to the COVID-19 pandemic, several countries have adopted policy measures to ensure sufficient supply on domestic markets and to avoid rising food prices, including cereals. In Ukraine, a decision to sell 160,000 tons of wheat was made, and at the beginning of spring 2020, the maximum export volume of this grain crop was set at 20.2 million tons (International Trade Centre, 2020). This discovery is all the more paradoxical when one considers global agricultural balances, which show an increasing surplus year on year (Table 1).

***Table 1**. Global estimate of global grain production during the COVID-19 pandemic*

	2018/2019	2019/2020	2020/2021	2021/2022 (based on H1 calculations)
Grain production (million tonnes)	2139	2172	2175	2223
Consumption (million tonnes)	2163	2193	2192	2226
Reserves (million tonnes)	625	604	608	605
Annual difference (million tonnes)	-12	-24	-17	-3
Major exports (million tonnes)	164	156	159	168

***Source:** (International Trade Centre, 2020)*

Restrictions that were introduced due to the spread of COVID-19 strongly affected a number of trade factors in underdeveloped countries. It is important to note that due to the introduction of these restrictions, a number of problems arose. As a result, the production of grain products decreased. In particular, these problems include the reduction of personnel and the difficulties associated with the transportation of grain. The most important factor that could help in overcoming this problem was international cooperation. In the context of a possible humanitarian disaster, governments had to put geopolitical interests aside in order to establish communication and find ways to resolve this situation. Grain has a great influence on the development of the entire trade in agricultural products, because grain itself is the basis for the manufacture of other products. It should also be understood that this branch of the economy is seasonal and depends on many objective factors, such as rainfall, which affect the harvest and the subsequent situation in this area. Also, government restrictions in other areas have a great impact on the development of this particular area; thus, the ban on crossing the border has a significant impact on cooperation in the field of agriculture.

Many instruments of export restrictions depend on trade as an engine of economic growth, production and sales in foreign markets. However, the COVID-19 pandemic has a strong impact on demand in key markets such as Europe and the US, and also on trade logistics with these markets. Many regions play a significant role in grain production for the northern hemisphere markets during the off-season, which can be a valuable source of export earnings. At the agricultural level, the COVID-19 crisis highlights the growing complexity of the food export equation. Between the desire to move some production processes to reduce dependence on global value chains and, on the other hand, to strengthen the resilience of these same chains so as not to weaken the supply of structurally dependent countries, the food prism reveals the difficulty of reconciling the two issues. The most appropriate option would undoubtedly be to promote a solution aimed at achieving a balance, i.e., ensuring both a minimum of food autonomy when agronomic and political conditions that allow it and the management of exchange flows necessary for the food security of the most vulnerable areas. This is the kind of agricultural policy mix strategy that could determine the future course of global food systems. Special attention should be paid to the border crossing restrictions for citizens of another country. Although these measures prevent the spread of the disease, they have a negative impact on poor countries, as they destroy already weak economies. In addition, developing countries are highly dependent on the export of grain, because in such countries the agricultural sector forms the basis of the entire economy (Palamarchuk *et al.*, 2021; Patyka *et al.*, 2021).

In this situation, it is important for the problem of export of agricultural and grain products in particular not to be under control of one single institution. Instead, all branches of management and politics should interact to resolve it. Thus, it is important to establish

cooperation between the governments of different countries in order to control and improve the situation in the field of grain trade. Not only government institutions, but also private ones, such as non-governmental organizations etc. should participate in this process. A close connection should also be established between these institutions. Their activities should be aimed at ensuring the transportation of goods, and sharing their own experience in this field in general. It is especially important that countries with strong economies support weaker partners in order to maintain the balance of world trade. Attention should also be paid to the information aspect, since the situation is developing at a rapid pace, the government needs to receive reliable data on the field of grain trade in a timely manner (Giordani *et al*., 2019). The purpose of the mentioned activity should be to ensure access of farmers to world trade, and to provide benefits therefor, with the aim of improving trade. All this can be done through the use and development of information technologies. Due to the remote format of meetings, politicians and heads of organizations can quickly make decisions without risking health.

The pandemic raises concerns about food security. Traffic restrictions, markets that can no longer be contained, cargo and trucks blocked at borders, complicated customs clearance. The health crisis and associated restrictions are weakening the distribution and production chains for agricultural products. With price volatility caused by shocks to food supply and demand, restrictions affect, in particular, the poorest people. After all, essential goods can, in theory, move freely despite restrictions on movement, but the health crisis has reduced the pace of exports and many countries are far from self-sufficient. Subjects of international trade in grain products, in order to maintain a favourable situation, should agree not to make restrictions hindering trade in agricultural goods. The focus should be on measures that will help prevent a global recession and thereby minimise further increases in food insecurity (Janssens *et al*., 2020). In order to fulfil this goal, the governments of countries must provide assistance of various levels and types, in particular, it can be additional investments, provision of social insurance for workers affected by the disease, and in general control of the epidemic situation at work. To prevent further weakening, questions of increased international aid and debt relief for the poorest countries should be raised in international bodies, but without allowing the mistakes of the past to be repeated.

