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Dynamics of hypotrophic changes in the morphological formations of the ovaries of heifers of mating age on the 7-9th day of luteogenesis

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Abstract. Real-time *in vivo* studies of the physiological or pathological state of the ovaries of cows and heifers are currently relevant because it is the morphological and functional state of the gonads of female cattle that directly affects the effectiveness of biotechnical procedures used in practice: fertilisation, stimulation, and synchronisation of sexual function. The purpose of this study was to find the quantitative and qualitative indicators of the morphological and functional state of the ovaries of Ukrainian red

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dairy heifers during the economic year under loose housing conditions of industrial dairy complex. The study used a modified differential diagnosis of the morphometric and functional state of the ovaries at a fixed time of the sexual cycle – from 7 to 9 days after ovulation of the dominant follicle (Day 0 of the cycle). To compare the results of transrectal examination of heifers in different seasons of the year, the technique of visualisation of palpation data *in vivo* was used. 175 heads of heifers of mating age were examined, among which on the 7-9th day of the sexual cycle, 55.42% were found to have a palpable corpus luteum in the ovary according to the stage of development, 26.96% had premature lysis of the corpus luteum, 8.57% had morphological and functional formations on the surface of the ovary, and 9.14% had symptoms of degenerative gonadal pathologies. Subsequently, animals with degenerative changes in the ovaries (follicular kytosis) did not take part in the study. The remaining heifers had ovaries with symptoms of in-depth hypotrophic changes in the tissues (30.00%) or signs of premature lysis of the corpus luteum (33.33%). During the economic year, a pronounced dynamics in the growth or decrease of hypotrophic phenomena in ovarian tissues was noted; the best indicators of morphometry and functional activity of heifers were observed in the warm season, which indicated a considerable effect of fresh air and solar insolation on the sexual function of heifers of mating age. It was found that the level of fertility of heifers during the year (an average of 40.02%) indicated the presence of errors in the technology of growing replacement stock in an industrial enterprise. The findings can be useful for optimising the schemes of hormonal synchronisation of the sexual cycle of replacement heifers and for the correct selection of hormonal therapy in case of symptoms of decreased sexual activity

Keywords: heifers; sexual cycle; corpus luteum; morphological and functional state; polymorphism; hypogonadism

INTRODUCTION

Decrease in fertility levels of dairy cows is an urgent problem in modern dairy farming. The infertility of the dairy herd substantially affects the profitability of dairy production, which is noted in numerous studies by Ukrainian and Western European authors. Thus, according to Ukrainian researchers, the prevalence of infertility in the dairy herd can reach 45-49%, and in 5-76% of cases, pathology of reproductive organs is the reason of sexual function disorders. According to O.O. Borshch *et al.* (2020), 53% of gynecological diseases were diagnosed with the persistence of corpus luteum, 42% – with ovarian hypofunction, and 5% – with cystic lesions of the gonads. A sufficiently long period of heifers until they reach physiological (economic) maturity is a biological feature of cattle as a type of productive animal. During this period, in industrial conditions young animals' organism is exposed to heterogeneous harmful factors, which can negatively affect the development and further functioning of gonads (Bors *et al.*, 2018; Bors & Bors, 2020).

It is well-known that the ovaries of heifers are characterised by essential polymorphism, which is associated with a significant physiological conditions (pregnancy, childbirth and the postpartum period). According to O.O. Borshch *et al.* (2020), A. Channo *et al.* (2022), the size of the ovaries in the follicular stage of the sexual cycle ranges from 2.0×1.5×1.5 cm to 4.0×3.0×3.0 cm. Furthermore, researchers point to high variability in heifer ovarian weight, which depends on the presence of the corpus luteum and the stage of its development or regression. Thus, according to L.H. Yevtukh *et al.* (2022), in the presence of the corpus luteum the ovary weight averaged 4.95-7.25 g, and in the absence of the corpus luteum – 4.78-4.98 g. According to transrectal palpation, these organs are oval in shape, with a firm elastic

consistency (Szelényi *et al.*, 2019). The findings obtained by R. Alvarez *et al.* (2023) suggest that the physiological and clinical state of the gonads during the phases of the physiological sexual cycle or gonadopathy of various etiology is understudied. The author states that this distorts the picture of the morphometry and linear dimensions of the cow's ovaries. The data varies considerably and depends on both the research methods (*in vivo* or *postmortem*) and the development of follicles and corpora lutea.

In cases of gonadopathy, F.X. Donadeu *et al.* (2020) found considerable deviations from normal morphology due to pathological formations, including follicular and luteal cysts, polycystic disease, etc. According to O.A. Baban *et al.* (2021), a decrease in the size of the ovaries of cows may be associated with hypotrophy, i.e., a decrease in the size of the cells of the tissues of the cerebral and cortical layers or hypoplasia. B.M. Jaśkowski (2019) explains this phenomenon by a decrease in the number of cells due to functional formations of different phases of the cycle, namely follicular or luteal. According to A.M. Getahun *et al.* (2021), there are no criteria for the differential diagnosis of these pathological phenomena in gonadal morphology at the macro level, which is determined by significant differences in the diagnosis of hypotrophic changes in the ovaries of cows, in particular, heifers of breeding age.

S.Y. Peng *et al.* (2020) provide signs of ovarian hypofunction that are used as diagnostic criteria for this pathology. This makes contradictory recommendations for the prognosis of pathological conditions of the gonads and methods of prevention and therapy of gynecological diseases. According to V. Rottgen *et al.* (2020), ovarian hypofunction is common among 10-68% of infertile cows and is characterised by anaphrodisia, normal ovarian size, reduce in vesicular follicles or their absence.

The polymorphism of gonads of heifers of mating age has been understudied, despite the functional influence of these genital organs on the productive characteristics of dairy cows during the reproductive use of females. That is why the purpose of this study was to determine the prevalence of hypotrophic ovarian changes on the 7-9th day of the sexual cycle of heifers of mating age during the year in different seasons.

MATERIALS AND METHODS

The study was conducted in 2021-2022 years. Animals were kept in loose housing conditions in an industrial-type dairy complex, which was part of agricultural holdings in the south of Ukraine (Odesa region), on a stock of Ukrainian red dairy heifers ($n=175$), which were examined before being put into groups for artificial insemination when the achievement of zootechnical parameters of breed standards

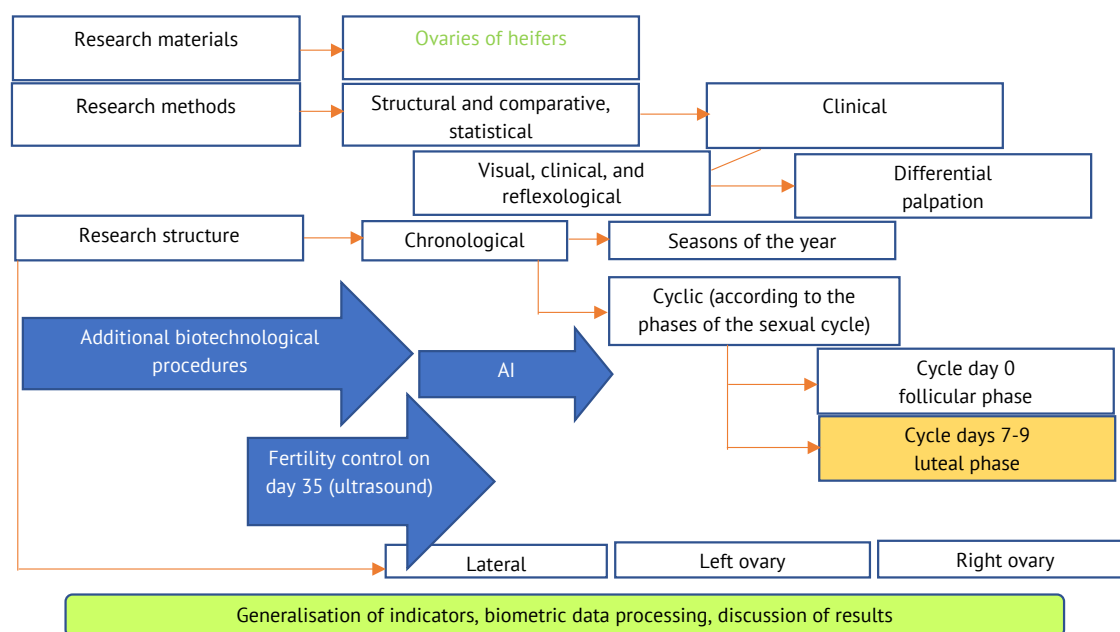


Figure 1. General methodological scheme of examination of the *in vivo* ovaries of heifers of mating age

Notes: fixed day of the study of the *in vivo* ovarian morphology for all examined heifers; AI is artificial insemination

Source: developed by the author

The conditions of maintenance of heifers met the requirements of current standards and were fed a mixed complete diet twice a day per the zootechnical standards for the content of the main ingredients. The animals were kept in zero grazing conditions. The animals were kept without pasture. A special fixing machine was used during the biotechnological procedures and transrectal examination, and no pain or damage was caused to any animal, following the applicable requirements. The different number of animals in the research and production experiment is explained by the regrouping of animals, their transfer to other divisions of the enterprise, and breeding sales to the public during the business year.

Only clinically healthy mature heifers with a live weight of 355-370 kg took part in the study. All cattle on the farm were covered by veterinary support and an immunisation programme against infectious diseases, which was pursuant to current veterinary guidelines. After transrectal examination, heifers without clinical contraindications and symptoms of gynecological diseases were transferred to the reproduction group, where on Day 0 of the sexual cycle artificial insemination was

performed according to the recto-cervical method with deconserved semen of foreign-bred red and white Holstein bulls in accordance with the breeding plan of the farm. 35 days after AI all heifers that did not show signs of sexual arousal during this time pregnancy was diagnosed by ultrasound scanning (using the KXV1 Kaxin device, made in China). The inseminated animals were accounted using the farm's computer database (Dairy Plan).

The innovation of the study consisted of several stages: 1 – using a fixed term for palpation of the ovaries of heifers of mating age in the luteal phase of the sexual cycle, when morphologically there are of a functionally active corpus luteum; 2 – the use of visualisation of palpatory data with a differential modified method of transrectal diagnosis of the ovaries of heifers (Sidashova *et al.*, 2020). Furthermore, the scientific basis for using the modified palpation technique was the anatomical features of heifer gonad development, namely, their essential polymorphism (Sidashova, 2019). Therefore, as a marker indicator of the severity of hypotrophic changes in ovarian tissues, the *in vivo* study considered not only linear sizes, but also the dynamics of corpus luteum formation, i.e., the

assessment of ovarian luteogenesis function at a fixed time of the luteal stage of the sexual cycle. During the study, the following indicators of palpation diagnostics were used (the abbreviations used hereinafter in the text, for all tables and diagrams):

- absence of morphological and functional formations on the surface of both ovaries, hypogonadism, severe hypotrophic changes in ovarian tissues (absence of CL – corpus luteum);
- presence of a functional corpus luteum on the surface of one of the ovaries with linear dimensions close to the species norms (CL);

- presence of a functional corpus luteum on the surface of one of the ovaries with linear sizes approaching the highest limit of the species norm and characterising high physiological quality of luteogenesis (CL+);

- presence of a non-functional corpus luteum on one of the ovaries, which has characteristic tactile signs of atresia and luteal cell disintegration, i.e., premature lysis of corpus luteum (CL lysis).

An example of the use of the method of visualisation of palpation data for assessing the physiological and morphological state of the ovaries of heifers is presented in Figures 2-4.

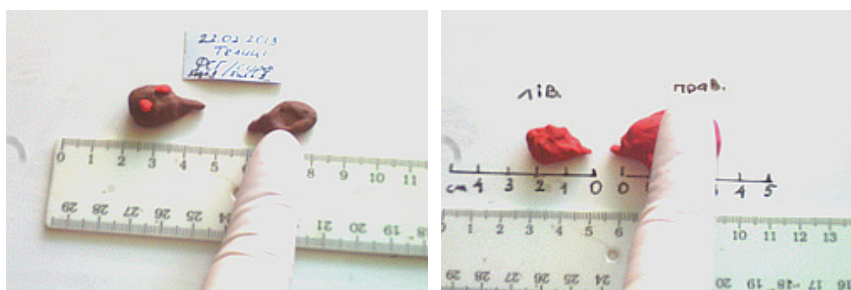


Figure 2. Application of palpation data visualisation technique to assess the morphological and functional state of heifer ovaries

Notes: real-time ovarian models demonstrate the method of measuring linear gonadal size with comparison of data by the size of the researcher's finger *in vivo*, with an assessment of the depth of tissue hypotrophy symptoms

Source: developed by the author



Figure 3. Ovarian morphometry of heifers

Notes: demonstration of palpatory assessment of ovarian morphometry in heifers using the method of visualisation of *in vivo* rectal diagnostic data

Source: developed by the author of this study



Figure 4. A model of heifer ovaries with morphometry close to the species norm

Notes: the ovaries are in transition to the active follicular phase (palpable lysed corpus luteum of the previous cycle and several antral immature follicles, less than 1 cm in diameter)

Source: developed by the author of this study

Apart from the chronological structuring by seasons (winter, summer, and transitional) of keeping heifers of mating age on the farm, the lateral structuring of gonads as paired organs was also applied. The left- and right-sided location of morphological and functional ovarian formations was considered, which significantly increased the accuracy of comparison of palpation parameters of each cows.

All experiments were conducted following the modern methodological approaches, requirements and standards that follow DSTU ISO/IEC 17025:2005 (2006). 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), and of the European Convention for the protection of vertebrates used for experimental and other scientific purposes (1986).

RESULTS AND DISCUSSION

The use of sequential procedures for differential palpation for each cows, considering the specific features of tactile perception of data, with the additional introduction of a technique for visualising rectal examination indicators using ovarian models in real time, helped to obtain a chronological seasonal profile of the morphology of the existing gonadal pathology of the livestock examined *in vivo*. The results of differential palpation diagnostics on Days 7-9 of the

ovarian sexual cycle of 175 heifers of mating age during different seasons of the 2021-2022 business year

showed the following morphological and pathological profile (Fig. 5).

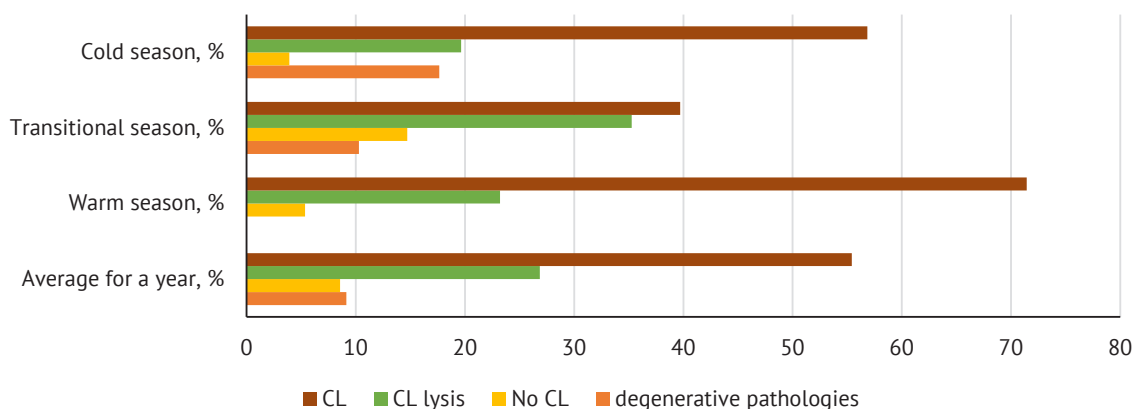


Figure 5. Morphofunctional/morphopathological profile of data on diagnostics of the ovaries of heifers on Days 7-9 of the sexual cycle in different seasons of 2021-2022, n=175

Source: developed by the author

The *in vivo* study of heifer ovaries showed that only 55.42% of females on Days 7-9 after ovulation of the dominant follicle had a functional luteal formation (corpus luteum), the morphometry of which corresponded to the species norm (Fig. 6). 44.58% of heifers' sexual cycles in 7-9 days ended in gonadopathies with various symptoms: cystic degeneration of follicles – 9.14%; lysed corpus luteum with typical signs of atresia – 26.96%; absence of functional formations on the ovarian surface with signs of severe organ hypotrophy – 8.57% (Fig. 7).

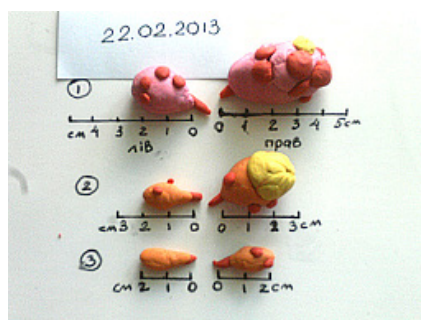


Figure 6. Profile of morphometric and morphofunctional parameters of the palpable diagnostics of heifers' ovaries at a fixed time of the luteal phase of the sexual cycle (Days 7-9)

Notes: 1) absence of CL, several antral follicles, normal ovarian size; 2) CL of the right ovary of satisfactory quality, hypogonadism of the left ovary; 3) severe hypogonadism of both ovaries, absence of morphological and functional formations



Figure 7. Ovaries of a 15-month-old heifer with signs of hypotrophic changes in the cortical and follicular layers

Source: developed by the author

Specifically, a substantial seasonal dynamic in the development of various functional or pathological conditions of heifer ovaries was established. Hypotrophic symptoms were observed most often in the transitional seasons of the year (spring-cold autumn), namely, in 66.67% (absence of CL) and 51.08% (premature CL lysis) of cases. A significant number of cases of hypoluteogenesis of ovarian tissues are noteworthy, in which, due to hypotrophic changes, premature atresia of the corpus luteum occurs by Days 7-9 of the cycle, which was already functioning in the first days of formation. The data of gonadopathies with degenerative tissue damage (single cysts or polycystic disease) were excluded from further analysis, and only the species norm and hypotrophic ovarian changes were considered, as presented in Table 1 (n=159).