COVID-19 has had a significant impact on the global food chain. Queues in grocery shops, shortages of staple foods and difficulties in shopping while respecting physical distancing measures have affected large parts of the world's population. Due to the lack of distancing options or adequate protection against the virus in grain-processing plants, many workers have quit their jobs and this has led to the closure of many plants. In addition, there has been an increase in food waste due to the shutdown of the commercial supply chain. Uncertainty over food availability can trigger a wave of export restrictions, causing global market shortages. International or regional trade makes a crucial contribution to global food security (Barrett, 2020). National governments need to support local communities and citizens in increasing local food production, including grains, through appropriate incentives – financial and in-kind – to increase food sustainability, minimise food waste and avoid over procurement to ensure equitable access to food for all. This unprecedented crisis is an opportunity to finally give global governance a real dimension, which is bound to lead to a deep questioning of international institutions.

As in many other export supply chains, food systems are complex and global, and are currently disrupted by a number of factors: disruption to harvesting, as agriculture is heavily dependent on cheap but skilled and flexible labour; production disruption, as production has been completely halted in various locations around the world due to outbreaks of virus among staff and difficulties in maintaining physical distancing measures; transport and trade disruption, as some ports do not operate normally, delivery may be delayed or cancelled, trains and trucks cannot cross certain borders, and some countries have suspended all flights. Although transportation is permitted, export restrictions can affect delivery and, consequently, reduced grain exports are affecting livestock farmers. Indeed, some of them have already experienced difficulties in finding enough food for their herds. Thus, the human dimension of this pandemic extends beyond people directly affected by the virus and seriously threatens the second Sustainable Development Goal, which is the elimination of hunger (Sustainable Development Goals, 2022). In some agricultural regions, droughts and locust invasions further exacerbate the situation. The pandemic confirms the need to stress-test the export supply chain, have an effective business continuity plan and build strong relationships with suppliers rather than relying on third parties (Falkendal *et al*., 2021).

According to D.G. Brewin (2020), as for the current high grain prices, they rose due to global supply-demand relationships and regulatory measures imposed by major exporters, such as additional duties and export restrictions, which are the result of the effects of COVID-19. Nor should one look for a surge in capital interest in investing in agricultural markets, because such activity has been ongoing for years and is likely to continue in the future. R.S. Gray (2020) considers that export restrictions operate against a backdrop of panic buying, which has left supermarkets with empty shelves not because of a lack of goods but because of logistical obstacles created by pandemic containment measures. Export restrictions on wheat and wheat flour increased the prices of essential commodities, including bread. R.F. Ceylan *et al.* (2020) convinced that with the onset of the crisis

triggered by COVID-19, the strain on public policy to strike the necessary balance between commitments and needs intensified and required going beyond traditional measures to guarantee the survival of the health system and the well-being of the supply chain, particularly cereals. These statements coincide with the results of the research, because authors found that the pandemic led to the imposition of mandatory quarantine measures and border closures by virtually all governments worldwide. These measures affected both production and trade flows. Trade distortions led to shortages and unexpected increases in the prices of major cereal exports.

The opinion by C.M. Galanakis (2020) that a slowdown in the movement of agricultural and food workers is blocking many farms is completely relevant. With the closure of borders due to the pandemic, they have all simultaneously become dependent on labour from elsewhere. Regarding the flexibility of customs processes, the multilateral system approaches non-tariff barriers to trade from different angles, where there is no overarching regulation on the issue. In general, J.E. Hobbs (2020) thinks that none of the instruments prohibit or discourage measures aimed at removing non-tariff barriers. On the contrary, they include provisions governing the application of such measures in a non-discriminatory and not overly trade-restrictive manner. According to W.A. Kerr (2020), the ban on exports and re-exports, however, was not absolute; as such transactions were allowed at the request of the party concerned, with prior authorisation, provided that two conditions were specified: that the supply of a particular product for the domestic market was sufficient; the availability of surplus resources had to be confirmed. In this sense, the evaluation carried out in each case must include a general and periodic analysis of the behaviour of the grain on the export market and also a specific study of the supply or production capacity of the inputs (Sohrabi *et al*., 2020; Xiao *et al*., 2020; Laborde *et al*., 2020).

Many regions are struggling to keep borders open and restore trade flows, several trade-related challenges have arisen (Ali *et al*., 2019). Increased air freight costs due to reduced commercial flights are hampering supply chain operations and the timely delivery of grain (Dey & Shekhawat, 2021). Measures limiting human mobility affect various production, trade and marketing processes. Also, delays in the transportation of grain at the borders, due to increased security measures, lead to additional financial costs. This process also takes time; in particular, S. Akter (2020), claims that such circumstances affect the supply of seeds and fertilizers to countries manufacturers, which creates a number of problems in the future. The global cereal harvest is expected to reach 2.175 billion tonnes by the end of the 2022 campaign and projections for the 2022/2023 season are for 2.223 billion tons – the richest season in the world, as far as figures allow (Wolfson & Leung, 2020; Boyacı-Gündüz *et al*., 2021). The ratio of available stocks to global consumption has stabilised between 27% and 30% over the last 5 years, but with increasingly large differences between cereal types (Hobbs, 2021). According to S. Kumar (2022), if the stock-to-consumption ratio of maize drops to 23%, due to a very strong increase in animal consumption, the ratio of wheat and rice, whose consumption is mainly for humans, will reach a record 37% by the next campaign. This is equivalent to an annual coverage of more than 4 months of global consumption of these two grains required for various diets around the world. At the height of the 2008/2009 crisis, this figure was not as high, with an annual coverage of barely 2 months, even as the price of grains and oilseeds skyrocketed, plunging populations in some countries into a dynamic of hunger riots (Kumar *et al*., 2022).

CONCLUSIONS

The pandemic has caused significant distortions in international trade and, in particular, in global grain value chains. Border closures have increased transport costs and, hence, the costs of international trade. In the same sense, mandatory quarantine measures, accompanied by the closure of production centres and a reduction in available labour, have led to shortages and higher grain prices. Similarly, the costs associated with customs processes, with fewer customs personnel and new bio-security procedures, have risen significantly. Possible ways to improve supply chains could be communication between different regions to share best practices; development of a trade promotion strategy by regional organizations; maintaining regular contacts of authorized bodies with industry, directly or through certification bodies; digital technology for exchanging information between countries.

When it comes to protecting health and well-being of citizens, it is important to ensure that all trade restrictive measures on grain exports do not disrupt food supply chain. It is precisely in times like these that international cooperation is a necessity, so that the response to the COVID-19 pandemic does not lead to unintended shortages of essential goods and exacerbates hunger and malnutrition. Also important is the development of digital technologies, by increasing the number and size of investments and creating opportunities for the development of talented young people working in this field. Beyond the human tragedy, this ongoing pandemic has deeply weakened economies, plunging many regions into severe recession, but above all it has disrupted the established global economic order. And, so far, the global economy has not fully recovered from this health crisis. The recovery from COVID-19 has created an imbalance between supply and demand, leading to severe pressure on grain prices, in particular due to bottlenecks in production chains, to which dizzying increases in transport and freight costs, rising energy prices, airport disruptions and shortages of some products have been added.

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Обмеження на експорт зерна під час COVID-19: особливості та шляхи вирішення

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Анотація. Світовий експорт сільськогосподарської та харчової продукції був актуальною темою в усі часи, оскільки регулював питання економічного та соціального розвитку багатьох країн. Однак ще більшої актуальності це питання набуло після початку пандемії COVID-19, коли ситуація в цьому секторі дуже ускладнилася. Метою дослідження є розгляд особливостей обмежень експорту зерна під час пандемії COVID-19 та шляхів їх подолання. Соціально-економічний метод застосовано для порівняння економіки з ринком та врахування множинності економічної поведінки. За допомогою функціонального методу встановлено теоретичні засади функціональної економіки, зосереджено увагу на еволюції термінології для позначення певних комбінацій обмежень на експорт зернової продукції та послуг під час пандемії COVID-19. Метод інституційного аналізу використано для оцінки якості інституційного базису економіки та політичних структур. Визначено, що зміни в торговельній сфері стимулювали розвиток виробництва в дефіцитних галузях. Встановлено, що усунення тарифних бар'єрів у торгівлі може бути корисним у подоланні кризи, що спричинена пандемією, а також дієвою ланкою зниження витрат міжнародної торгівлі. Інший висновок полягає в тому, що варто зберігати доступ до продовольства, а не обмежувати експорт у країнах, де люди можуть страждати від голоду. Практична значущість роботи полягає у визначенні особливостей доцільних обмежень експорту зерна під час пандемії та виокремленні основних шляхів вирішення цієї проблеми

Ключові слова: сталий розвиток, пандемія, продовольча безпека, макроекономічне середовище

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Analysis of Modern Instruments for Strategic Management Accounting at Agricultural Enterprises

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Abstract. In modern realities of enterprises functioning, methods that keep management records play an important role. Therefore, it is always relevant to assess effectiveness of existing methods, find opportunities for their improvement or consider new accounting instruments. In this work, the emphasis is on the analysis of strategic management tools within the framework of the Republic of Tajikistan and the country's agricultural sector, the reason for which is its significant role in the effective development of the state's economy. Thus, the purpose of the work is to show the principles of choosing strategic accounting tools at agricultural enterprises in Tajikistan and to assess their effectiveness. Analysis was the main research method in the research; in addition, modelling, abstraction, historical approach and other tools played an important role. The work analysed the main features of accounting in the Republic of Tajikistan in the context of modern strategic management at agricultural enterprises. The article also briefly describes the current state of the agricultural sector, its role in the functioning of the country's economy and its development prospects. In addition, the features of three main strategic methods of cost management were analysed, namely, "standard costing", "direct costing" and Activity Based Costing; the work describes advantages and disadvantages a company can receive using one of these methods. The article adds new knowledge to the concepts of modern strategic management, in particular in the context of agricultural enterprises, and also allows better understanding of the peculiarities of Tajikistan's economic development and methods of accounting in this country