Table 1. Profile of the morphological state of heifer ovarian luteogenesis on Days 7-9 of the sexual cycle according to differential palpation diagnostics, n=159

Indicators	Seasons, surveys						Total/average	
	Winter		Transitional		Warm		Heads	% (M±m)
	Heads	%	Heads	%	heads	%		
Number of animals	42	26.42	61	38.36	56	35.22	159	100.00
CL	30	30.63	27	27.84	40	41.24	97	33.34±4.05

Table 1. Continued

Indicators	Seasons, surveys						Total/average	
	Winter		Transitional		Warm		Heads	% (M±m)
	Heads	%	Heads	%	heads	%		
CL lysis	10	21.28	24	51.06	13	27.66	47	33.33±9.05
Absence of CL	2	3.33	10	66.67	3	20.00	15	30.00±18.95

Notes: * and ** – explanations in the text (hereinafter – for all tables and diagrams)

Source: developed by the author

Observations during the year showed that in August the clinical condition of the ovaries of heifers were divided approximately equally, namely functional corpus luteum of the ovary on Days 7-9 day of the sexual cycle (33.34%) and those showing hypotrophic changes in ovarian tissues at the same time, specifically with pronounced signs of hypotrophy and lack of corpus luteum formation (30.00%) and with symptoms of premature lysis of the corpus luteum due to changes in the blood supply of the ovaries (33.33%) ($P>0.05$). The absence of essential correlations between the data indicates a considerable number of paratypical factors that affect

the process of ovarian luteogenesis of heifers of mating age in industrial production.

Specifically, significant differences in the number of heifers with severe ovarian hypotrophy in summer and transitional seasons indicate a substantial impact of climatic conditions, namely the temperature factor, on the sexual function of heifers (the number of cases of absence of the corpus luteum in winter is 20 times less than in spring and autumn months). Graphically, the dynamics of ovarian hypotrophic changes in different seasons of the year is presented in the diagram (Fig. 8).

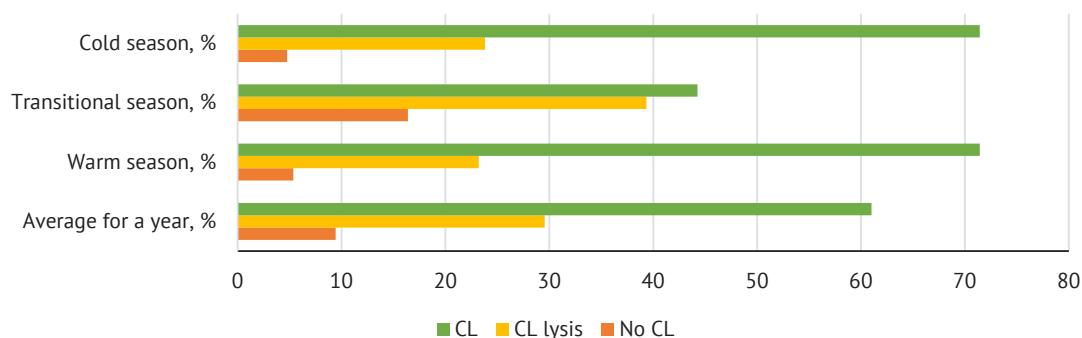


Figure 8. Dynamics of hypotrophic changes in luteal formations of heifers' ovaries on Days 7-9 of the sexual cycle (2021-2022), n=159

Source: developed by the author

The substantial impact of seasonal factors on the ovarian luteogenesis of heifers of mating age prompted a more detailed consideration of the morphology of corpus luteum, which had clear functional characteristics.

The dynamics of the physiological process of luteogenesis in heifers of mating age in different seasons of the year, characterised by high-quality corpus luteum, is presented in Table 2.

Table 2. Results of differential palpation assessment of the quality of functional ovarian corpus luteum on Days 7-9 of the reproductive cycle in heifers of mating age, n=74

Indicators	Presence of CL				All functional CLs, % (M±m)	
	incl. CL		incl. CL+		heads	% (M±m)
	heads	%	heads	%		
Number of animals	49	100.00	25	100.00	74	100.00
Periods: Winter	12	24.49	13	4.00	13	17.57
Transitional	16	21.21	21	20.00	21	28.38
Summer	21	42.86	40	76.00	40	54.05

Source: developed by the author

According to the study, the highest number of high-quality corpora lutea, i.e., the least signs of hypoluteogenesis due to hypotrophic changes in the

follicular and cortical tissues of the ovaries, was observed in the summer (76.00% of all detected functional active corpora lutea). The winter period showed

the worst results in terms of the quality of corpora lutea on Days 7-9 of development in heifers of mating age: only 4.00% of quality quality yellow bodies were found, which indicated a violation of haemodynamics in the gonads, a decreased vitality of cells and hormo-

nal activity of ovaries. Figure 9 presents the ovary of a clinically healthy 15-month-old heifer with a well-developed corpus luteum on Day 9 after ovulation. In palpation diagnostics, these corpora lutea have characteristic morphometric and tactile features.



Figure 9. Heifer ovary with a well-developed corpus luteum of the sexual cycle

Notes: a small physiological cavity is visible inside the temporary gland in the dissection

Source: developed by the author of this study

During the study, the ovaries of heifers were observed as a paired organ, and the hypotrophic phenomena in

the gonads was determined according to lateral localisation. The data obtained are presented in Figure 10.

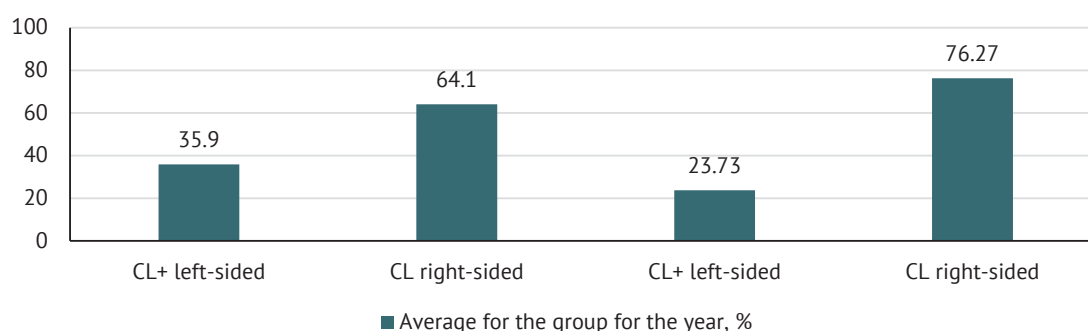


Figure 10. Dynamics of lateral localisation of functional corpora lutea on Days 7-9 of development in heifers in different seasons of 2021-2022, n=98

Source: developed by the author

The analysis of the fertility rate of heifers of mating age in the farm, presented in Table 3, suggests that on

average for the year the total fertility of heifers was 40.02% of all inseminations ($P>0.05$).

Table 3. Effectiveness of artificial insemination of heifers of mating age during the business year, n=558

Season	AI* of heifers performed, total	Became pregnant (M±m)		incl. of the 1 st AI, %	incl. from the 2 nd and more AIs, %
	heads	heads	%		
Winter	60	20	33.33	39.00	31.11
Transitional	111	51	45.55	41.14	48.03
Warm	387	151	31.02	39.91	29.98
Total for the year	558	222	36.63±4.51*	40.02±0.62**	36.37±5.84**

Note: * – ** – $\hat{\sigma}=1.074$; $CV=2.684$; $td=0.743$; $P>0.05$

Source: developed by the author

The absence of essential connections between the indicators suggests a considerable impact of economic factors on the effectiveness of reproduction, while there is a tendency to increase the fertility of heifers of mating age in seasons with more comfortable temperatures for cattle, namely in spring and

autumn, when the overall level of pregnancy increased to 45.55%, and by repeated inseminations – to 48.03%. The applied methodological approach to the differential palpation of the morphological and functional *in vivo* structures of the ovaries of heifers of mating age increases the predictability of biotechnological

procedures for the reproduction of dairy cattle and the selection of adequate schemes for the prevention and treatment of hypotrophy of the gonads of cows.

The observed phenomenon of heterogeneity of the morphological structure of the corpora lutea of the heifer's ovaries by differential palpation *in vivo*, has become an opportunity to explain the pathogenesis of hypotrophic changes in tissues due to sclerotisation of the walls of blood vessels, both arterial and venous, which was histologically confirmed by O.A. Baban *et al.* (2021). Microanatomical examination of the ovaries of heifers with symptoms of anaphrodisia revealed a characteristic decrease in the density of follicular cells in the primary follicles around the oocytes and the formation of gaps between them. The appearance of such formations was explained by a decrease in the secretory activity of follicular cells. According to L.H. Yevtukh *et al.* (2022), the phenomena of follicular cell detachment and destruction with their collagenisation are associated with the processes of follicular atresia against the background of venous hyperaemia and fatty infiltration. S. Dhara and M. Sharma (2020) observed comparable changes in 30-66% of follicles with signs of atresia.

H.P. Hryshchuk *et al.* (2023) noted that the pathogenesis of the formation of basophilic inclusions in the form of droplets near the layer of vesicular follicles is still unclear. E. Dirandeh and J. Ghaffari (2018) found that the cow ovarian medulla in hypofunction was represented by connective tissue elements and permeated with a great number of blood vessels of different diameters. Collagen fibre formation was observed in these vessels, which could lead to a decrease and loss of their elasticity, and therefore the function of normal haemodynamics and nutrition of ovarian tissues. J. Ma *et al.* (2022) conducted pathomorphological studies of the ovaries of cows with hypofunction, with a history of infertility and anaphrodisia. The authors found a range of deviations from the normal morphology of the luteal phase of the cycle, namely: no functionally active corpus luteum, atretic corpus luteum with follicular cell remnants, a decrease in the number of follicular cells and their layers in vesicular follicles, and the formation of collagen fibres in blood vessels.

The present study experimentally proved the expansion of the scope of palpation diagnostics of the morphological and functional state of the ovaries by applying the transrectal data visualisation technique and using a fixed day of the sexual cycle as the palpation period. This approach made it possible to anatomically substantiate palpable indicators and their connection with the specific features of gonadal morphometry *in vivo*. In F.S. Lima *et al.* (2019), the shape and consistency of the gonads were normal in heifers with ovarian hypofunction. Real-time research contradicts these findings. It is probable that the difference in the appearance and structure of organs *in vivo* compared to pathological specimens should be taken considered.

A range of microanatomical studies have reported observations of premature or delayed atresia of vesicular follicles on Days 12 and 15 of the estimated sexual cycle *in vivo*. The experiments performed on a fixed day of the spontaneous sexual cycle interpreted these findings, due to numerous cases of premature corpus luteum atresia by Days 7-9, which can be explained by the development of hypotrophic changes in ovarian tissues. V. Rottgen *et al.* (2020) suggest that there are several mechanisms for the development of pathological phenomena in the ovaries of heifers in the early stages of development of preimplantation zygotes, namely: neurohumoral disorders, especially the synthesis of progesterone and luteotropic hormone; latent endometrial inflammatory processes and possible, yet accounted reasons.

The observed folding of the follicular layer, detected on histological preparations of ovarian tissues from heifers with gonadal hypofunction, is explained by the fact that the inner follicular cells lose their ability to secrete, while the basal cells secrete in the intervals between them. In such follicles, the theca layer is thinned due to a decrease in the density of theca cells. F.S. Lima *et al.* (2019) also observed detachment of follicular cells and their free location in the follicular fluid, destruction of oocytes and follicular cells with the formation of pyknotic nuclei. Such processes indicate a loss of functional capacity of the organ. Using a modified method of palpation diagnostics, the studies revealed clinical conditions of significant gonadal tissue hypotrophy in heifers *in vivo*, which provides a higher prognostic result in production conditions.

N.P. Mazur *et al.* (2020) believe that the differential diagnosis of ovarian hypotrophy and hypoplasia is possible only by investigating pathological changes. According to their histological data the blood vessels were localised mainly in the area of the ovarian gate and around the vesicular follicles. Groups of blood vessels in ovarian hypoplasia, compared to hypofunction, were very weakly expressed, and collagen fibres were found in the vessel walls. The formation of collagen fibres in blood vessels can lead to a decrease in their elasticity, function, and nutrition of ovarian tissue. Such claims require additional research with comparisons of data using different methods.

In a range of studies, R. Alvarez *et al.* (2023) revealed patterns of functional asymmetry in the ovaries of heifers and cows, which was confirmed by the analysis of data using lateral fixation of palpable indicators. According to the researches of N. Mimoun *et al.* (2021) based on histological studies of the ovaries of cows with a permanent infertility due to anaphrodisia, it was found that 2-60% of lactating cows had a history of disorders in maintenance, keeping and feeding of heifers. Thus, by expanding the diagnostic capabilities of palpation examination of the ovaries, the possibility of the use of prophylactic and therapeutic schemes for stimulation and synchronisation of the sexual function of

heifers under commercial heifer rearing was increased. A. Channo *et al.* (2022) confirm the data on the increase in gynecological diseases, specifically, gonadopathies in both lactating cows and heifers during the cold transitional seasons of the year.

The researches show the way to optimise the conditions of keeping heifers of mating age, under which the most desirable morphometric parameters of the gonads during luteogenesis are observed, which determine the hormonal regulation of the formation of the muscular and mucous tissues of the uterus, resulting in increased survival of early embryos and pregnancy rate.

CONCLUSIONS

Approximation of technological conditions for keeping of heifers of mating age to the naturally occurring biological requirements for the formation of animals in the environment will contribute to the development of high-quality gonads in terms of morphometric parameters, which will function according to the stages of the sexual cycle. This opens an additional biological resource for increasing the fertility rate of mating heifers, calf yields, and the planned rotation of primiparous cows into the dairy herd. These issues of industrial milk production are still highly relevant and need to be the right approach.

Studies using the modified palpation technique provided an additional resource for explication the morphometric and functional parameters of the gonads according to the lateral localisation of the luteal stage of the sexual cycle. The study confirmed the patterns of functional asymmetry of the ovaries of mating heifers as paired organs. The prevalence of hypotrophic phenomena in heifers of mating age is much more pronounced to the left, namely: 64.0% in the transitional seasons, 76.27% in the warm season.

Based on a year-long palpation examination of heifers of mating age ($n=175$), it was found that on average 30.00% of ovaries had severe symptoms of hypotrophic changes in the absence of any morphological and functional structures on Days 7-9 of the sexual cycle; 33.33% – the presence of corpus luteum with signs of premature lysis due to hypotrophy; 33.34% –

ovaries had a functionally active corpus luteum with linear sizes within the species norms ($P>0.05$). Significant seasonal dynamics of ovarian hypotrophy in heifers of mating age on Days 7-9 of the reproductive cycle was revealed: the highest rate of hypogonadism with the absence of morphofunctional corpus luteum was observed in transitional (spring, autumn) seasons – 66.67%, the lowest – in winter – 3.33%. It was experimentally established that signs of hypogonadism with symptoms of hypoluteogenesis and premature corpus lysis were found in transitional seasons (51.06), and the highest level of functional corpus luteum was observed in the summer season (41.24%), and specifically, the highest number of morphologically high-quality corpus luteum – 76.00%.

The effectiveness of artificial insemination of heifers of mating age of the experimental herd averaged 40.02% for the business year with minor seasonal fluctuations, which indicated a substantial effect of economic paratypical factors on the fertility of the heifers of mating age ($P>0.05$). The use of a modified technique of differential palpation with a fixed examination period increases the predictability of diagnosis and selection of effective schemes for the prevention and treatment of hypotrophic ovarian pathologies and requires further research. The results confirm to the presence of a biological resource of generative and hormonal function in heifers of mating age of dairy breeds, which require detailed investigation to use the results to optimise hormonal and medicinal schemes for stimulation and synchronisation of the sexual cycle of the heifers of mating age under industrial housing technologies.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Динаміка гіпотрофічних змін морфологічних утворень яєчників телиць парувального віку на 7-9 день лютеогенезу

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Анотація. Дослідження фізіологічного або патологічного стану яєчників корів та телиць в режимі реального часу *in vivo* є на сьогодні актуальними з причини того, що саме морфофункціональний стан гонад самиць великої рогатої худоби безпосередньо впливає на ефективність застосованих на практиці біотехнічних процедур: запліднення, стимуляцію та синхронізацію статевих функцій. Метою роботи було визначення кількісно-якісних показників морфофункціонального стану яєчників телиць української червоної молочної породи за перебігу господарського року за безприв'язного утримання в умовах промислового молочного комплексу. Впродовж дослідження було використано модифіковану диференційну діагностику морфометричного і функціонального стану яєчників у фіксований термін статевих циклу – з 7-го по 9-й день після овуляції домінуючого фолікулу (0-й день циклу). Для порівняння результатів трансректального обстеження телиць у різні сезони року було використано методику візуалізації пальпаторних даних *in vivo*. Було обстежено 175 голів телиць парувального віку, серед яких на 7-9-й день статевих циклу у 55,42 % встановлено пальпаторно жовте тіло яєчника відповідно стадії розвитку, у 26,96 % – передчасний лізис жовтого тіла, у 8,57 % – відсутність морфофункціональних утворень на поверхні яєчника, у 9,14 % – симптоми дегенеративних патологій гонад. В подальшому тварини з дегенеративними змінами яєчників (фолікулярна кістозність) не брали участь у дослідженні. Інші телиці мали яєчники з симптомами глибоких гіпотрофічних змін в тканинах (30,00 %) або ознаки передчасного лізису жовтого тіла (33,33 %). Впродовж господарського року відмічена виражена динаміка у наростанні або зниженні гіпотрофічних явищ в тканинах яєчників; найкращі показники морфометрії і функціональної активності телиць мали у теплий сезон, що свідчило за значний вплив на статеву функцію телиць парувального віку свіжого повітря і сонячної інсоляції. Було встановлено, що рівень заплідненості телиць впродовж року (в середньому 40,02 %) свідчив за наявність похибок у технології вирощування ремонтного поголів'я в умовах промислового підприємства. Отримані результати можуть бути корисні для оптимізації схем гормональної синхронізації статевих циклів ремонтних телиць та коректного добору гормональної терапії за симптомів зниження статевої активності.