Keywords: agricultural sector, the economy of Tajikistan, accounting, cost calculating systems, entrepreneurship

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INTRODUCTION

Due to the abundance of economic crises in the last few years, international economic processes, as well as functioning of each individual economic system, have undergone huge changes (Guenette *et al*., 2022; Jackson *et al*., 2021). Therefore, it is not surprising that irreversible developments have also affected the economy of Tajikistan, which has undergone significant changes in recent years, affecting almost all spheres of economic activity. These changes require a review of the principles of economic management at companies, including agricultural enterprises, and cause the need to improve the means and methods of managing them. Under modern conditions, the main task of managing agricultural enterprises is to increase profits and achieve the highest efficiency of agricultural production. Agricultural sector is dominant in the national economy of the Republic of Tajikistan; therefore well-being of the country's population depends to a greater extent on the state and efficiency of economic entities in this sector (Khushvakhtzoda & Oyev, 2020).

Economists agree in their reasoning that accounting, and in particular, management reporting, is required not only for the state (to evaluate the enterprise regarding the legality of its activities and levying taxes that are fair, in the opinion of the state), but also for the company itself, which thus gets the opportunity to monitor development and make management decisions during its activity (Ystrom, 2019; Gardi *et al*., 2021). Thus, discussion of modern strategic management tools at agricultural enterprises of the Republic of Tajikistan remains important. A significant number of scientists have worked on the analysis of this and related problems. In particular, K. Pawlak and M. Kolodziejczak (2020) described the peculiarities of preparation of accounting statements in the Republic of Tajikistan, pointed out disadvantages of the existing reporting system, and also emphasised the importance for the country to transition to international financial reporting standards in their work. In turn, T.F. Plotaeva and V.A. Yakushina (2013) worked on studying the advantages and disadvantages of the "direct costing" system. In their work, they described the features of this system in sufficient detail, and also emphasised the purposes which it is best used for. C. Ratnasih and R.A. Sulbahri (2022) also worked on this matter, focusing on the effectiveness of this model based on empirical indicators at Indonesian enterprises. At the same time, W.K. Shihab (2022) describes the ABC (Activity Based Costing) system in the same manner outlining its positive and negative features.

The objective of the research is the overview of enterprises of the agricultural sector as such, as well as their methods for choosing tools of strategic management accounting. The novelty of the work consists in a detailed coverage of the features of the main cost accounting systems at enterprises ("standard costing", "direct costing" and ABC), as well as in description of the benefits and negative effects received by companies from their use.

Thus, *the purpose of the work* is to analyse the methods of choosing strategic management tools for enterprises in the agricultural industry, and to determine the principles of this choice.

MATERIALS AND METHODS

As part of the research, great attention is paid to the problems of choosing cost accounting systems at agricultural enterprises. In particular, the "standard-costing", "direct-costing" and ABC systems are considered. Such choice was made because the system of instruments of strategic management accounting itself is quite extensive. This makes it almost impossible to consider all its components in one paper. Thus, it was decided to concentrate only on one of its components.

The main sources for writing the article were the works of other authors. In addition, some statistical sources were used, among which it is worth highlighting the Statistical Yearbook of the Agency on Statistics under President of the Republic of Tajikistan (2022). An important role was also played by regulatory acts used in the work: Order of the Ministry of Finance of the Republic of Tajikistan No. 124 "On Approval of the Instruction on the Procedure for Filling in the Forms of Quarterly and Annual financial statements by business entities" (2001), Resolution of the Government of the Republic of Tajikistan No. 210 "On Approval of the Regulations on Calculating the Cost of Products (works, services) at enterprises and organizations of the Republic of Tajikistan" (1999) and Order of the Ministry of Finance of the Republic of Tajikistan No. 41 "On Approval of the Chart of Accounts for Accounting of the Financial and Economic Activities of Economic Entities and the Guidelines for the Application of the Chart of Accounts for Accounting of the Financial and Economic Activities of Economic Entities" (2011).

Thus, theoretical abstract-logical methods of research became the basis of the work. The main method was analysis, the reason for which is the data processed in the work and used in the formation of the main conclusions of this research. With the help of modelling, the influence of different types of cost accounting on the development of the enterprise to determine their advantages and disadvantages was formed; abstraction was also used to achieve the same goals. Also, the work uses the method of deduction, which allows forming a complete vision with the help of individual data on the state of the strategic management accounting instruments in Tajikistan. Historical method, which made it possible to assess the history of the development of the agricultural sector in the country, was used as well. In addition, a significant number of statistical research methods were used, among which it is worth highlighting the graphic one, allowing qualitatively demonstrating, order and analysing disarranged data sets.

Therefore, the entire work can be divided into several main stages. The first one briefly describes the general current state of the economy of Tajikistan, namely, the country's agricultural sector. At the second stage, the features of the operation of the three main methods of cost accounting ("standard costing", "direct costing" and ABC) when using them during enterprise accounting are described; possibilities of cost estimation at companies are analysed, based on the features of accounting in the country. At the third stage, the obtained results were compared with data from the works of other scientists.

RESULTS

Effective functioning of a modern agricultural enterprise implies a combination of various interrelated production sectors and farms (crop production, animal farms, processing of uncultivated agricultural products) (Ninson, 2020; Liu, 2021). In some cases (for example, when summarising and presenting statistical information on certain macroeconomic indicators), hunting, forestry and fisheries are added to the agricultural sector. Today, agricultural enterprises of the Republic of Tajikistan are a key link in the process of food production and play an important role in ensuring the food security of the country. This trend can be seen below in Figure 1.

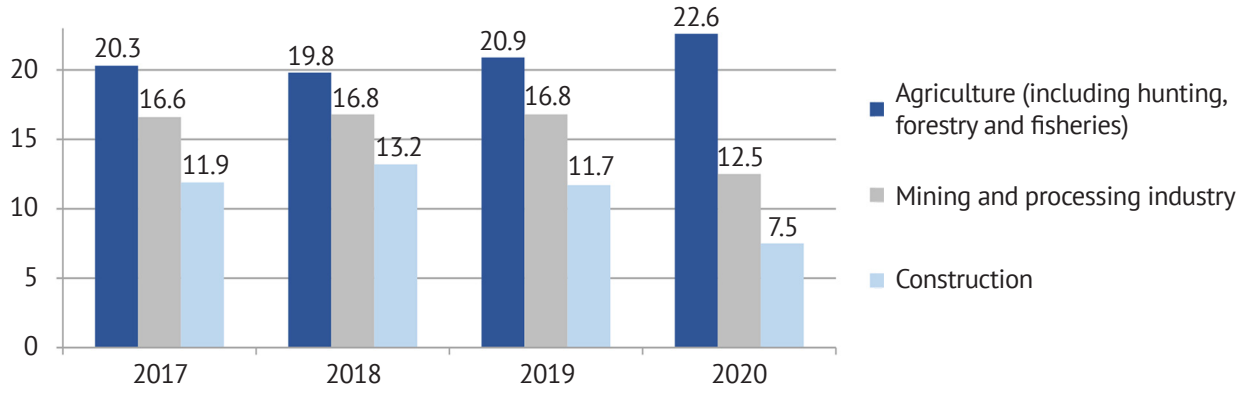

***Figure 1**. Share of the main industries in the production of gross domestic product of the Republic of Tajikistan, %*
***Source:** compiled by the author based on data from the Statistical Yearbook of the Agency on Statistics under President of the Republic of Tajikistan (2022)*

As can be seen from Figure 1, the country has a significant share of all agricultural products in the structure of gross domestic product (GDP) production. So, if in 2020 the volume of GDP production in the country amounted to 82543.0 million somoni, then 18659.8 million somoni of this (or 22.6%) falls on the agricultural sector and production areas close to it (hunting, forestry and fisheries). With the increasing importance of agricultural production in the national economy, special attention is paid to improving the management system of agricultural enterprises (Ferreira *et al.*, 2022; Fan & Rue, 2020). Working in difficult market conditions, agricultural enterprises are trying to ensure the efficiency of their production through the use of modern management tools. As evidenced by the experience of management activities in the country and abroad, one of the factors that most influence the efficiency of the production activities of agricultural enterprises is the cost of their products (Kumudasari & Saroso, 2020). The quality, timeliness and relevance of information on the cost of production used to manage agricultural enterprises affect all indicators of their performance (Dagmara & Cichoń, 2017; Sener *et al.*, 2019). Reliable and timely information on the expenditures and costs of agricultural production determines the validity of management decisions made by managers of agricultural enterprises, and also contributes to an increase in the overall efficiency of their production activities. Such information is formed in the management accounting system, one of the most important components of which is strategic accounting.

Strategic management accounting provides directors and managers of modern agricultural enterprises with an effective and diverse toolkit, among which the leading place is occupied by methods and tools for accounting and managing production costs (Nik Abdullah *et al.*, 2022). At the same time, the choice of the most appropriate methods and means from the totality of the proposed tools is a serious problem of strategic management accounting in modern conditions. The quality of the information base for strategic planning, control and analysis, and, consequently, the level of efficiency of the entire system of strategic management accounting depend on the optimality of this choice (Shalaeva, 2014). Special literature on management accounting discusses the means and methods, realisation of which can technically implement various accounting procedures for preparing information for cost management in strategic management accounting (Pronyaeva & Fedotenkova, 2015). The methodology of strategic management accounting in terms of managing expenditures and product costs can be implemented on the basis of traditional elements of the accounting method (documentation, inventory, accounts and double entry, balance

sheet generalisation, evaluation, costing), economic analysis (grouping, comparison, vertical analysis, horizontal analysis, factor analysis, coefficient analysis) and management (planning, forecasting, development and application of norms and limits, control, regulation, etc.) Strategic management accounting, applying and adapting these methodological components, creates its own methodological toolkit, its choice of elements depending on the set management goals and specific management tasks to be solved. Traditional accounting, economic analysis and management play a key role in the formation of management accounting tools.