Ключові слова: ремонтні телиці; статевий цикл; жовте тіло; морфофункціональний стан; поліморфізм; гіпогонадізм



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Histomorphological changes in atheroma of the dog (morphological study on the example of 12 clinical cases)

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Abstract. Pathological processes in the skin of domestic animals are one of the most common diseases of domestic animals both in the Kyrgyz Republic and throughout the world. In connection with the complex differential diagnosis of oncological and non-neoplastic diseases in animals based only on clinical signs, the purpose of this work was to conduct a statistical and histomorphological analysis of cases of atheroma development in dogs. Clinical, histological and statistical methods were used during the research. The research was conducted on a controlled group of domestic dogs in

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the amount of 12 animals, which were followed for several years. When collecting the anamnesis, in addition to clinical signs, the breed, age and concomitant diseases of the dogs were recorded. During surgical removal of atheroma, histological preparations were prepared to clarify the diagnosis and study histomorphological changes in the tissues. Atheroma of the skin in dogs was most often registered in old animals, which were more than 9 years old at the time of diagnosis. At the same time, such pathology was most often localized in dogs in the area of the head and neck. Among the breeds, the Boxer turned out to be the most susceptible to the formation of sebaceous gland cysts. Also, the incidence among females was 75% of all cases of registered diseases. Histological studies revealed the prevalence of cases with the formation of multiple cysts – 67%, when a number of smaller formations are found near one huge atheroma, while cases of purulent inflammation were only 16%. Surgical removal of the atheroma was an effective method of treatment, while no cases of re-development of cysts were observed during the long postoperative follow-up. The obtained results make it possible to expand knowledge on the clinical manifestation, diagnosis and treatment of non-neoplastic skin diseases in domestic animals

Keywords: surgical and biopsy material; sebaceous gland cyst; morphological diagnosis; non-neoplastic skin pathology; operative treatment

INTRODUCTION

Skin diseases in domestic animals are quite common in world practice and the Kyrgyz Republic and arise due to numerous aetiological factors. Therewith, one of the main pathological skin problems in dogs is various oncological and tumor-like diseases that develop in all layers of the skin. In Kyrgyzstan, according to S. Ishenbaeva *et al.* (2023), pathological changes in the skin of epithelial and mesenchymal nature are most often established in domestic animals, lymphoreticular formations were less common, and melanomas were in last place in terms of prevalence. The same authors note that in the morphological diagnosis of skin diseases, benign neoplasms prevailed – 52%, malignant tumors were detected in 30% of cases, and atheromas and other tumor-like formations – 18%. In other domestic animals, and cats, in particular, in studies conducted by E. Wouters *et al.* (2022), the number of skin diseases of non-tumor aetiology was observed slightly less frequently than in dogs.

Among the skin formations, atheroma is also widespread in dogs. Most researchers indicate that atheroma is a neoplasm of a cystic nature, which is formed as a result of blockage of the sebaceous gland duct. Pathological changes of oncological and non-tumor nature in the skin can develop regardless of the area of the body and, according to J. Munday *et al.* (2022), externally appear the same, in the form of rounded elevations above the skin. Therefore, for the differential diagnosis of such formations of a non-tumor nature, it is necessary to conduct histomorphological differentiation. According to clinical signs, cysts have slow growth and contain fluid inside that has accumulated as a result of blockage of the duct. B. Hassan *et al.* (2022) indicate that sebaceous gland cysts are quite common in dogs and do not cause much discomfort to animals. Initially, according to M. Sbaraglia *et al.* (2021), cystic formations are not an oncological pathology, but unlike the opinion of the previous author, they are extremely rare in the body of animals.

In appearance, this type of pathology is difficult to distinguish from other benign skin tumors in dogs,

which, according to J. McKenzie *et al.* (2023), belong to basal cell formations and is also quite often diagnosed in cats and dogs. One of these pathologies may be an adenoma of the sebaceous gland – a benign tumor, which, as a result of not timely treatment, may carry certain risks to animal health due to the possibility of further transformation into a malignant neoplasm. Since, externally, at the initial stage, the cyst and adenoma are practically indistinguishable, it is difficult to determine the process of developing in the body and timely provide the necessary assistance to the animal, and only the histological method allows determining with high information the nature of the course of the atypical process in the epithelial cells of the skin. In addition, the contradictory results of studies on the prevalence of cystic skin pathology among dogs, given in the analysed literature, are insufficient criteria for forecasting. Therefore, the analysis of a number of cases of pathological skin morbidity in dogs in Kyrgyzstan is an urgent study to accumulate information and expand the knowledge base on the prevalence of cystic pathology in dogs of different breeds.

The purpose of this study was to conduct statistical and morphological examinations of the development and course of the pathological process in the skin of dogs in histologically confirmed cases of atheroma in animals.

LITERATURE REVIEW

Skin diseases in pets are common diseases in veterinary practice. One of them is an atheroma or cyst of the sebaceous gland, which develops due to mechanical blockage of the excretory duct. According to S. Paterson (2009), there are atheromas ranging in size from a pea to a chicken egg. These formations are usually painless, have a rounded shape and elastic consistency. Most often, atheroma is preceded by traumatic skin damage. Also one of the reasons for the formation of atheromas, according to J. Swanson *et al.* (2019), is a decrease in the expression of Keratin 79 and Gata6 factors that

regulate the expansion of the follicular duct. In most cases, according to D. Wiener (2021), cysts in dogs affect several layers of skin at once, including sebaceous glands. In this regard, there are single and multiple skin cysts. In most cases, according to A. Neff and H. Horch (2023), these are benign, expansively growing structures that are relatively common in the jaw region, less often, they are localised in the soft tissues of the face and neck. Similar areas of sebaceous gland lesions on the scalp are described by S.N. Ishenbaeva *et al.* (2012) and J. Jyothi *et al.* (2023), and also in the abdomen and chest. A frequent localisation of sebaceous gland atheroma was the soft tissues of the face, and in studies by A. Shakirova *et al.* (2020). Cysts lined with epithelium are called true cysts, and those that do not contain epithelium – pseudocysts. The growth of atheromas is relatively slow, inflammation occurs during infection, and suppuration is often observed (Chele & Mădălina, 2023).

The study by M. Patel *et al.* (2019) indicates that the pathology of the sebaceous glands accounted for 4.04% of cases of all skin diseases in dogs, while out of 23 cases, three adenocarcinomas and one hyperplasia (atheroma) were diagnosed in 19 cases. In other studies, V. Kukulj *et al.* (2021) indicate that pathological changes in the sebaceous glands had a slightly different proportion. Thus, out of 2432 skin biopsies in dogs, a cyst was detected in 35% of cases, and an adenoma – only in 4.4%. Similar results are reported by S. Ishenbaeva *et al.* (2023) in studies in which cat atheroma was diagnosed in 30% of cases of all skin diseases.

The breed dependence of cystic skin diseases in dogs is indicated by S. Hobi *et al.* (2023), B. Lancellotti *et al.* (2020). Most studies indicate that atheroma and other skin neoplasms are observed in dogs of all breeds, but the Labrador retriever was identified to be the most susceptible breed, followed by the Pomeranian and mongrel dogs. In addition, a high incidence of skin diseases of the head was observed in pugs and bulldogs. No less than the breed predisposition, the age of animals is also a factor in the morbidity of dogs. In the studies by H. Aupperle-Lellbach *et al.* (2022), the highest incidence was recorded at the age of 9 to 12 years, and it was 39.1%. D. Devathasan *et al.* (2022) and Yu. Dubinchuk (2022) also indicate an increase in the incidence of animals with ageing.

There are also no clear recommendations for the treatment of atheroma. Thus, in the study of J. Chitty (2022), it is indicated that the cyst of the sebaceous gland does not cause discomfort in animals and they need to be treated only in case of suppuration as a result of their infection.

MATERIALS AND METHODS

The studies were conducted on pathological material obtained from domestic dogs with signs of formations in the skin, whose owners turned to the veterinary clinics “Samson” and “Doctor Zoo” in Bishkek. For this

purpose, dogs were selected, regardless of breed and age, with clinical manifestations in the form of tumors or tumor-like changes in the skin. For such animals, a complete anamnesis was collected, if possible, with a detailed recording of the course of the clinical process, morphological and histological examinations, and the results of medical procedures. In total, for the period from 2020 to 2022, 12 dogs took part in monitoring studies, which at one time were given the primary diagnosis of skin atheroma, and subsequently, it was confirmed by histological results.

The procedure for conducting research and collecting information was as follows: initially, an examination of the pathological formation was conducted with detailed consideration of such parameters as shape, size, colour, the presence of a coat on the surface of the neoplasm, and the place of its localisation. Through palpation, the consistency, mobility, and soreness of the formation were determined. In the future, detailed information about the age, breed, living conditions, and other factors was collected about the animals that fell into the target group. During the observation of the development of the pathological process, changes were recorded by photographing them during the entire controlled period. During surgical treatment, histomorphological examination was performed to clarify the final diagnosis and microphotography for documentary confirmation. If the first part of the study on the examination and observation of animals was conducted in the clinic, at the place of the patient's treatment, then the histological preparation was made, and its analysis was performed in the histology laboratory of the Department of Veterinary and Sanitary Examination, Histology and Pathology of Kyrgyz National Agrarian University named after K.I. Skryabin.

In veterinary clinics, during the surgical removal of tumors and tumor-like formations, 11 samples of pathological material were taken from dogs, and one biopsy was performed for the manufacture of histological preparations. Biopsy and surgical material delivered from clinics was fixed in a 10% aqueous solution of neutral formalin. Dehydration of tissue samples with atheromas from dogs was performed at room temperature under normal conditions. After fixing the pathological material to prepare histological sections, it was poured into paraffin according to the generally accepted method (Vareniuk & Dserzhynsky, 2019). For this purpose, a set of histological equipment from the German company Thermo Scientific was used, namely an automatic device for histological tissue processing Microm STP 120 and Microm EC 350 for the preparation of paraffin blocks. Skin sections from the blocks were conducted on a rotary microtome Microm HM 340 E (Thermo Scientific, Germany) with a thickness of 4-6 microns. Washing and staining of the prepared pathological material from paraffin was conducted using an automatic Microm HMS 70 station (Thermo Scientific, Germany). Histological sections were stained

with hematoxylin and eosin. Microscopic studies were conducted using an Olympus BX51 light microscope (Japan). A mandatory method of documenting the results obtained was microphotography using a digital photo-system for processing video images of DSM-510 with pre-installed ScopePhoto computer software that ensures the objectivity of morphological analysis. In addition, records indicating the surgical or biopsy method of taking pathological material, which were also recorded in the laboratory journal, were amenable to mandatory documentation. The results obtained were amenable to analytical and mathematical processing using descriptive statistics methods, while the distribution of animals was conducted depending on their breed, age, and sex.

RESULTS

Pathological problems occurring in the skin of pets are primarily detected by their owners, and therefore, such diseases have the highest frequency of diagnosis in veterinary clinics in comparison with other cases of neoplasms in the body. Surface placement and characteristic clinical signs contribute not only to the early diagnosis of skin problems in animals but also allow for their effective treatment at an early stage, which guarantees a fairly high therapeutic effect. Among the pathology of the skin, there are both oncological problems in the form of benign and malignant formations and diseases of a non-tumor nature – cysts, atheromas, and other inflammatory processes. In most cases, at the initial stage, almost all skin diseases occur with the same symptoms – the formation of a small swelling, mobile, painless, with a low intensity of growth. Such similar symptoms reduce the effectiveness of differential diagnosis of neoplasms to clinical signs. Therefore, in most veterinary clinics, when characteristic symptoms are

detected, surgical removal of such tumors is performed to protect animals from possible further metastasis in the event of the development of a malignant neoplasm. There is also no possibility of differentiation of pathological skin problems depending on the place of formation of swellings. This is due to the fact that the skin in all areas has the same morphological structure, and any of the above pathological processes can develop in it. During the preparation of the study, cases of atheroma development were examined to conduct a comprehensive analysis of the clinical signs of the course of the pathological process in the bodies of dogs, morphological changes in tissues, and the consequences of surgical intervention for therapeutic purposes. Based on such circumstances, all cases of skin atheroma in dogs whose owners applied to veterinary clinics in Bishkek to analyse the possible manifestation of clinical symptoms in such pathology were selected to identify histological changes occurring in the skin. A total of 12 dogs of different breeds and ages with a preliminary diagnosis of atheroma were examined. All cases were documented and are given for the purpose of using the information for subsequent research.

The first case in the study was a mongrel dog named Bublik. It is a male, about two years old, with a black coat colour. At the time of diagnosis, it had a height at the withers of 38 cm and a weight of 12 kg. The pathological process was localised on the left side in the neck area. Therewith, a single swelling of 2-3 cm in size, mobile and painless, was felt. According to the dog's owner, the tumor was discovered about a year before going to the clinic, and the growth is insubstantial, about one centimetre per year. After surgical removal of the formation, histological preparations were prepared, the image of which is shown in Figure 1.

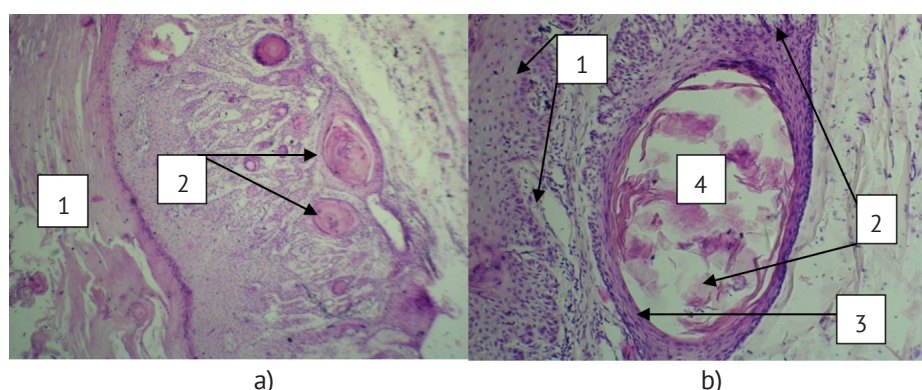


Figure 1. Histological changes in atheroma of the skin in dogs: a) magnification $\times 40$; b) magnification $\times 100$

Note: 1 – skin; 2 – atheroma; 3 – the wall of the atheroma; 4 – the cavity of the atheroma with the contents. Staining with hematoxylin-eosin

When analysing histological preparations, a diagnosis was made – multiple atheroma of the skin. After surgery, the animal's condition is good, no further relapses were observed. The second case, German boxer breed dog, named Rex, male, 8.3 years, brown colour,

weight 26 kg, and height 54 cm. The neoplasm was located on the right front paw between the 1st and 2nd phalanges of the fingers. The cyst is 1.5-2 cm in size, painless. It was discovered a few weeks ago no growth was noticed during this period. The histological picture

during microscopic examination corresponded to an atherosclerotic lesion (Fig. 2). After surgical removal of the atheroma, repeated neoplasms were not observed.

Third case. German boxer breed dog named – Rizza, female, 7.4 years. The tumor was located on the skin of the lumbar region, 1.5-2 cm in size, mobility

is limited, painless (Fig. 2). It was discovered about a month ago, during the observation period, the growth is not substantial, the increase is only a few millimetres. The general condition of the dog is good. The diagnosis after histological examination is atheroma of the skin.

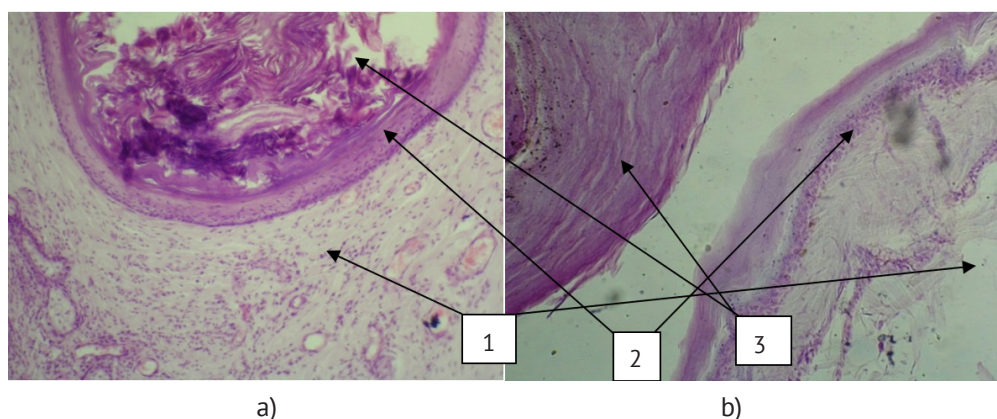


Figure 2. Histological changes in skin atheroma in dogs: a) 2nd case; b) 3rd case

Note: 1 – connective tissue that surrounds the atheroma; 2 – the wall of the atheroma; 3 – the contents of the atheroma. Staining with hematoxylin-eosin, $\times 100$ magnification

Fourth case. Dog named Linda, breed – German Shepherd, female, 8.1 years. Numerous tumors throughout the body of small and medium sizes have been formed over the past three years. The swelling is limited, almost all are mobile and not painful. The primary diagnosis of atheroma of the skin. After suppuration, two atheromas were surgically removed, the first was in the withers and the second – in the sacrum. No new swellings were established at the site of the removed

formations, and no accelerated growth was observed in the remaining atheromas. The dog is active after surgery, no noticeable deviations in behaviour and general condition were established. Histological examination of the pathological material not only confirmed the primary diagnosis but also allowed detecting small atheromas in the skin that were not detected during a clinical examination (Fig. 3). The final diagnosis, based on microscopy, is multiple atheroma of the skin.

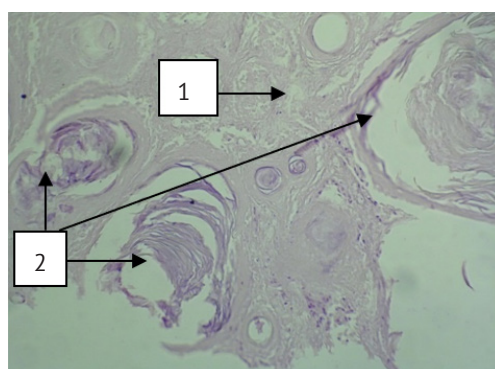


Figure 3. Histological change in atheroma

Note: 1 – connective tissue that surrounds the atheroma; 2 – atheromas. Staining with hematoxylin-eosin, $\times 40$ magnification

Fifth case. Breed – boxer, age – 13 years, colour – fawn, name – Sasha, female. Two tumors were found in the dog. The first one was located in the temple area, dense, rounded shape, measuring 6×4 cm. The tumor is hard and painful on palpation; the local temperature is higher than the surrounding tissues. The growth period is 1.5-2 months. The second neoplasm, about 1 cm in size,

was identified in the mammary gland and was painful on palpation. After removing the tumor in the temple area, a tumor formed on the ear three months later, and in February 2022, the dog died of cancer. Microscopic examination of the pathological material from the removed tumor from the temporal region of the head identified multiple atheromas of the skin with signs of inflammation (Fig. 4).

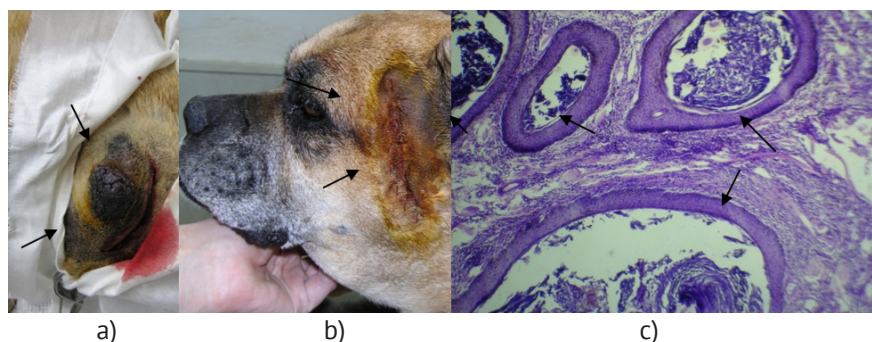


Figure 4. Fifth case: a) clinical manifestation of atheroma before surgery; b) after removal of atheroma; c) histological manifestation of atheroma in pathological material

Note: the arrows indicate the atheroma and the area of the skin after its removal. Staining with hematoxylin-eosin, $\times 40$ magnification

Sixth case. Breed – Pekingese, name – Sherry, age – 2.3 years, colour – red. The dog is not sterilised. A neoplasm of 1-2 cm in size was located on the skin of the forearm. The tumor is mobile, pale pink, painless. The

shape is rounded. The consistency is dense. The growth period is unknown; it was discovered four days before going to the clinic. After removal and histological examination, an atheroma of the skin was diagnosed (Fig. 5).

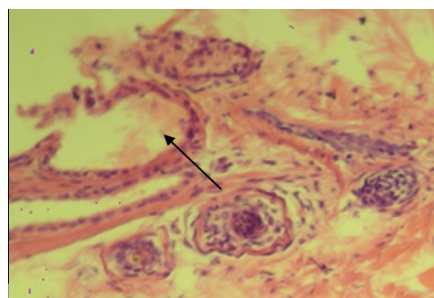


Figure 5. Microscopic changes in atheroma of the skin

Note: the arrow indicates the atheroma cavity, hematoxylin-eosin staining, $\times 100$ magnification

Seventh case. Mongrel dog named Sarah – female, about 9 years. On examination, two tumors were identified on the skin. The first is in the area of the left iliac region of the abdomen, rounded shape, dense consistency, painless, mobile, size 4 $\times$ 2.5cm. The growth was observed

for about a year. The second one is on the left shoulder, with a rounded shape, dense consistency, and 1.5-2 cm in size. Both tumors were removed. During histological examination, the following diagnoses were made – skin atheroma and low-grade adenocarcinoma (Fig. 6).



Figure 6. 7th case: a) clinical changes in atheroma; b) histological change in atheroma

Note: the arrow indicates the atheroma cavity, hematoxylin-eosin staining, $\times 100$ magnification

Eights case. The dog of the breed is a dachshund, named Sarah. Female. Age – 13.2 years. Therewith, three tumors in the mammary gland were established

in the animal during examination at the clinic. Their growth was monitored for three years. Tumors of different sizes, from 2.5 to 6 cm.

They were slightly visibly different. As a result of the accelerated growth of one of the tumors, surgical intervention was performed. The results of the

histological examination were followed by the following diagnoses – atheroma, lipoma and low-grade adenocarcinoma (Fig. 7).



Figure 7. Eighth case: a) macroscopic change in atheroma; b) histological change in atheroma

Note: hematoxylin-eosin staining, $\times 100$ magnification

Ninth case. A German Shepherd dog, named Magla, aged 2 years and 3 months. The coat is black-and-white. A tumor in the neck, 0.5 cm in size. Discovered about a month ago. Neoplasm of oblong shape, grey colour, soft consistency. Due to the small size of the tumor, a

skin biopsy was performed. 2.5 months after the manipulation, the tumor disappeared, no re-growth was detected. The general condition of the animal after the biopsy is excellent. Histological diagnosis – skin atheroma with suppuration (Fig. 8).

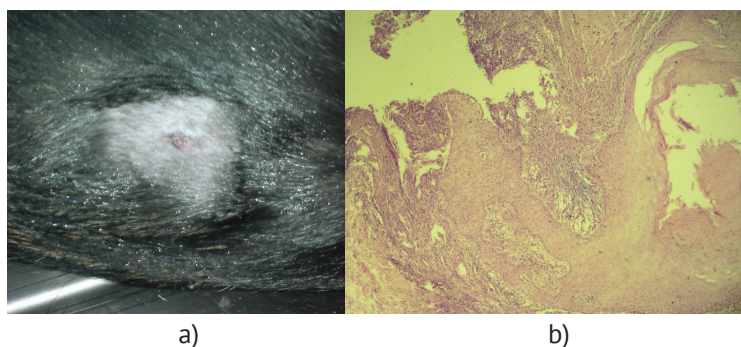


Figure 8. Ninth case: a) clinical manifestation of atheroma; b) histological change in atheroma

Note: hematoxylin-eosin staining, $\times 40$ magnification

Tenth case. An adult dog of the pit bull breed named Missiya, aged 4.5 years. The coat is red with white spots. A tumor on the scalp between the ears. The tumor is dark in colour, bumpy consistency, oval in shape, 3 $\times$ 2 cm in size. The growth of the neoplasm

was observed for four years. After surgical removal and histology of the pathological material, a diagnosis was made – atheroma of the skin (Fig. 9). After surgery, the animal's condition was excellent, and no relapses were observed.

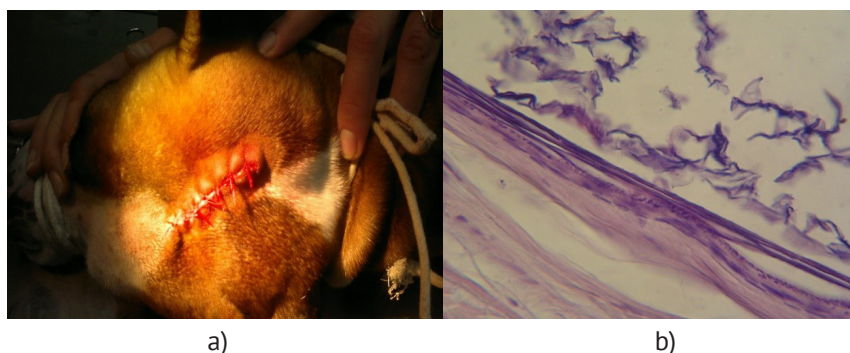


Figure 9. Tenth case: a) post-operative wound in atheroma; b) histological change in atheroma

Note: hematoxylin-eosin staining, $\times 100$ magnification

Eleventh case. A dachshund dog, named Matilda., aged 12 years and 3 months. The coat is red. The examination identified a single neoplasm on the skin of the lateral wall of the abdomen measuring 3×3 cm. The tumor is of a dense consistency, rounded shape, with a slight pain when palpating. During surgical intervention, a cavity filled with pus was found, which corresponded to an infected cyst. Subsequently, the histological diagnosis confirmed the presence of atheroma with suppuration (Fig. 10).

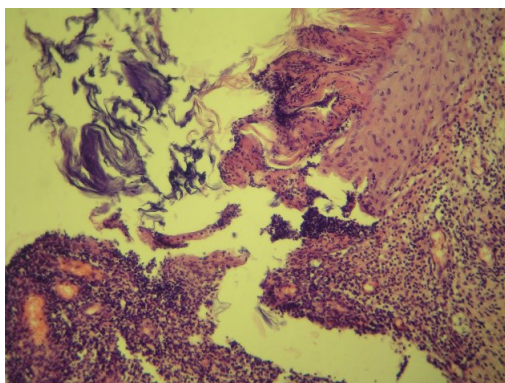


Figure 10. Histological change in purulent atheroma
Note: hematoxylin-eosin staining, ×100 magnification

Twelfth case. A German Shepherd dog named Graf, aged 5 years and 7 months, male. The coat is black-and-white. Examination of the tumor identified the presence of purulent exudate on the surface of the neck skin. The swelling is rounded, 2.5×1.5 cm in size, dense consistency. Before removal, the tumor was observed for about 1.5 months. After the shell rupture and pus discharge, a preliminary diagnosis of a furuncle was made. Histological examination identified multiple atheromas (Fig. 11).

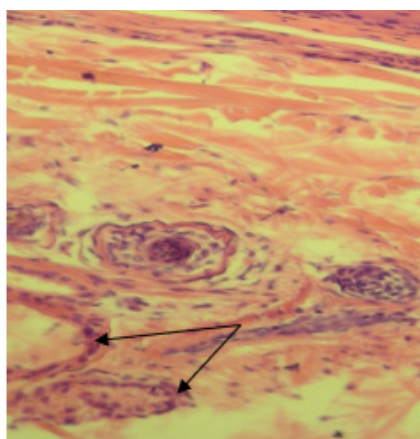


Figure 11. Histological change in atheroma
Note: the arrow indicates the atheroma cavity, hematoxylin-eosin staining, ×100 magnification

Analysing even the few results of the study of the occurrence of atheromas in dogs, an increase in cases

of the development of the pathological process in old dogs is clearly traced. Histological examination showed the formation of multiple atheromas, of which only one grows to a larger size. Surgical intervention with the surgical removal of such skin formations has a favourable prognosis and in most cases ends with a complete recovery of the animals.

DISCUSSION

Due to the surface location of pathological processes in the skin, neoplasms of any type are primarily detected by pet owners and, in this regard, occupy one of the prevailing places among oncological diseases in the statistics of veterinary clinics. Considering the almost identical clinical picture of the manifestations of various pathological processes of the skin, especially in the initial phases, confirmed in the studies of researchers such as J. Willcox *et al.* (2019) and M.A. Hasiri *et al.* (2019), and the absence of characteristic sites of formation of different types of tumors and formations of a non-tumor nature on the body of animals – S. Kim *et al.* (2022), makes the histological method the only way of differential diagnosis of skin diseases. As a consequence of the relatively low pathological effect of atheromas on the animal's health, which is associated with the non-oncological nature of the disease, the greatest immediate danger to the body is the possible suppuration of the cyst contents. The big threat to the animal from this disease is that they can mask the development of other, more dangerous oncological diseases and thereby pose certain risks to the life of animals.

In the conducted examinations, pathological formations of a non-tumor nature were diagnosed in different places of the animal body, but the formation of atheromas was most often observed in the head and neck area. Such localisation of sebaceous gland cysts in dogs accounted for 42% of the few cases used in the study. Atheromas were also identified in the abdominal wall of the abdomen and extremities – 20% each. In addition, isolated cases were diagnosed in the back and mammary gland zones. In one case, a generalised form of atheromatosis of the skin was diagnosed, when cysts of different sizes were detected throughout the body in one animal, some of them had signs of purulent inflammation. The study by J. Parmar *et al.* (2019) also confirms the main localisation of disorders in the sebaceous glands in dogs in the head area, and in this paper, the emphasis is placed on the predominant manifestation of the disease in old animals. Analysis of the results of the examinations conducted within the framework of this study also identified a substantial increase in the manifestation of atheroma in older animals. In such dogs, atheromas were observed in combination with other tumors, including malignant ones, in addition, atheromas in older dogs were of a larger size. This was not due to a longer period of their growth during the life of the animal, but, possibly, was the result of a

decrease in the body's defences in old age. The intensity of atheroma growth in young animals, even in a small population, was substantially lower than in older dogs. Similar results were obtained in the studies of E. Racine (2022), in which the main number of animals with symptoms of atheroma were over the age of 8 years. The influence of sex on the development of sebaceous gland cysts within the research group showed a substantially higher probability of developing atheroma in female dogs. Thus, in the sample of dogs that succumbed to treatment in the network of veterinary clinics in Bishkek, 75% of the animals were females. Similar studies, conducted on cats in Kyrgyzstan, showed similar results. Cat diseases were diagnosed in 79.3% of cases, whereas in dogs, only in 20.7%.

A small sample of animals included in the study did not allow determining the reliable influence of the breed on the frequency of cystic processes in the skin of dogs, although a small number of mongrel dogs may indicate that purebred dogs get sick much more often. Another side of this factor may be the fact that due attention is not paid to the health of mongrel dogs, which manifested itself in a small number of such animals. Among ten purebred dogs, three animals belonged to the boxer breed, which may indicate a substantial susceptibility to cystic diseases in this breed. This assumption is confirmed by the results obtained in the study by D. Wiener (2021), where boxers belong to the number of breeds with the maximum manifestation of this type of pathology. However, the main symptoms allow differentiating atheroma from other skin formations in animals can only be detected by microscopy of histological preparations obtained during biopsy or as a result of surgical removal of the neoplasm. In all diagnosed cases of atheroma in dogs, histomorphological changes occurring in the skin indicated the presence of one large and, in some cases, several small cavities filled with a cell-free mass. The cyst was formed due to an increase in the cavity of the sebaceous gland. Histological preparations show a wall lined with epithelial cells. The contents of the cyst do not contain cellular structures, and when stained with hematoxylin-eosin, it is stained with less intensity than the surrounding tissues. Similar characteristics of the histomorphological structure of atheroma are suggested in the methodological manual by F. Cian and P. Monti (2019). On all histological preparations from sick animals, one large cavity with dense contents is always established. But in most cases, in particular, up to 67% smaller atheromas were observed near the large one. Such placement of secondary atheromas may indicate a systemic skin disease in domestic animals and not be a random process that develops as a result of a simple blockage of the ducts of the sebaceous gland, as explained in previous studies. The probability of a simultaneous process in a number of sebaceous glands is quite low. It has not yet been possible to find a physiological explanation

or characteristic pathological factors that could lead to the development of such a process in literary sources, so this area may be of interest for further research.

In the absence of treatment, according to most researchers, the final stage in the development of atheroma is its infection with the development of purulent inflammation, and the histological preparation will have inherent changes characteristic of such a process (Jyothi *et al.*, 2023). Namely, nuclear cell structures appeared in the cyst cavity, represented by phagocytes and neutrophils, which are visualised due to the differential colouration of nuclear material from cytoplasmic structures and cystic contents. A substantial part of the cystic wall epithelium on a microscopic preparation is subject to desquamation or necrosis. Single or multiple fibrinous filaments are visible in the cyst cavity. Partial destruction of the atheroma wall with signs of purulent inflammation was observed on histological preparations obtained from pathological material from only two dogs. Infection and purulent inflammation in the animals of the research group were identified to be a rather rare phenomenon, which manifested itself only in 16% of cases, and this despite the fact that in some animals, the observation period for the development of atheroma was several years. This indicates that such a finale in the development of atheroma is a rather rare phenomenon, which may be associated with mechanical injury to the cyst wall and the entry of microorganisms there, whereas most atheromas retain growth for a long time.

Treatment of sebaceous gland cysts in dogs and other pets consists in surgical truncation of such a formation. Of the 12 controlled animals, 11 underwent surgical removal of this pathology. As a result of the manipulations performed, the condition of the animals did not differ from the condition before the surgical intervention. Observation of operated animals for a long period showed the absence of relapses of cyst formation, even due to mechanical injuries, which were surgical manipulations, which once again confirms the inaccuracy of assumptions about the traumatic nature of the cause of atheroma. Therefore, further studies on the causes of the development of cystic skin pathology in pets, and factors contributing to its occurrence are an urgent subject and will be continued in future studies.