After having explored the literature on management accounting, as well as taking into consideration the peculiarities of the organisation of production in the agricultural sector of the Republic of Tajikistan, the following conclusion was drawn: the basis for the development of modern tools for strategic management accounting at agricultural enterprises in order to manage the cost of agricultural products and analyse the behaviour of costs in this industry can be integration of positively proven management accounting systems. Among such systems, the systems of "standard costing" (normative cost accounting), "direct costing" (accounting for direct variable costs) and ABC (Activity Based Costing) or "activity based costing", etc. can be considered. The cost accounting system "standard-costing" is a system for calculating the cost at standard expenditures (Eisenberg, 2016). The term standard costing means "pre-set cost" and the cost accounting method based on it is that the accounting reflects not what actually took place, but what should happen (Avdeeva *et al*., 2020). When it is applied, deviations of actual costs from normative (standard) are separately reflected in accounting. All costs incurred in accounting are correlated with the normative; an analysis of the identified deviations is carried out when comparing the actual costs with the normative ones. The purpose of this cost accounting system is to identify losses and deviations in the profits of the enterprise. The system is based on the preliminary rationing of costs for the manufacturing of products.

The essence of the "direct costing" system is the division within its framework of all costs of the enterprise into fixed and variable (Caril & Canavari, 2013). In it, fixed costs (related to the reporting period and not dependent on production volumes, sales or other variable indicators) are not included in the cost of manufactured products, and when calculating the cost price, only variable costs are taken into account (directly dependent on the volume of output, sales or other variable indicators). Finished goods and work in progress are taken into account only in the amount of variable production costs; the fixed costs of the reporting period are generally attributed to the financial result of the enterprise and are not allocated to specific types of products (Prüggler *et al*., 2011). Under the conditions of using the "direct costing" system at agricultural enterprises, the scheme for constructing a profit or loss statement becomes multi-stage. A distinctive feature of this cost accounting system is that it makes it possible to explore the relationship between production volume, costs and profit. The problem of using the "direct costing" system is the difficulty in identifying and differentiating variable and fixed costs due to the difficulty of assigning them to a specific category of costs.

According to the national accounting rules (Order of the Ministry…, 2001; Resolution of the Government…, 1999) and in accordance with the chart of accounts for the financial and economic activities of economic entities of the Republic of Tajikistan (Order of the Ministry…, 2011), all variable costs of an agricultural enterprise (wages of workers associated with the production of agricultural products, costs of raw materials, other materials, etc.) are accumulated on the synthetic account 10730 "Work in progress" and then, as products are released, they are attributed to the balance of finished products and work in progress. Agricultural enterprises can also record variable expenses in the accounts of category 55100 "Expenses for the production of biological assets", depending on the types of agricultural activities, types of biological assets and the harvested crops.

The fixed costs of an agricultural enterprise (rent of fixed assets, interest on a bank loan, advertising and sales promotion expenses, etc.) are reflected in the accounts of the category 55200 "Sales expenses", 55300 "General and administrative expenses" and are debited at the end of the reporting period debit account 70000 "Summary of income and expenses". The results of the activities of agricultural enterprises are recognised at fair value less the estimated costs of selling finished products and are reflected in the accounts of category 10750 "Agricultural products from biological assets" (10751 "Finished crop production", 10752 "Finished livestock products", 10753 "Finished industrial products", 10754 "Finished products of auxiliary production and other agricultural services", 10755 "Products purchased from the population") and the corresponding accounts of category 11400 "Biological assets" (11410 "Animals – consumable biological assets", 11420 "Animals – fruit-bearing biological assets", 11430 "Plants – consumable biological assets", 11440 "Fruit-bearing plants", etc.). The difference between identified amount and the cost of identified biological assets – profit or loss from agricultural activities is reflected in the accounts of category 44100 "Income from biological assets" (44110 "Profit (loss) from the initial recognition of biological assets, 44120 "Income from the collection of agricultural products", 44130 "Profit (loss) from changes in the fair value of biological assets" (Order of the Ministry…, 2011).

Today, many agricultural enterprises, when calculating the cost of production, face a significant problem in choosing the method of allocating overhead (indirect) costs between types of products (Lizot *et al*., 2021). For a long time, in their management accounting

system, they have been accepting direct labour costs for the production of a unit of output as the basis for the distribution of overhead costs. However, in modern market conditions, this procedure has lost its effectiveness, and agricultural enterprises resort to finding a new procedure for distributing overhead costs. The prerequisites for the search for new methods of distribution of overhead costs were the following circumstances:

– expansion of the range of types of agricultural products and crops produced, as well as an increase in the volume of products and costs for their production;

– a decrease in the share of direct labour costs in the cost structure and a proportional increase in indirect overhead costs as a result of greater involvement of mechanisation and automation in the production of crops and products;

– an increase in the expenditure of costs not related to the production activities of agricultural enterprises (costs for logistics, advertising and sales promotion, costs for the preparation, adjustment and maintenance of mechanisation and automation).