CONCLUSIONS

Based on the materials of the analysis of veterinary records on the dynamics of development and clinical manifestation of pathological processes in the skin of dogs of a non-tumor nature and histological examinations of pathological material obtained during surgical removal of such atheromas, the following conclusions and suggestions can be made for future research. Despite the small sample of controlled animals, the majority of sick animals belonged to the group of old dogs, which at the time of the study were 9 or more years old. In addition, females were more susceptible to skin

diseases, the proportion of which was 75% of all animals. Among the presented dog breeds, boxers were more often identified to have atheromas than representatives of other breeds. The main place of localisation of cystic pathology in dogs was the area of the head and neck in up to 42% of all cases, atheromas in the extremities and abdominal wall were less often detected. In histological examination of pathological material obtained by truncation of areas with cystic skin lesions, cases of multiple atheromas prevailed. When a number of small ones were established next to the main, larger cyst. In percentage terms, such cases were more than 66%, which indicates the systemic development of this pathology in dogs.

The development of atheroma in the animal's body is a long process, in some cases, the dynamics of the development of this skin disease in dogs have been observed for 2-4 years, and only in some cases, None.

in particular, in these studies, only 16% of cysts are infected, which manifests itself in purulent inflammation with the formation of abscesses. The use of the surgical method of atheroma treatment allowed getting rid of the disease without cases of relapse. The results obtained in the framework of these studies do not coincide with a number of conclusions of other authors, in particular, regarding the main traumatic cause of atheroma formation and predominant suppuration of cysts. Therefore, these studies are planned to continue with the involvement of a large number of animals in a controlled group.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

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Гістоморфологічні зміни при атеромі у собак (морфологічне дослідження на прикладі 12 клінічних випадків)

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Анотація. Патологічні процеси в шкірі домашніх тварин є одним з найбільш поширених захворювань домашніх тварин як у Киргизькій Республіці, так і в усьому світі. У зв'язку зі складною диференціальною діагностикою онкологічних хвороб та захворювань непухлинного характеру у тварин лише за клінічними ознаками, метою цієї роботи стало проведення статистичного та гістоморфологічного аналізу випадків розвитку атерому у собак. Під час проведення досліджень використовували клінічний, гістологічний та статистичні методи. Дослідження проводилися на підконтрольній групі домашніх собак у кількості 12 тварин, спостереження за якими тривало протягом кількох років. При зборі анамнезу фіксували, крім клінічних ознак, породи, вік та супутні захворювання у собак. При проведенні хірургічного видалення атером готували гістологічні препарати для уточнення діагнозу та вивчення морфогістологічних змін у тканинах. Атерома шкіри у собак найчастіше реєструвалася у старих тварин, яким на момент встановлення діагнозу було більше 9 років. При цьому найчастіше така патологія локалізувалася у собак у ділянці голови та шиї. Серед порід боксер виявився найбільш сприйнятливим до утворення кіст сальних залоз. Також захворюваність серед самок становила 75 % від усіх випадків зареєстрованих хвороб. Гістологічні дослідження виявили превалювання випадків з утворенням множинних кіст – 67 %, коли біля однієї величезної атерому виявляється ряд дрібніших утворень, при цьому випадки розвитку гнійного запалення становили лише 16 %. Ефективним методом лікування було оперативне видалення атерому, при цьому випадків повторного розвитку кіст не спостерігалось протягом тривалого постопераційного спостереження. Отримані результати дозволяють розширити знання з клінічного прояву, діагностики та лікування захворювань шкіри непухлинної природи у домашніх тварин

Ключові слова: хірургічний та біопсійний матеріал; кіста сальної залози; морфологічна діагностика; непухлинна патологія шкіри; оперативне лікування



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Effectiveness of probiotics in growing broiler chicken

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Abstract. Antibacterial preparations are used to prevent bacterial diseases in poultry when raising broilers but given the negative factor of their residual accumulation in meat and the acquisition of resistance by pathogens, it became necessary to find alternative means. The purpose of this study was to determine the effectiveness of various concentrations of *Bacillus coagulans* on the growth and development of broiler chickens. Methods employed: microbiological; physiological to determine the state of health and safety of chickens; zootechnical;

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pathological; statistical. The chickens in the experiment had a higher live weight at Day 35: in Group 1 – by 11%, in Group 2 – by 15.4%, and in Group 3 – by 18.4%, as opposed to the control. The average daily body weight gain of chickens in groups with *B. coagulans* was higher, in Group 1 – by 10.8%, in Group 2 – by 15%, and in Group 3 – by 18.3%. The preservation rate in all experimental groups, regardless of the probiotic concentration, was 100%, while in the control group – 80%. There was an increase in live weight in the following groups: Group 1 – by 11%, Group 2 – by 15.5%, Group 3 – by 19%. Feed conversion was lower in Group 1 by 5.3%, in Group 2 – by 3.4%, and in Group 3 – by 2%, compared to the control. At the end of the study, the level of *Lactobacillus* sp. in the intestines of chickens in Group 1 was 33.78% higher, in Group 2 – by 50%, in Group 3 – by 78.37%; a decrease in the content of *Enterobacteriaceae* sp. in Group 1 – by 51.48%, in Group 2 – by 65.11%, in Group 3 – by 90.67%; *Staphylococcus* sp. in Group 1 – by 15.04%, in Group 2 – by 35.44%, in Group 3 – by 51.47% ($p \leq 0.05$), in contrast to the control. The average bursal weight in Group 1 was 4.82% higher, in Group 2 – 30% higher, in Group 3 – 37.53% higher, and the bursal index was 15%, 25%, and 30% higher, respectively, compared to the control.

Keywords: antibiotic resistance; *B. coagulans*; intestinal microflora; feed conversion; immunocompetent organs; live weight; preservation

INTRODUCTION

Broiler farming on an industrial scale creates a great concentration of livestock in a small area. During the cold season, poultry are kept indoors, which creates favourable conditions for infectious diseases to develop. Producers need to prevent the occurrence and spread of bacterial infections among chickens. The ban on the use of antimicrobials as growth promoters and preventive measures for bacterial infections has led to a search for alternative means of preventing them. The relevance of subject under study lies in the use of the probiotic strain *B. coagulans* ALM-86 to prevent bacterial diseases in broiler chickens.

G.R. Gibson *et al.* (2017) found that live probiotic strains of microorganisms in therapeutic doses improve the productive qualities of animals. The studies indicate that the doses of probiotic strains should be clearly defined and confirmed by toxicological studies regarding the safety of the specified strain of microorganism for animals. Therefore, the use of each probiotic must be clearly substantiated and its positive effect on animals must be proven. Furthermore, not only animal health is a vital indicator for the farm, but also production indicators such as live weight gain and feed conversion. Modern broiler crosses have a fast growth rate and accelerated metabolism, and therefore the results of using probiotic products can be clear in a shorter period of time.

A. Grant *et al.* (2018) found that probiotic strains of *Bacillus* have gained popularity for use in broiler breeding to provide safe and high-quality products. The advantages of these microorganisms are the synthesis of biocides, the formation of the microbiome, positive immunological and morphological changes in the gastrointestinal tract of chickens. However, different strains of *Bacillus* have cultural differences, and the mechanism of influence on poultry performance is not clearly defined.

J.M. Ngunjiri *et al.* (2019) found a direct correlation between beneficial and pathogenic microflora in the gut and lungs at all production stages of chicken rearing.

Scientists believe that advances in this research could lead to the development of an effective method based on intervention in the microbiome to improve productivity and control disease in poultry. Multidrug-resistant bacterial pathogens, which become resistant due to the use of antibiotics, are one of the most arduous challenges in poultry production. T.J. Johnson *et al.* (2018) investigated over two thousand samples from different broiler farms. The basic bacterial microflora, *Lactobacillus*, was identified by sequencing the 16S rRNA gene. The researchers believe that it is necessary to continue experiments towards maintaining the isolated microbiota using probiotic strains of microorganisms.

Y. Wu *et al.* (2018) found that the use of *Bacillus coagulans* in poultry affected by necrotising enteritis reduced the level of *C. perfringens*. The use of the broiler probiotic had a positive effect on productive performance and increased alkaline phosphatase activity. The experiment was limited to the use of a single pathogenic microorganism, *Clostridium perfringens*, and therefore there is a need to expand the range of possible antimicrobial activity of *B. coagulans*. K. Sasaki *et al.* (2020) concluded that *B. coagulans* restores the beneficial microflora in the human gut and reduces the number of *Enterobacteriaceae* in the colon. However, the effect of *B. coagulans* on the small intestinal microbiome and possible morphological changes is uncertain. C. Liu *et al.* (2022) indicated that *B. coagulans* in the poultry diet had a positive effect on the metabolism of protein and other metabolites. No studies have been conducted on the effect of probiotics on the immunocompetent organs of poultry.

The findings of W. Zheng *et al.* (2023) suggest that *B. coagulans* has prominent qualities as a probiotic. The researchers propose to use it for livestock production as a substitute for growth promoters and feed antibiotics. Furthermore, *B. coagulans* showed low levels of acute and chronic toxicity. There is a need to conduct research

in this area to determine the effectiveness of the introduction of *B. coagulans* in livestock. That is why the *purpose of this study* was to determine the effectiveness of *B. coagulans* ALM 86 in preventing bacterial diseases and the productive performance of broiler chickens.

MATERIALS AND METHODS

The study was conducted in the vivarium of the Faculty of Veterinary Medicine of Sumy National Agrarian

University in October 23. Broiler chickens (Cobb-500 cross) were selected as the object of study, of which 4 experimental groups and one control group of 25 birds each were formed. From the first to 36 days, the birds were kept on the floor on deep litter and fed with compound feed according to the standard indicators. Poultry in the experimental groups were supplemented with the probiotic *Bacillus coagulans* ALM-86 in various concentrations (Table 1).

Table 1. Research design

Groups	Concentration of <i>Bacillus coagulans</i> ALM-86
Experimental No. 1	1×10^5 , CFU/g
Experimental No. 2	1×10^7 , CFU/g
Experimental No. 3	1×10^9 , CFU/g
Control	water

Notes: CFU is a colony forming unit

Source: compiled by the authors of this study based on personal findings

Study of the properties of *Bacillus coagulans*. The adhesive properties of *Bacillus coagulans* ALM-86 were determined according to the Brilis method (Brilis *et al.*, 1986) using the mean adhesion index (MAI), erythrocyte participation rate (EPR), and erythrocyte adhesion index (EAI).

Study of physiological parameters of chickens. The weight of the chickens was determined by weighing them on the second, third, fourth, and fifth week of the experiment. Feed conversion and chicken survival were also measured.

Research on the microbiome in chickens. The composition of the bacterial microflora of the intestine of chickens was determined after slaughter of five birds from each group at the age of 2 and 5 weeks. Average samples weighing 1 g were taken from the contents of the small intestine (duodenum), transferred to a phosphate-buffered solution of 9 cm³, diluted from 10^{-1} to 10^{-10} and inoculated onto MPA (meat-peptone agar), Endo, Sabouraud, and vegetable media. They were incubated in a thermostat with an exposure time of 24-48 hours at 37°C, followed by counting the faecal CFU.

Determination of the effect of probiotic on the immunocompetent organs of broiler chickens. At the age of 5 weeks after slaughter, five poultry heads were examined for the mass of immunocompetent organs (bursa, thymus). The bursal index was also determined using the following formula:

$$BI = m / M \times 1000, \quad (1)$$

where BI is the bursal index, m is the mass of the immunocompetent organ, M is the mass of the chicken.

Statistical analysis. Statistical calculations of the data obtained were carried out using the Fisher-Student method (Fisher & Mosteller, 1948) when comparing the digital data of the control and experimental groups. The study considered the statistical significance

of indicators ($p < 0.05$) over 95%. Animal studies were conducted according to the methodology of DSTU EN ISO/IEC 17025:2019 (2021), the requirements and rules of bioethics and humane treatment of vertebrates 2010/63/EU (Hartung, 2010). The animals were kept, and all manipulations were conducted following the provisions of the Procedure for conducting tests and experiments on animals by scientific institutions (Law of Ukraine No. 249, 2012) and the European Convention (1986).

RESULTS AND DISCUSSION

Results of the study of *Bacillus coagulans* and its properties. At the first stage, the culture of *Bacillus coagulans* ALM-86 was prepared by growing it on vegetable medium, preparing smears and examining it under a microscope (Fig. 1).

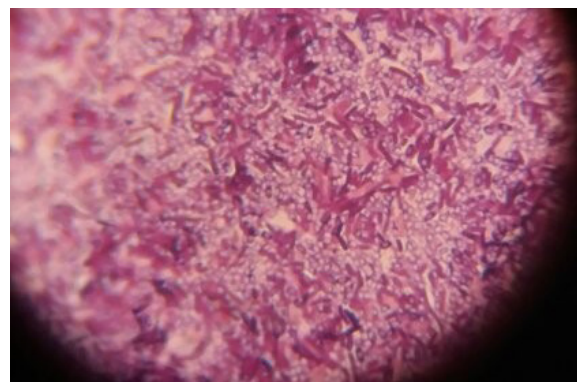


Figure 1. Light microscopy of *B. coagulans* ALM-86 (magnification $\times 4000$)

Source: compiled by the authors

The degree of adhesion to rooster erythrocytes was also determined to establish the virulence of the strain (Fig. 2).

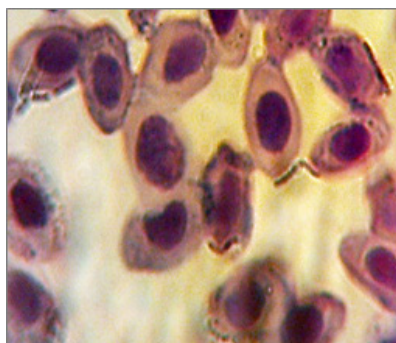


Figure 2. The result of adhesion of *B. coagulans* ALM-86 on rooster erythrocytes ($\times 1000$)

Source: compiled by the authors

When investigating the adhesiveness index of *B. coagulans* ALM-86 erythrocytes, it was found that it was 2.35 ± 0.12 . This is the average indicator according to the Brilis method (Fig. 2), the adhesion index – 1.80 ± 0.05 , and the adhesion coefficient –

85.34 ± 1.12 .

Results of determining the productive performance of chickens. At the beginning of the experiment, the body weight of broiler chickens, their preservation and feed conversion were found (Table 2).

Table 2. Physiological and productive parameters of broiler chickens using *Bacillus coagulans* ALM-86, $n=25$

Indicators	Experimental groups of chickens			
	1 Experimental <i>B. coagulans</i> 1×10^5 , CFU/g	2 Experimental <i>B. coagulans</i> 1×10^7 , CFU/g	3 Experimental <i>B. coagulans</i> 1×10^9 , CFU/g	control
Body weight per 1 head Day 1, g	50.20 ± 0.61	50.12 ± 0.37	49.80 ± 0.24	49.91 ± 0.36
Day 7, % to control	178.21 ± 1.50 101.10	180.21 ± 1.15 102.30	179.12 ± 1.34 101.70	176.30 ± 1.22 100.00
Day 14, g % to control	474.64 ± 2.25 97.90	498.68 ± 1.41 101.60	496.35 ± 1.28 101.20	490.04 ± 1.45 100.00
Day 21, g % to control	997.10 ± 1.57 105.90	$1,014.30 \pm 3.69^*$ 107.70	$1,020.5 \pm 2.99^*$ 108.40	941.10 ± 2.18 100.00
Day 28, % to control	$1,631.92 \pm 28.78$ 101.60	$1,679.30 \pm 37.51$ 104.60	$1,751.20 \pm 42.67^*$ 109.20	$1,605.31 \pm 39.70$ 100.00
Day 35, g % to control	$2,472.41 \pm 8.83^*$ 111.00	$2,569.30 \pm 17.53^*$ 115.40	$2,642.92 \pm 25.34^*$ 118.40	$2,226.92 \pm 30.30$ 100.0
Average daily live weight gain, g % to control	$72.07 \pm 0.66^*$ 110.80	$74.84 \pm 0.54^*$ 115.00	$76.93 \pm 0.60^*$ 118.30	65.05 ± 0.23 100.00
Poultry mortality (heads) preservation, %	0 100.00	0 100.00	0 100.00	5 80.10
Live weight gain, kg	$60.55 \pm 0.65^*$ 111.00	$62.98 \pm 0.86^*$ 115.50	$64.92 \pm 0.72^*$ 119.00	54.50 ± 0.63 100.00
Feed consumption, kg	128.20 ± 0.65 94.68	130.82 ± 0.45 96.60	132.61 ± 0.86 97.90	135.42 ± 0.33 100.00
Feed consumption per 1 kg of live weight gain, kg % to control	2.11 85.10	2.07 83.50	2.04 82.20	2.48 100.00

Notes: $*P < 0.05$ – relative to the control

Source: compiled by the authors of this study based on the data obtained

The results show that different concentrations of the probiotic had distinct effects on weight gain and feed conversion during broiler chicken rearing. The body weight of day-old chickens in the experimental and control groups was the same. At one to two weeks of age, chickens had analogous body weights

in all groups. Body weight increased ($p \leq 0.05$) in the groups of chickens on Day 21 of the experiment: in Group 1 – by 5.9%, in Group 2 – by 7.7%, in Group 3 – by 8.4%, in contrast to the control. On Day 28 of the study, a significant increase in weight was obtained in the broiler groups: in Group 1 – by 1.6%,

in Group 2 – by 4.6%, and in Group 3 – by 9.2%. The use of *Bacillus coagulans* ALM-86 has a positive effect on the productive qualities of poultry. At the end of the experiment on Day 35, the weight of chickens in Group 1 increased significantly by 11%, in Group 2 – by 15.4%, and in Group 3 – by 18.4%. Furthermore, the average daily weight gain of broilers in the groups where the poultry took the probiotic was significantly higher: in Group 1 – by 10.8%, in Group 2 – by 15.0%, and in Group 3 – by 18.3%, in contrast to the control.