In addition, the use of computer technology and new technology for processing accounting information has made it possible to apply more advanced and sophisticated methods for processing primary accounting information. The above circumstances were the reason for the use of ABC system in cost accounting and calculation of the cost of agricultural products. This system implies that production costs arise as a result of performing certain types of activities or works (operations). At the same time, it is considered that products are not the cause of costs, but the cause of operations or works that result in costs (Egorova & Yudanova, 2015). Manufacturing of each product requires performance of certain operations (works) that demand certain resources. This circumstance contributes to the cost accounting methodology based on the process approach. It is the processes that help to objectively evaluate and explore the relationship between output and costs. The main difference between ABC system and other cost accounting systems is the special procedure for distributing indirect (overhead) costs. Under the conditions of application of this system, the cost of agricultural products is defined as the cost of direct expenditure plus the share of indirect expenditure of each type of activity included in the cost of this product. In turn, the share of indirect expenditure is defined as the product of the of cost carriers value (cost carrier is an indicator of measuring the activity of a particular type of activity, reflecting the essence of this type of activity and interconnected with the object whose cost is being calculated) of this type of activity by its quantitative value, correlated with a specific object cost calculation.

DISCUSSION

In general, as noted in the work of I. Boryshkevich (2014), the term "strategic management" came into use at the turn of 1960s and 1970s, to emphasise the difference between the current management at the production level and the control that was carried out at the highest level to predict various kinds of events affecting company development in the future. Thus, this phenomenon is relatively new in terms of modern business. In turn, interpretation of the concept of "strategic management accounting tools", which is one of the components of "strategic management", remains ambiguous, since it can be described differently by different scientists. A.V. Glushchenko and E.N. Samedova (2012) define it as a complex mechanism for coordinating and integrating specific tools for the formation of accounting and management information. Its variable use enhances the ability to achieve goals, and the tool as a means of practical implementation of one or a combination of its methods. As follows from this definition, the tools of strategic management accounting contribute to obtaining information that meets the management needs of various users about the production activities of an agricultural enterprise. Under the tools of strategic management accounting at agricultural enterprises is understood a set of means and methods for obtaining, processing, summarising and presenting information necessary for making valid and effective management decisions, as well as evaluating their implementation.

It should be also noted that the accounting reporting standards of Tajikistan are not effective enough, because they possess many attributes that have remained since the existence of the Soviet Union. Therefore, in Tajikistan, as in most post-Soviet states, an attempt was made to switch to International Financial Reporting Standards (IFRS). However, there were some problems. N.A. Prodanova *et al.* (2016) admit that, for a qualitative transition to new audit standards, the country lacks the knowledge and experience for such a transformation nationwide. In addition, there are financial, informational and resource shortages. In addition, entrepreneurs themselves lack motivation to shift to these standards. A serious problem also turned out to be the contradiction between the regulatory framework of Tajikistan and IFRS. However, in case of a gradual transition to the principles of international standards, the country's enterprises will not only be able to interact with creditors much easier, but will also receive new opportunities for more innovative accounting, which will increase their efficiency.

It was already mentioned that in the Republic of Tajikistan, agricultural sector played an important role for the functioning of the economy as a whole: this was due not only to macroeconomic indicators, but also to some others. In particular, S.A. Kurbonov (2011) argues that agriculture in the country remains and would probably remain the principal component in the structure of the economy along with industry and other sectors. The scientist describes the industry as very promising in the subsequent development of the country and believes that

it should become its basis. This is especially relevant in today's realities, given the momentum the global famine problem is beginning to gain (Pollard and Booth, 2019). At the moment, there are many problems in the country's agriculture. Without going into too much depth, entrepreneurs should probably focus on intensive expansion methods for their decisions, since, as K. Pawlak and M. Kolodziejczak (2020) note; such methods are more efficient than extensive. The work describes the essence of the functioning of several cost accounting systems, the first of which is the "standard-costing" system. During its analysis, conclusions were drawn that it had significant advantages, which were based on the timely identification and prevention of adverse events in the process of forming the cost of production and the profit of the enterprise. It should be noted that its effectiveness is noticed by some scientists from other countries using empirical data as an example. However, other scientists highlight some of the shortcomings of this system. In particular, Pyper *et al.* (2022) writes about the complexity of these standards (difficulties in determining the balance of finished products in the warehouse and work in progress in the workshops due to changes in market prices under inflationary conditions).

As for "direct costing" system, it also has its advantages and disadvantages in comparison with others. It should be noted that in general there are two varieties of this system: direct (basic, classical) "direct costing", as well as developed, differentiated multi-stage "direct costing" (variable costing), however, it will be considered only their general features (Ratnasih & Sulbahri, 2022). T.F. Plotaeva and V.A. Yakushina (2013) evaluate the benefits received by the company when usin direct costing system. Among them is the ability to quickly reorient production in response to rapidly changing market conditions and find the types of products or services with the highest profitability (thus getting the opportunity to switch exclusively to their production). In other words, a feature is the ability to calculate variable costs separately for each product. Scientists describe many other advantages of the system, as well as its disadvantages, but point out that they are few compared to the benefits. It can be only noted that the main disadvantage of this system is that it can be very difficult to accurately separate the variable and fixed costs at the enterprise, which is why the process of accounting formation can be laborious.