Therewith, the preservation rate in all experimental groups, where different concentrations of the probiotic were used as an additive, was 100%. In the control group of 25 chickens, 5 chickens died from a bacterial infection. Chickens in the control group showed a decrease in weight on Day 35. It was found that the main cause of death of the chickens was catarrhal inflammation of the small intestine (Fig. 3). During the bacteriological examination of the pathological material, the causative agent of escherichiosis was isolated.

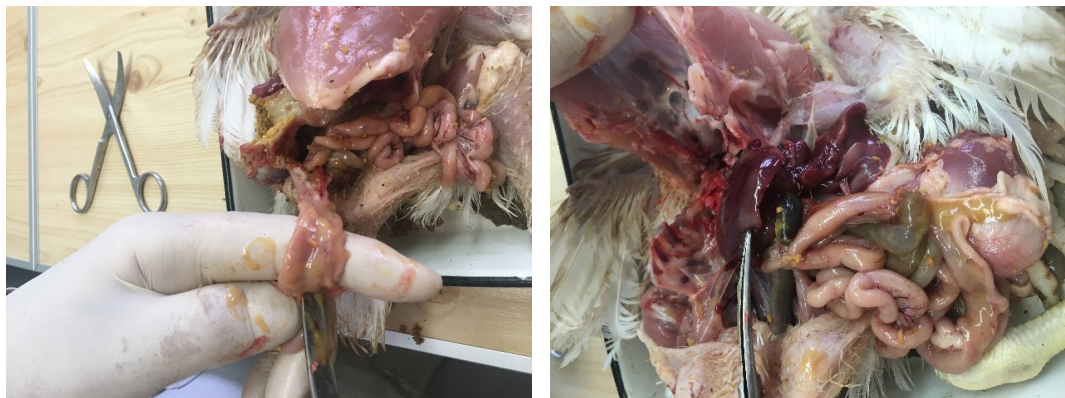


Figure 3. Catarrhal inflammation of the small intestine in chickens of the control group

Source: compiled by the authors of this study based on the data obtained

As a result of the experiment, there was an increase in body weight gain by group: in Group 1 – by 11%, in Group 2 – by 15.5%, and in Group 3 – by 19.0%. Feed consumption for the entire study period was 5.3% less in Group 1, 3.4% less in Group 2, and 2% less in Group 3, which is also a plus to economic profit compared to broiler chicken rearing without probiotics. This fact is also confirmed by the indicator of feed consumption per 1 kg of live weight gain, which was 14.9% lower in Group 1, 16.5% lower in Group 2, and 17.8% lower in Group 3 than in the control group.

Results of determining the composition of gastrointestinal microflora in chickens. The health and productivity of poultry depend on the state of the intestinal bacteriocenosis. The next significant variation is in the content of microorganisms in the small intestine of broiler chickens of different groups at the beginning of poultry rearing. During this period, the bacterial content in the intestines of chickens in Groups 1 and 2 was approximately 1.5-2.0 times higher than in the control group.

Table 3 presents quantitative changes in the intestinal microflora of chickens at 2 and 5 weeks of age.

By Week 2 of the study, the colonisation of the beneficial intestinal microflora with *Lactobacillus sp.* increased in Group 1 by 1.48%, in Group 2 – by 31.94%, and Group 3 – by 64.78%. By Week 5 of the study in broiler chickens, the amount of *Lactobacillus sp.* in the intestinal contents was 33.78% higher in Group 1, 50.0% higher in Group 2, and 78.37% higher in Group 3 than in the control. Furthermore, a potential increase in *Lactobacillus sp.* in chickens of the experimental groups should be noted compared to Week 2 of the study. The family *Enterobacteriaceae sp.* includes bacteria that include well-known pathogens such as *Escherichia coli*. Therefore, the decrease in the level of pathogenic bacteria in the intestine of chickens was a sign of the competitiveness of *Bacillus coagulans* ALM-86 as a probiotic. By Week 2 of the study, a significant decrease in the content of *Enterobacteriaceae sp.* bacteria was found in Group 1 by 34.5.9%, in Group 2 – by 37.27%, and in Group 3 – by 53.16%. By Week 5 of the experiment, the number of *Enterobacteriaceae sp.* in the duodenum in Group 1 decreased by 51.48%, in Group 2 – by 65.11%, in Group 3 – by 90.67% compared to the control.

Table 3. Composition of the microflora of the small intestine (duodenum) of chickens on Weeks 2 and 5 of rearing, n=5

Groups	<i>Lactobacillus sp.</i>		<i>Enterobacteriaceae sp.</i>		<i>Staphylococcus sp.</i>		Associated microflora	
	Week 2	Week 5	Week 2	Week 5	Week 2	Week 2	Week 2	Week 5
1st Experimental B. coagulans 1×10 ⁵ , CFU/g	(10.27±0.28) ×10 ⁴	(19.83±0.32)* ×10 ⁷	(13.02±0.34)* ×10 ⁴	(14.73±0.24)* ×10 ⁷	(8.64±0.42)* ×10 ⁴	(6.04±0.30) ×10 ⁷	(13.18±0.42)* ×10 ⁵	(14.91±0.41)* ×10 ⁸

Table 3. Continued

Groups	<i>Lactobacillus</i> sp.		<i>Enterobacteriaceae</i> sp.		<i>Staphylococcus</i> sp.		Associated microflora	
	Week 2	Week 5	Week 2	Week 5	Week 2	Week 2	Week 2	Week 5
2nd Experimental <i>B. coagulans</i> 1×10 ⁷ , CFU/g	(13.4±0.35)* ×10 ⁴	(22.25±0.39)* ×10 ⁷	(12.47±0.28)* ×10 ⁴	(10.59±0.36)* ×10 ⁷	(6.28±0.32)* ×10 ⁴	(4.59±0.43)* ×10 ⁷	(13.90±0.40)* ×10 ⁵	(12.77±0.52)* ×10 ⁸
3rd Experimental <i>B. coagulans</i> 1×10 ⁹ , CFU/g	(16.64±0.29)* ×10 ⁴	(26.41±0.35)* ×10 ⁷	(9.31±0.35)* ×10 ⁴	(2.83±0.18)* ×10 ⁷	(4.85±0.29)* ×10 ⁴	(3.45±0.29) ×10 ⁷	(12.98±0.58)* ×10 ⁵	(11.99±0.50)* ×10 ⁸
control	(10.08±0.26) ×10 ⁴	(14.87±0.28) ×10 ⁷	(19.88±0.25) ×10 ⁴	(30.36±0.55) ×10 ⁷	(14.56±0.24) ×10 ⁴	(7.11±0.38) ×10 ⁷	16.71±0.37 ×10 ⁵	16.23±0.36 ×10 ⁸

Notes: *P<0.05 – relative to the control

Source: compiled by the authors of this study based on the data obtained

By Week 2 of the test, the growth of *Staphylococcus* sp. was inhibited in Group 1 by 40.65%, in Group 2 – by 56.86%, in Group 3 – by 66.68%, compared to the control. In Group 1, the level of *Staphylococcus* sp. at the end of the tests was significantly lower than in the control group by 15.04%, in Group 2 – by 35.44%, and in Group 3 – by 51.47%. The associated microflora included unidentified microorganisms that affect the overall level of bacteria in the duodenum of broiler chickens. The content of associated microflora on Week 2 of the experiment was lower in Group 1 by 21.12%, in Group 2 – by 16.81%, and in Group 3 – by 22.32% compared to the control. At Week 5 of the study, the level of associated microflora in Group 1

was 8.13% lower, in Group 2 – 21.31% lower, and in Group 3 – 26.12% lower.

The results of determining the effect of probiotic on the immunocompetent organs of chickens. To establish the possible toxic effects on the chickens' body and immune system, necropsies and studies of immunocompetent organs were performed (Table 4). The average thymus weight of broiler chickens in the groups was almost identical, and therefore the thymus index was not calculated. The conducted studies found that the average weight of the bursa in Group 1 and the control group was lower than in the other groups. The average weight of the organ in Group 1 was 4.82% higher, in Group 2 – by 30.0%, and in Group 3 – by 37.53%.

Table 4. Weight of immunocompetent organs of chickens at the age of 5 weeks, n=5

Groups	Average thymus weight, g	Average weight of the bursa, g	Bursal index
1st Experimental <i>B. coagulans</i> 1×10 ⁵ , CFU/g	7.81±0.20	3.91±0.27	2.30±0.12
2st Experimental <i>B. coagulans</i> 1×10 ⁷ , CFU/g	8.31±0.34	4.85±0.41*	2.50±0.21
3st Experimental <i>B. coagulans</i> 1×10 ⁹ , CFU/g	8.15±0.28	5.13±0.45*	2.60±0.12
control	8.03±0.45	3.73±0.21	2.10±0.14

Notes: *P<0.05 – relative to the control

Source: compiled by the authors of this study based on the data obtained

According to the results, the bursal index was 15% higher in Group 1, in Group 2 – 25% higher, and in Group 3 – 30% higher compared to the control group. The experiment suggests that chickens in the experimental groups where *Bacillus coagulans* ALM-86 was used as a dietary supplement had greater body weight, safety, and immune status compared to the control group.

The determination of the properties of *B. coagulans* ALM-86 showed that the strain has an average adhesion index, which indicates the avirulence of the bacterium to the organism. O. Shkromada et al. (2022) proved the positive effect of the *Bacillus* probiotic on the gastrointestinal tract microflora and milk production in cows. According to the results of body weight determination, an increase in broiler chicken weight was found in the experiment on Days 21-35

of rearing, where the probiotic was used. The best results were obtained in Group 3, where *B. coagulans* was fed at a dilution of 1×10⁹ CFU/g. The findings of Y. Zhou et al. (2020) also reported an improvement in the performance of broiler chickens upon using *B. coagulans*. R. Jäger et al. (2018) and Z. Liu et al. (2022) confirm that *B. coagulans* promotes protein metabolism in animals.

During the research, it was found that the safety of chickens in all experimental groups, where different concentrations of the probiotic were used as an additive, was 100%. In the control group, chickens died due to intoxication caused by a bacterial infection. C. Liu et al. (2022) proved that one of the bacterial pathogens for poultry is *S. pullorum*, which leads to a high mortality rate caused by acute enteritis. Thus,

B. coagulans inhibits the growth and reproduction of opportunistic microflora in the intestine, which is confirmed by the study of the composition of the duodenal microbiome in chickens of two and five weeks of age. The test results are confirmed by K. Amoah *et al.* (2019) and S.S. Xing *et al.* (2019), who found that *B. coagulans* reduced intestine damage and restored the microbiome.

The number of beneficial microflora *Lactobacillus sp.* was significantly greater in chickens of the experimental groups, while *Enterobacteriaceae sp.* and *Staphylococcus sp.* were lower. S. Xie *et al.* (2022) and W. Zhen *et al.* (2018) confirmed that *B. coagulans* helps to form the intestinal microbiome, increase the body's resistance, and contribute to protection against pathogens such as *S. enteritidis* in chickens. Thus, *B. coagulans* can be added to the poultry diet as a growth stimulant and biocide, especially for young poultry, due to the inhibition of pathogenic microflora. Furthermore, studies have established the presence of a stimulating effect of *B. coagulans* ALM-86 on immunocompetent organs – the bursa, which gives grounds for its use as an immunomodulator. T.V. Bomko *et al.* (2017) and Z. Wang *et al.* (2022) found that in mice with streptomycin-induced colitis, the introduction of *B. coagulans* led to the cessation of diarrhoea, normalisation of gastrointestinal motility, and complete restoration of immunity merely using the probiotic.

In addition, due to the improved feed digestibility in chickens of the experimental groups, there was a decrease in feed conversion, which reflects the economic feasibility of using *B. coagulans* as an additive to broiler chickens. The high intensity of growth and metabolic processes in broiler chickens makes it necessary to search for safe growth and immunity stimulants. As an alternative, the *B. coagulans* ALM-86 probiotic for industrial use proved to be a desirable choice in the study.

CONCLUSIONS

Studies have shown that chickens treated with probiotics had a higher body weight on Day 21: in Group 1 – by

5.9%, in Group 2 – by 7.7%, in Group 3 – by 8.4%; on Day 28: in Group 1 – by 1.6%, in Group 2 – by 4.6%, in Group 3 – by 9.2%; on Day 35: in Group 1 – by 11%, in Group 2 – by 15.4%, in Group 3 – by 18.4%, compared to the control. When using *B. coagulans* in chickens, the average daily body weight gain was higher in Group 1 by 10.8%, in Group 2 – by 15.0%, and in Group 3 – by 18.3%, with an increase in body weight gain by 11%, 15%, and 19%, respectively, compared to the control. The survival rate of the chickens in the experiment was 100%, as opposed to 80% in the control group. Feed consumption per 1 kg of live weight gain was 14.9% lower in Group 1, 16.5% lower in Group 2, and 17.8% lower in Group 3; feed conversion was 5.3%, 3.4%, and 2% lower, respectively.

At Week 2 of the study, the content of *Lactobacillus sp.* in the intestines of poultry in Group 1 was 1.48% higher, in Group 2 – 31.94%, and Group 3 – 64.78%; at Week 5 of the study: Group 1 – 33.78%, Group 2 – 50.0%, Group 3 – 78.37%. At Week 2 of the study, a decrease in the content of *Enterobacteriaceae sp.* was found in Group 1 by 34.5.9%, in Group 2 – by 37.27%, in Group 3 – by 53.16%; at Week 5: in Group 1 – by 51.48%, in Group 2 – by 65.11%, in Group 3 – by 90.67%, significantly compared to the control. By Week 2, the study found an inhibition of *Staphylococcus sp.* growth in the groups as follows: in Group 1 – by 40.65%, in Group 2 – by 56.86%, in Group 3 – by 66.68%; by Week 5, in Group 1 – by 15.04%, in Group 2 – by 35.44%, and in Group 3 – by 51.47%. The study of the effect of *B. coagulans* on immunocompetent organs showed that the average bursa weight in Group 1 was 4.82% higher, in Group 2 – 30.0% higher, in Group 3 – 37.53% higher. Further prospects of the research are to investigate the effect of *B. coagulans* ALM-86 on the intestinal morphology of broiler chickens.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Ефективність застосування пробіотиків при вирощуванні курчат-бройлерів

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Анотація. При вирощування бройлерів з метою профілактики бактеріальних хвороб птиці застосовують антибактеріальні препарати, але, враховуючи негативний фактор накопичення їх залишкової кількості у м'ясі, та набуття збудниками заразних хвороб резистентності виникла необхідність пошуку альтернативних засобів. Метою дослідження було визначити ефективність різних концентрацій *Bacillus coagulans* на ріст та розвиток курчат-бройлерів. Використані методи: мікробіологічний; фізіологічний для визначення стану здоров'я та збереженості курчат; зоотехнічний; патологоанатомічний; статистичний. Курчата в експерименті мали більшу живу масу на 35 добу: в групі № 1 на 11 %, в групі № 2 – на 15,4 %, в групі № 3 – на 18,4 %, на відміну від контролю. Середньодобовий приріст маси тіла курчат у групах з *B. coagulans* був вище, в групі № 1 на 10,8 %, в групі № 2 – на 15 % та в групі № 3 – на 18,3 %. Збереженість у всіх дослідних групах не залежно від концентрації пробіотику, склала 100 %, в контрольній – 80 %. Відбувалось збільшення живої ваги в групах: групі № 1 – на 11 %, групі № 2 – на 15,5 %, групі № 3 – на 19 %. Конверсія корму була нижча у групі № 1 на 5,3 %, у групі № 2 – на 3,4 %, у групі № 3 – на 2 %, на відміну від контролю. По завершенню дослідження рівень *Lactobacillus sp.* у кишечнику курчат групі № 1 групи був вище на 33,78 %, групі № 2 – на 50 %, групі № 3 – на 78,37 %; зниження вмісту *Enterobacteriaceae sp.* у групі № 1 на 51,48 %, групі № 2 – на 65,11 %, у групі № 3 – на 90,67 %; *Staphylococcus sp.* у групі № 1 групи на 15,04 %, в групі № 2 – на 35,44 %, в групі № 3 – на 51,47 % ($p \leq 0,05$), на відміну від контролю. Середня маса бурси в групі № 1 була більша на 4,82 %, в групі № 2 – на 30 %, в групі № 3 – на 37,53 %, відповідно бурсальний індекс на 15 %, 25 %, та 30 %, порівняно з контролем

Ключові слова: антибіотикорезистентність; *B. coagulans*; мікрофлора кишечника; конверсія корму; імунокомпетентні органи; жива маса; збереженість



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Creation of nurseries and veterinary preventive measures for saigas of Betpakdala and Ural populations in Kazakhstan

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Abstract. Preservation and maintenance of biological diversity of Earth's life is one of the key tasks of mankind. Therefore, the search and substantiation of methods that will be able to stabilise and increase the number of endangered species of animals and plants, a key direction of modern science, consisted in the development of organisational and veterinary preventive approaches in the breeding of saigas of the Betpakdala and Ural populations in the conditions of the nurseries "ASAR Live"

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and “Akboke” in the Republic of Kazakhstan. The nursery stock was formed by the internment of new-born saigas removed from the natural population. Control over the growth and development of saigas was carried out by periodic weighing and determining the average daily weight gain. Dimensional characteristics of young animals were determined by selecting the measurements of the sexes of the body. The state of health of the animals in the premises was monitored by determining biochemical and haematological indicators – the content of protein, glucose, cholesterol. Approbation of the developed scheme of feeding new-born saigas in nurseries turned out to be quite effective, which was confirmed by high average daily weight gains at the level of 167-180 g up to 4 months of age and a 6-fold increase in live weight during this period. At the same time, the preservation of the animal population in nurseries was at the level of 97.3%. Accounting for all the necessary parameters to create optimal conditions for saigas in the nursery allowed increasing the population of saigas from 150 to 230 units already in the first year, considering that at the time of the formation of the herd it consisted of new-born animals. A comprehensive approach to solving the problem of preserving the local population of saigas in Kazakhstan allowed developing an effective system of measures tested in nurseries for the preservation and breeding of animals of the Betpakdala and Ural populations. The developed methods of preserving the endangered Tatar saiga population in nurseries, in addition to the reputational image of Kazakhstan as a country that solved the problem with the autochthonous species of fauna, contain recommendations for the development of a population of wild animals with minimal stress and adaptation consequences

Keywords: internment; feeding scheme; preservation of biodiversity; biochemical indicators; body measurements

INTRODUCTION

Humanity, as a social structure, has only recently come to understand that the preservation of plant and animal diversity on the planet is the main guarantor of the existence of all aspects of organic life on Earth. However, industrialisation and the active use of natural resources by humans reduce the habitat of most species of autochthonous representatives of flora and fauna around the world, including the Republic of Kazakhstan. One of the main factors of the decline in the population of Red Book species in the West Kazakhstan region is significant man-made changes in the natural landscape, and, accordingly, in the composition of vegetation that grew in these territories. This provoked both a change in the migration routes of wild animals and a decline in their populations due to a decrease in their range.

No less dangerous factor in preserving the diversity of animals in Kazakhstan is criminogenic phenomena, in particular, poaching in a number of regions and settlements in the range of animal populations, which are already on the verge of extinction. Therefore, special attention is paid to the conservation of biodiversity as one of the key areas of protection against the extinction of rare and endangered species of plants and animals in Kazakhstan. There are the draft laws adopted by the government on the protection of biodiversity, in particular Law of the Republic of Kazakhstan No. 160-І “On Environmental Protection” (1997), Law of the Republic of Kazakhstan No. 175-ІІІ “On Specially Protected Natural Areas” (2006), Law of the Republic of Kazakhstan No. 70-VІІ “On Ratification of the Protocol on the Conservation of Biological Diversity to the Framework Convention for the Protection of the Marine Environment of the Caspian Sea” (2021), and other regulations.

Special attention should be paid to autochthonous species, for which the Kazakh steppes are the main habitat, while preserving biodiversity. One of these species, which is included in a number of international conventions and lists of environmental organisations, is the saiga. *Saiga tatarica* is a species of animal from the family of pronghorn antelopes that inhabit the steppe and semi-desert expanses of Eurasia, they have biological characteristics that help them adapt to the harsh conditions of the Eurasian steppes. Of commercial interest are saiga antlers and derivatives, which are especially appreciated in the culture and medicine of Southeast Asian countries. All countries of the saiga breeding range banned any commercial export of these products more than ten years ago. But despite this ban, the number of offers for the sale of saiga antlers at auctions in countries is quite high, which does not exclude a high percentage of poaching in saiga breeding sites. As of 2023, the Kazakh saiga population makes up the bulk (95%) of the total global population of this species. Therefore, the development of methods and approaches aimed at preserving the Kazakh saiga population is an urgent area of research. The message of A. Serikbayeva *et al.* (2023) indicate that the International Union for Conservation of Nature (IUCN) has identified saigas as an endangered species. One of the most effective approaches to the conservation of endangered species is the creation of sanctuaries, nurseries, and other reserves for their breeding.

This approach, according to M. Nurushev *et al.* (2022), allowed to increase the number of national parks from 1 to 11; create 4 natural reserves; achieve the inclusion of 140 species of fauna to protected animals, of which 22 were listed in the Red Book of the Republic of Kazakhstan. In the West Kazakhstan

region, the main distribution area of saigas, according to K. Kenesarina (2019), in recent years there has been an active development of the gas and oil industry, which has led to changes in the landscape and biological diversity of the local territory. The construction of pipelines, roads, railways, and other infrastructure has reduced the population of local mammals, including Red Book animals. In addition, the year 2003 was critical for the saiga population, when the number of animals of this species did not exceed 21.3 thousand units. The main reasons for this change in numbers, according to K. Mukantayev *et al.* (2022), was poaching. From other reasons, A. Serikbayeva *et al.* (2023) note the loss of habitats through human industrial activity, snowy winters, lack of food, diseases, and competition with farm animals. But epizootics turned out to be the most dangerous for the saiga population. Diseases of pasteurellosis, teileriosis, ruminant plague, pasture mites, and other parasitic diseases led to a decrease in the number of animals by 200 thousand units per year according to S. Fereidouni *et al.* (2019).

In connection with the obligations assumed by the Republic of Kazakhstan within the framework of the ratified International Convention on Biological Diversity and the Memorandum of understanding concerning conservation, restoration and sustainable use of the saiga antelope (*Saiga tatarica*) (2021), the conservation of the animal population of this species is important for the image of the country. Due to the significant incidence of saiga antelopes and poaching, the breeding of these animals in captivity is considered as one of the alternative methods of preserving this species. Therefore, the purpose of this study was to work out the organisational conditions for the creation of nurseries for breeding saigas in captivity, and the development of veterinary and sanitary measures in nurseries to prevent the death of animals.

LITERATURE REVIEW

Kazakhstan is home to the Betpakdala, Ustyurt, and Ural groups of saigas, which belong to the Tatar (Eurasian) subspecies (*Saiga tatarica*), and the Mongolian subspecies (*Saiga tatarica mongolica*), which, according to A. Serikbayeva *et al.* (2023), is distributed in Mongolia. G. Turlybekova *et al.* (2017) indicates that 95% of the population of the Tatar subspecies of saiga is located in Kazakhstan, while in the Betpakdala and Ural groups there was a 45% increase in livestock, while the Ustyurt population practically disappeared. According to M. Shpigelman (2023), the number of saigas in Western Kazakhstan was estimated at 1.13 million units.

E. Milner-Gulland *et al.* (2020) observed a decrease in the number of offspring (from 1.27 ± 0.25 calves to 0.92 ± 0.39) due to female infertility. Therefore, the only method of preserving the population and maintaining its genetic diversity remains the creation of sanctuaries where optimal conditions for breeding saiga are

maintained. K. Kushaliyev and Zh. Usenov (2022) highlight the importance of veterinary and preventive measures when keeping saigas in captivity, V. Havrylenko *et al.* (2022) focus on the regulatory function of humans when working with the population in the reserve, and L. Dimeyeva *et al.* (2022) are convinced that maintaining high-quality pastures in nature reserves can stimulate the growth of the number of animals. J. Grachev (2022) concludes that when creating sanctuaries for saiga, as a migratory animal, it is necessary to create conditions for desert, semi-desert, and steppe zones. Because these areas are important for wintering, calving, and summer feeding. But the main factor in the increase in the number of saigas in sanctuaries is the systematic prevention of poaching, as indicated by D. Blank and Y. Li (2021). According to O. Baitanaev *et al.* (2014), the main reason for the reduction of the saiga population by 97.9% (from 1 million to 21.3 thousand units) from 1990 to 2003 was related to poaching.

In nature reserves, due to the high crowding of animals, special attention is paid to veterinary safety issues. In particular, pasteurellosis, since, according to O. Baitanaev *et al.* (2014), a significant part of the saiga population died from this disease in the 1980s. S. Fereidouni *et al.* (2019) estimate the number of saigas killed by foot and mouth disease (FMD) and pasteurellosis over a 60-year observation period in Kazakhstan to be 100,000 and 805,700 saigas, respectively. In study by U. Taubaev *et al.* (2022) indicates that almost all adult animals are pasteurellosis carriers. Among other infectious diseases, a significant incidence was observed in the Mongolian population from ruminant plague (Pruvot *et al.*, 2020). Parasitic diseases are also frequently reported among saiga. A. Abdybekova *et al.* (2021) found 55 species of endoparasites and about 10 species of ectoparasites of saigas in Kazakhstan. The spread of invasive diseases is also reported by A. Abdybekova *et al.* (2023), M. Khanyari *et al.* (2022). K. Kushaliyev *et al.* (2023) indicate a significant mortality of saigas from parasitic diseases up to 15-17% from ctenosomiasis and 7-9% from moniziosis. Saigas also acted as a source of invasion in pastures for farm animals, which makes sanctuaries not only a method of stabilising the saiga population, but also preventing morbidity among animals.

MATERIALS AND METHODS

The creation of nurseries for breeding and preserving the gene pool of the Tatar subspecies of saiga (Ural and Betpakdala populations) was carried out in West Kazakhstan and Ulytau regions of the Republic of Kazakhstan as part of the programme for breeding and domestication of saigas. Organisational approaches to the maintenance and feeding of new-born saigas were based on the results of scientific research by the staff of the Zhangir Khan University and practical developments of employees of LLP "ASAR Live" (Fig. 1).



Figure 1. Removal of new-born saiga fawns from the natural environment

Source: authors's photo

The approbation of the developed organisational approaches for the adaptation of animals in captivity and veterinary measures to preserve the health of saiga was carried out in the established nurseries "ASAR Live" in Zhanaarka district, Ulytau region and "Akbroken" in the West Kazakhstan region for the breeding of saiga. Before the construction of the nurseries, epizootic monitoring of their territory was carried out to identify pathogens of infectious diseases: anthrax, blackleg, brucellosis, rabies. In the spring of 2022, 150 caught saigas were placed in the "ASAR Live" nursery for breeding saigas of the Betpakdala population, according to the permission received for the use of animals for scientific purposes. The nursery was organised jointly with the "ASAR" farm, located in the habitat of the Betpakdala saiga population and occupies an area of 370 ha (Fig. 2).



Figure 2. "ASAR Live" nursery

Source: authors's photo

In 2023, the second "Akbroken" nursery was opened in the West Kazakhstan region for saigas of the Ural population. It is equipped 20 km from Uralsk, in the area of

operation of the Ural agricultural experimental station and is located on an area of 56 ha. 200 new-born young animals were interned from the natural environment for breeding and keeping in the "Akbroken" nursery (Fig. 3).



Figure 3. "Akbroken" nursery

Source: authors's photo

In order to reduce the impact of stress on saigas during their internment, it was decided to move only new-born animals to nurseries. The levels and feeding technology were developed by the university staff. All saiga young stock, regardless of sex, were kept in groups of 20 fawns and tamed to bottle feeding. In the early days of life, calves were fed a formula based on Nestogen baby food and cow's milk with fish oil added. From the second month, the young stock was gradually accustomed to eating grain (barley). Control over the growth and development of saigas was carried out by periodic weighing and taking measurements: at birth, at one and four months of age. At birth, weighing was performed at the site of saiga antelope capture using a hand scale with an electronic sensor (Fig. 4a), subsequent measurements were made using an electronic floor scale (Fig. 4b).



Figure 4. Weighing of saiga calves: a) at capture; b) at the age of one month

Source: authors's photo

Health monitoring was carried out by determining the biochemical parameters of the blood system, while blood for haematological studies was taken from the jugular vein in the morning before feeding. The

indicators were determined using automatic analysers Chem Well 2910 and Mindray BC-2800 Vet in the conditions of the veterinary clinic of the Zhangir Khan University (Fig. 5).



Figure 5. Blood collection from saigas for haematological and biochemical analyses

Source: authors's photo

During rearing, periodic check-ups were also carried out to detect diseases of various aetiologies in saigas. But the main focus was on infectious and invasive diseases.

Diagnostic and coprological studies were carried out on the premises of the Institute of Veterinary Medicine and Animal Husbandry at the Zhangir Khan University.

RESULTS

Monitoring studies. One of the areas of work on the conservation of the saiga population is regular monitoring studies. The dynamics of the number of saigas in different populations over the past 3 years has been consistently positive. According to the materials of the Kazakhstan Association for the Conservation of Biodiversity, by far the largest saiga population in the country is the Ural (Table 1).

Table 1. Number of saigas in Kazakhstan in 2016-2023

Year	Population size, units			
	Ustyurt	Ustyurt	Betpakdala	Total
2016	70,200	1,900	36,200	108,300
2017	98,200	2,700	51,700	152,600
2018	135,000	3,700	76,400	215,100
2019	217,000	5,900	111,500	334,400
2020	Quarantine (no monitoring studies have been conducted)			
2021	545,000	12,000	290,000	842,000
2022	801,000	28,000	489,000	1,318,000
2023	1,130,000	39,700	745,300	1,915,000

Source: developed by the authors

The Ural and Betpakdala populations have been steadily growing in recent years. The increase in the current year amounted to more than 45.0% compared to 2022. The high growth was facilitated by favourable food and weather conditions of the past two years and fairly effective protection, which has improved markedly over this period. But the main reason for the increase in the number of saigas is their multiplicity (twins and sometimes triplets). On average, there are 1.5 calves per mother. Such a high multiplicity made it possible, without prejudice to both the population and without violating the maternal instinct during subsequent calving, to wean calves from the twin for the

increase of numbers of heads in newly formed nurseries. This approach has been successfully tested in the creation of nursery stock for two saiga populations – Betpakdala and Ural.

Organisational arrangements. In the nurseries “ASAR Live” and “Akbroken”, the technology of feeding calves was developed and tested on a large number of new-born saigas. Since the normal growth and development of saiga calves depends on compliance with feeding norms and intervals, energy balance, and veterinary standards of animal maintenance and condition. Table 2 shows the scheme of feeding new-born calves by the author's team and used in the above-mentioned nurseries.

Table 2. Feeding scheme for saiga young stock

Age, days	Volume of milk or milk formula, l	Number of feedings per day	Age, days	Volume of milk or milk formula, l	Number of feedings per day
1-3	0.05-0.1 (Nestogen)	5	61-90	0.4 (CMR)	3
4-10	0.1-0.15 (Nestogen)	4	91-105	0.45 (CMR)	3
11-40	0.25 (whole cow milk)	4	106-120	0.5 (CMR)	2
41-60	0.25-0.3 (CMR)	4	121-150	500 (CMR)	1

Notes: CMR – calf milk replacer

Source: developed by the authors

The frequency of feeding of calves was determined depending on the appetite and physiological state of the young stock, their activity, the amount of green feed consumed, hay, concentrates, and other factors. In the first ten days of the dairy period, calves were fed with a mixture based on Nestogen infant

formula, then feeding was carried out with cow's milk. From the age of one month, milk replacer with the addition of vegetable proteins has already been used to feed young animals. The feeding regime of young animals during the entire dairy period is presented in Table 3.

Table 3. Feeding regime of calves in the dairy period

Age of young stock							
1-3 days		4-60 days		61-105 days		106-120 days	
No. of feeding	Time	No. of feeding	Time	No. of feeding	Time	No. of feeding	Time
1st	06:30	1st	06:30	1st	06:30	1st	08:30
2nd	11:30	2nd	11:30	2nd	14:00	2nd	20:00
3rd	14:30	3rd	16:30	3rd	21:00	-	-
4th	18:30	4th	21:30	-	-	-	-
5th	21:00	-	-	-	-	-	-

Source: developed by the authors

The sucking reflex was actively manifested in the captured new-born calves, which allowed them to be adapted quite quickly to artificial feeding. Already during the first weeks, it was necessary to increase the dose of the mixture. After 2 months of the suckling period, calves were able to consume from 400 to 500 ml of milk formula in one feeding. During the use of infant formula, there were no signs of gastrointestinal disorders. From the first days of drinking milk, 2 ml of fish oil was added to it once a day. From the second month, calves began to be gradually accustomed to feeding barley grain, and from the age of 4 months, sources of inorganic calcium (feed chalk) were included in the diet. At first, it was

added to milk, and in later periods it was added to concentrates. The daily feed requirement of 4-5 month-old saigas consisted of 2.5 kg of coarse feed (hay, straw), 300 g of concentrated feed (medium grinding), salt lick and water without restrictions. Notably, while feeding milk, calves got used to the service staff, but as they grew older, they had an increased instinct for self-preservation. Control over the development of saiga calves in captivity was carried out by weight and height indicators. To do this, animals were systematically weighed and body measurements were determined at birth, at one and four months of age. The results of the weigh-in from birth to 4 months of age are presented in Table 4.

Table 4. Dynamics of changes in the live weight of calves of the Ural and Betpakdala populations ($M \pm m$), ($n=270$), kg

Age, days	Sex	
	Males	Females ($M \pm m$)
New-borns	3±0.2	4±0.2
30 days	9.1±0.2	9±0.2
120 days	21±1.5	20.1±0.9

Source: developed by the authors

The live weight of new-born saiga antelopes in the Ural population was 3.16 kg, whereas the saiga antelopes of the Betpakdala population were significantly

larger – 3.96 kg ($P<0.05$), although the body parameters of new-born animals practically did not differ (Table 5).

Table 5. Exterior parameters of new-born saigas, $M \pm m$ ($n=100$), cm

Measurements	Ural population ($n=50$)	Betpakdala population ($n=50$)
Oblique length of the body	32.5 $\pm$ 1.7	33.5 $\pm$ 2.1
Chest depth	12.4 $\pm$ 0.9	13.5 $\pm$ 1.1
Height at withers	41.4 $\pm$ 2.5	41.8 $\pm$ 3.7
Height at hips	40 $\pm$ 2.5	40.9 $\pm$ 3.9
Chest width	7.7 $\pm$ 0.9	7.9 $\pm$ 1
Width at hip bone	6.38 $\pm$ 0.8	6.91 $\pm$ 1
Chest circumference	33.1 $\pm$ 1.9	33.8 $\pm$ 2.2
Pastern circumference	4.41 $\pm$ 0.5	4.59 $\pm$ 0.7

Source: developed by the authors

According to the dynamics of the live weight gain of saiga antelopes of two populations, the gains of males were higher than those of females during the entire control period. According to the average daily increase, the difference between animals of different sexes was 4-7 g (Table 6).