W.K. Shihab (2022) describes ABC costing system in great detail in his work. In the article, the scientist defines a whole set of positive and negative aspects of the system. Among the advantages, the scientist highlights the following: the system determines the goods and services that make the greatest or least contribution to the business, calculates costs more accurately, which gives greater control over indirect costs, provides information for making strategic decisions, is applicable to all types of organisations, allows linking costs with their cause, measuring the performance of both workers and departments, creating financial forecasts. Among the flaws, the scientist writes about the high level of the reliability of information role (much higher than that of others), possible difficulties in determining cost factors; in addition, the system requires primary cost sharing, is focused on cost optimisation, leaving aside the organisation's systemic vision, and also does not adequately account for unforeseen expenditures in the cost. It is difficult to unequivocally answer what kind of cost accounting system at an enterprise as a whole can be the most effective for conducting agricultural business. Complicating this task is the fact that all methods have their strengths and weaknesses, many of which are important for the functioning of agriculture. However, according to the author, the ABC system can still be the most effective for companies, given the positive factors that it brings, in particular, the ability to regulate indirect costs, the ability to evaluate the performance of separate costs and departments, determine the type of product that brings the most business benefits (in this case, the type of culture), etc. It should be noted that among existing methods there is definitely no best one, since each of them can be more useful in different situations.

CONCLUSIONS

In the work, some features of submitting financial statements in Tajikistan were briefly considered, pointing out some of their shortcomings and the need for a subsequent transition to international auditing standards. In addition, the article describes the current state of the agricultural sector in the country. In the context of agriculture, it is noted that the sector is quite promising for subsequent progress in the country, and moreover, for becoming its foundation.

The article analyses three main types of cost accounting for products, namely, "direct costing", "standard costing" and ABC. It was shown that all of them had their own strengths and weaknesses and were suitable for solving a certain range of problems. Thus, "direct costing" can be the best choice if the company wants to be able to accurately estimate the amount of costs per unit of production; ABC – to evaluate the performance of individual departments in the company; "standard-costing" – to evaluate deviations in the results obtained from the planned ones. Therefore, the choice of strategic management tools for effective cost accounting and calculation of production costs for a particular agricultural enterprise will differ depending on the goals pursued by the company. Many performance indicators of the company, as well as its ability to make the right management decisions, will depend on how correctly the management accounting tool is chosen. The system of cost accounting and calculation of the cost of production chosen by the enterprise should be fixed in the accounting policy of the agricultural enterprise. However, according to the author, in general, the most effective for companies in the agricultural

sector, among all the models described in the paper, is the ABC system.

A promising direction for further research is the formation and compilation of practical advice for entrepreneurs in the agricultural sector to determine the optimal strategic management accounting tools for the most efficient enterprise management. In addition, it is important for future research to consider in more detail other components of the strategic management accounting toolkit.

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Аналіз сучасних інструментів стратегічного управлінського обліку на сільськогосподарських підприємствах

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Анотація. У сучасних реаліях функціонування підприємств важливу роль відіграють методи ведення управлінського обліку. Тому завжди актуальною є оцінка ефективності існуючих методів, пошук можливостей їх удосконалення або розгляд нових облікових інструментів. У даній роботі акцент зроблено на аналізі інструментів стратегічного управління в рамках Республіки Таджикистан та аграрного сектору країни, причиною чого є його значна роль в ефективному розвитку економіки держави. Мета роботи – показати принципи вибору інструментів стратегічного обліку на сільськогосподарських підприємствах Таджикистану та оцінити їх ефективність. Основним методом дослідження в роботі був аналіз, крім того, важливу роль відіграли методи моделювання, абстрагування, історичний підхід та інші інструменти. У роботі проаналізовано особливості бухгалтерського обліку в Республіці Таджикистан в контексті сучасного стратегічного управління на сільськогосподарських підприємствах. У статті також коротко охарактеризовано сучасний стан аграрного сектора, його роль у функціонуванні економіки країни та перспективи розвитку. Крім того, проаналізовано особливості трьох основних стратегічних методів управління витратами, а саме: «стандарт-костинг», «директ-костинг» та «Activity Based Costing», описано переваги та недоліки, які може отримати підприємство, використовуючи один з цих методів. Стаття додає нові знання до концепцій сучасного стратегічного управління, зокрема в контексті сільськогосподарських підприємств, а також дозволяє краще зрозуміти особливості економічного розвитку Таджикистану та методи ведення бухгалтерського обліку в цій країні

Ключові слова: аграрний сектор, економіка Таджикистану, бухгалтерський облік, системи калькулювання собівартості, підприємництво

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