Table 6. Dynamics of live weight gain of calves born in 2023

Age period	Sex	
	Males	Females
Absolute gain, kg		
From birth to 1 month	5.95	5.08
1-4 months	11.9	11.1
From birth to 4 months	16.89	16.02
Average daily body weight gain, g		
From birth to 1 month	180	173
1-4 months	131	127
From birth to 4 months	142	138

Source: developed by the authors

As a result of the conducted research, it can be stated that the growth and development of calves had high indicators. The increase in live weight from birth to 4 months of age averaged 140 g per day. This indicates that the conditions created in nurseries can ensure the normal development of animals from the first days of

life. In the "ASAR Live" nursery, when keeping saigas in captivity, females were able to mate in the first year, and males reached puberty at a later age.

Veterinary and preventive measures. The results of the biochemical study of blood serum are presented in Table 7.

Table 7. Biochemical parameters of saiga blood in the spring and autumn period when growing them in the nursery "ASAR Live" ($n=10$)

Indicator	$M \pm m$	Lim
Total protein, g/l (spring)	76.7 $\pm$ 7	67.2-89.1
Total protein, g/l (autumn)	75.8 $\pm$ 2.4	58.2-95.1
Parts by weight Albumins, g/l	53.9 $\pm$ 1.4	33.7-79.4
Parts by weight Globulins, g/l	42.02 $\pm$ 4.4	29.4-73.2
Protein coefficient	1.6 $\pm$ 0.1	0.7-2.3
Glucose, mg/dl (spring)	57.4 $\pm$ 2.3*	47.2-69.3
Glucose, mg/dl (autumn)	77.9 $\pm$ 4.3*	61.4-101.2
Cholesterol, mg/dl (spring)	186.66 $\pm$ 10.1*	139.5-214.6
Cholesterol, mg/dl (autumn)	149.7 $\pm$ 13.8*	95.2-178.4
Parts by weight Triglycerides, mg/dl	101.78 $\pm$ 20.1	94.9-109.7
Iron, mg/dl	93.4 $\pm$ 5.84	42.1-177.6

Notes: * – $P < 0.05$

Source: developed by the authors

The protein content in the blood of the animals, regardless of the season, was within the physiological norm – from 60 to 115.5 g/l, and in terms of the ratio of albumins to globulins corresponded to animals with active growth. While the concentration of glucose and cholesterol significantly differed between seasons. This may be evidence of the transition of calves feeding from a dairy diet to the use of plant-based feed. Thus, the study of the biochemical parameters of the blood of saigas in the conditions of the nursery revealed an excess concentration of most indicators compared to animals of analogous age of the European population contained in natural conditions. This may indicate the best conditions for animals in the created reserves.

In the nurseries “ASAR Live” and “Akboke” all diagnostic and preventive manipulations are carried out according to the plan of veterinary and preventive measures. Veterinary control of the saiga's health is carried out by clinical examination and analysis of haematological and biochemical blood parameters of the entire animal population. This approach allowed detecting early signs of diseases in animals and carrying out treatment in a timely manner. Thus, due to this approach, immediately after the removal of newborn calves, they showed signs of psoroptosis (scabies), which was eliminated by twice treating the animals with ivermectin. There have been isolated cases of mortality of saiga calves with signs of dyspepsia. Mainly signs of digestive disorders were observed in the first two weeks after their removal from the natural environment, which was a likely consequence of stress in a number of animals. Thus, the problem of stress leads to a malfunction of the immune system, its adaptive and other protective mechanisms. Therefore, in order to reduce the stress state of young animals, they were kept in cages with plastic mesh, 15-20 units in each, which allowed excluding cases of injury. Of the diseases of non-infectious aetiology in nurseries, there were: phlegmon, bronchopneumonia, injuries, limb fractures, enteritis during the preweaning period, hoof diseases, and subsequently, some females had obstructed labour. As a result of keeping saigas in a limited area and the absence of the need for them to move long distances in search of food, the animals in the nurseries had an overgrowth of the hoof horn. As a veterinary measure, it has also been necessary to clean the hooves of saigas.

The most common diseases in wild mammals in Western Kazakhstan are parasitic diseases, in particular, moniezia. Since insects are involved in the development cycle of this parasite, the peak incidence is manifested in the summer, during their swarming. It is not possible to protect animals from insects on the territory of the nursery. To do this, preventive deworming was performed using ivermectin or albendazole preparations (Fig. 6). To do this, the first use of drugs was carried out at 30-45 days of age, repeated after 15 days and the final

treatment of animals was carried out after 25-30 days. This allowed reducing the incidence among animals.



Figure 6. Lifetime diagnosis of moniezia

Source: authors's photo

Summing up, all the organisational and veterinary preventive measures carried out during the creation and operation of nurseries for saiga in Kazakhstan ensure the preservation of the genetic diversity of the population by preventing the incidence of animals of various aetiologies, poaching, and the spread of pasture invasions in significant territories. As of today, the nurseries have received good results of joint cooperation with the involvement of University faculty, undergraduates and students of universities of Kazakhstan, zoo specialists, and local media, which increases the effectiveness of conservation of autochthonous, relict species of flora and fauna of Kazakhstan.

DISCUSSION

Since the purpose of creating nurseries for the breeding of wild saiga fauna is to preserve the unique gene pool of the oldest animal species, which is represented almost only on the territory of Kazakhstan, such work becomes not only a national task but also important for the image of the country. The use of nurseries and sanctuaries as a model for the conservation of animal species that are on the verge of extinction is one of the most humane approaches in preserving the biodiversity of living organisms. Modelling of natural and landscape conditions in the reserves allowed creating optimal conditions for the restoration of the number of saigas of the Betpakdala and Ural populations. This approach to preserving the entire biological diversity of living objects characteristic of a particular region is

consistent with the opinion of T. Atemasova *et al.* (2023) and will allow, in addition to animals, to preserve the plant diversity inherent in this territory.

When creating nurseries “ASAR Live” and “Akbroken” for breeding saiga in the territory of West Kazakhstan and Ulytau regions of the Republic of Kazakhstan, most of the factors necessary for successful breeding and reproduction of saiga were considered. For this purpose, the nurseries were geographically located in the landscape conditions of the steppe, semi-desert, and desert, since each of them serves as a habitat for saigas during certain seasonal periods. This is consistent with the opinion of B. Chimeddorj *et al.* (2023), that when creating sanctuaries, it is necessary to consider the attachment of interned animals to the conditions of the environment in a natural state and, if possible, create them. In addition, an equally important factor in the creation of nurseries is the size of its territory, especially if further introduction of animals into natural conditions is planned. Chinese researchers Z. Jiang *et al.* (2020) concluded that the small area of reserves for saigas does not allow restoring the number of animals as migratory species. Therefore, this factor must be considered when organising the work of the reserve. This approach of recreating the habitat conditions with floodplain meadows and forests, and the constant increase in the nursery area in the floodplain of the Syrdarya River was tested by M. Suleymenova *et al.* (2017) in Kazakhstan during the reintroduction of Bukhara deer brought from the Karachingil hunting farm.

The period of adaptation of the animals was long and only in 16 years allowed to increase the number of deer in the nursery from 8 to 103 individuals. As saigas were captured within the confines of the nurseries when they were established, there was no adaptation period, but the effects of stress could have a long-term impact on the physiological abilities of the interned saigas. In order to reduce the impact of stress, it was decided to form a nursery population with new-born animals. This approach solved the problem of stress in animals and made it relatively easy to select saiga antelopes for the nursery. This is due to the physiological characteristics of migrating animals, when the mother leaves her new-born offspring for a relatively long period in search of food. The approach used in the development of the livestock of the “ASAR Live” and “Akbroken” nurseries is unique, since such reports could not be found in the scientific literature. The relatively easy artificial feeding of young ruminants allows using this method of forming the livestock of sanctuaries in further work while preserving other species. The use of industrially produced whole milk replacers has enabled the feeding of saiga calves to be harmonised and a healthy saiga population to be established in the two reserves. The results of the growth and development of saiga calves were monitored by periodic weighing and taking measurements.

The search for reference values of biochemical and haematological parameters in the scientific literature for captive saigas, depending on sex, age, and physiological condition (disease, stress), did not yield positive results. Therefore, the obtained indicators of the main biochemical and haematological studies of saiga from nurseries were compared with the parameters of new-born saiga in the natural environment, which were measured in study by X. Liu *et al.* (2019). This indicates that the conditions that have been created in the nurseries correspond to the natural needs of saigas. This was also confirmed by later results of the reproductive ability of animals that were kept in captivity. Young females became sexually mature already at 8-9 months and the next year brought offspring. Similar results of the development of reproductive ability were observed in animals in the natural population in the studies by D. Moon (2023). According to the researcher, females became sexually mature at 7-8 months and were ready to mate in the first year of life.

The most dangerous factor in creating an artificial population of animals in a nursery or wildlife sanctuary is the narrowing of genetic diversity in such a group. Similar results were obtained by Z. Jiang *et al.* (2020) and M. Nurushev *et al.* (2020). When, as a result of a decrease in the genetic diversity of the herd, there was a decrease in reproductive ability and immunity in animals. In a broader study of the entire saiga population, A. Rey-Iglesia *et al.* (2022) indicate low genetic variability in the Tatar and Mongolian saiga species, both species also have a low percentage of heterozygosity in the main genes. These studies indicate a significant narrowing of diversity in saiga populations and a further increase in monozygosity may lead to the degeneration of the species. Therefore, when creating a herd in the nurseries “ASAR Live” and “Akbroken”, the selection of calves was carried out in significant territories of adjacent regions of Kazakhstan in order to expand genetic diversity. No more than two new-born animals were selected from the distribution area of one family. What could guarantee the absence of kinship ties between individuals in the livestock of the nursery. The other side of this approach was to ensure the veterinary well-being of nurseries from infectious problems.

Given the fact that the outbreak of pasteurellosis at one time almost destroyed the saiga population, a plan of veterinary and preventive measures was drawn up in the nurseries, which provides for the study of livestock for particularly dangerous diseases – brucellosis, FMD, which are indicated in their studies by W. Beauvais *et al.* (2019). Regular coprological examination for the presence of helminth eggs has also become a necessary area of research, since the parasites themselves are able to reduce the immunological status of animals and contribute to morbidity in the population. Thus, the nurseries at the Zhangir Khan University are a modern base for scientific and practical work on the

conservation of the biodiversity of local fauna, which is equipped with modern resources and facilities for conducting veterinary and environmental research. This allows conducting the entire range of work on the study of saigas of the Betpakdala and Ural populations in the conditions of established nurseries. It is a practical school for Kazakh scientists, undergraduates, and doctoral students of national higher educational institutions, where scientific research of ecological, biological and genetic directions is carried out, in compliance with the conditions of zoohygienic standards for the growing of animals in captivity. In this regard, the development and implementation of mechanisms for the conservation and regulation of the number of saigas is an urgent and in-demand task of modern Kazakh science.

Currently, work on the study of saigas in captivity continues. Recently, the main focus has been the investigation of molecular genetic DNA markers in the Betpakdala and Ural saiga populations. In the near future, it is planned to set up a modern genetic laboratory at the Zhangir Khan University to carry out diagnostic tests for diseases of various aetiologies, and to solve the following problems: genomic selection and genetic certification of saiga; identification and evaluation of markers of disease resistance; studying, evaluating, and adjusting the composition of the saiga microbiota, selecting the most effective diet for keeping in nurseries; studying and preserving the gene pool of the population of *Saiga tatarica*; creation of national genomic databases.

CONCLUSIONS

Based on the materials of the conducted research on organisational approaches to the creation of nurseries "ASAR Live" and "Akbroken" to preserve the gene pool of the Betpakdala and Ural saiga populations (*Saiga tatarica*) and the development of healthy livestock, the several conclusions and suggestions can be made for future scientific work. An integrated approach to solving the problem of conservation of the saiga

population has allowed the authors to develop a system of measures to regulate the number of migratory wild animals in the western regions of Kazakhstan. An analysis of the number of animals in the Ural and Betpakdala populations indicates stable growth in recent years. The increase in livestock in 2022 was more than 45% compared to their number in 2021. The developed mechanism for removing new-born saigas from the natural environment with the subsequent technology of feeding them allowed creating breeding stock with minimal adaptation and stress loads. And the developed feeding scheme for saigas allows obtaining average daily live weight gains in young animals of both populations at the level of 167-180 g from birth to 4 months.

The results obtained indicate high rates of growth and development of saiga calves in nursery conditions, at a level even higher than in natural conditions. The live weight of calves from birth to 4 months of age increased 6 times in both males and females. The developed veterinary and preventive approaches to the maintenance of saigas in the established nurseries allowed maintaining the number of interned animals at the level of 96.4-98.2% in the Betpakdala and Ural saiga populations, respectively. The created conditions for the animals of the Betpakdala population in the "ASAR Live" nursery allowed increasing the population of saiga from 150 units in 2022 to 230 units in 2023, given the fact that the herd consisted of new-born animals. Such results indicate the compliance of the created conditions for the breeding of saigas. In future studies, it is planned to investigate the genetic diversity of the saiga population in nurseries and develop molecular genetic markers characteristic of the rapid typing of animals of the Betpakdala and Ural populations.

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CONFLICT OF INTEREST

None.

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Створення розсадників та ветеринарно-профілактичні заходи для сайгаків бетпакдалінської та уральської популяції в Казахстані

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Анотація. Збереження та підтримання біологічного розмаїття життя Землі одне з ключових завдань людства. Тому пошук та обґрунтування методів, які зможуть стабілізувати та збільшити чисельність видів тварин і рослин, що зникають, ключовий напрямок сучасної науки, полягали у розробці організаційних та ветеринарно-профілактичних підходів при розведенні сайгаків бетпакдалінської та уральської популяції в умовах розсадників «ASAR Live» та «Akboke» в Республіці Казахстан. Поголів'я розплідників формувалося шляхом інтернування вилучених із природної популяції новонароджених сайгаків. Контроль за зростанням та розвитком сайгачат проводили шляхом періодичного зважування та визначення середньодобового приросту ваги. Габаритні характеристики молодняку визначали шляхом добору промірів статей тіла. Стан здоров'я тварин в розпліднику контролювали шляхом визначення біохімічних та гематологічних показників – вміст білка, глюкози, холестерину. Апробація розробленої схеми годівлі новонароджених сайгачат у розплідниках виявилася досить ефективною, що підтвердилося високими середньодобовими приростами ваги на рівні 167-180 г до 4-х місячного віку і збільшенням живої ваги за цей період в 6 разів. При цьому збереження поголів'я тварин у розсадниках перебувало на рівні 97,3 %. Облік всіх необхідних параметрів для створення оптимальних умов для сайгаків у розпліднику дозволив вже в перший рік збільшити популяцію сайгаків зі 150 до 230 голів, враховуючи, що на момент формування стада воно складалося з новонароджених тварин. Комплексний підхід у вирішенні проблеми збереження місцевої популяції сайгаку в Казахстані дозволив розробити ефективну систему заходів, апробовану в розплідниках щодо збереження та розведення тварин бетпакдалінської та уральської популяції. Розроблені методи збереження зникаючої популяції татарського сайгака в розплідниках, крім репутаційного іміджу для Казахстану, як країни, що вирішила проблему з автохтонним видом фауни, містять рекомендації щодо формування популяції диких тварин з мінімальними стресовими та адаптаційними наслідками

Ключові слова: інтернування; схема годування; збереження біорізноманіття; біохімічні показники; проміри тіла



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Study of the efficiency of growing maize for silage for processing into biogas and digestate

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Abstract. Given the growing area of maize cultivation in Ukraine and globally, research into the possibilities of using silage as a component of biogas plants to produce biogas as an alternative fuel and digestate as a bio-organic fertiliser is of high relevance and production necessity. The purpose of this study was to investigate the chemical composition of green mass of maize and its changes depending on the growing season for its use in biogas production. Field, laboratory, laboratory-field, and statistical research methods were used in the study. The findings of this study showed that the quality of maize silage depends on soil and climatic conditions, elements of growing technology, genetic characteristics of a particular hybrid, its maturity group, harvesting time, etc. The genetic characteristics of the hybrid had the greatest influence on the dry matter content, specifically, the mid-early hybrid Amaros stood out in terms of dry matter content on 10-14 August 2020 (26.61%). The highest crude fibre content was observed in the hybrid P9170 – 6.32% and 26.86%, respectively, in natural and absolutely dry matter. This hybrid belongs to the mid-season ripeness group. The shift in the harvesting time of the green mass of the maize hybrids under study also affected the characteristics of the chemical composition and the carbohydrate-lignin complex of the green mass of the maize hybrids under study. The yield of green mass of maize

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hybrids substantially depended on the maturity group of maize hybrids and was highest in the mid-season maize hybrids Burito – 78.1 t/ha and P9170 – 73.1 t/ha, while in the group of mid-early hybrids it was 55.3 t/ha and 68.9 t/ha in Amaros and P9071. Hybrids with a long growing season have higher green mass yields and dry matter yields per unit area. The findings can be used by farms that grow silage maize and have the opportunity to use the vegetative mass as a component of biogas plants for biogas production and digestate production

Keywords: bioorganic fertilisers; silage maize; dry matter; starch; lignin; structural carbohydrates; crude protein

INTRODUCTION

Maize is one of the most productive cereals and uses many resources, including soil, moisture, light, nutrients, etc., to generate its productivity. In the context of energy shortages, global warming and military aggression, the issue of using biomass from crops, including maize, to produce alternative fuels (biogas and bioethanol) has become quite acute. Research on the cultivation and use of silage maize as a substrate for a biogas plant is very effective, as silage maize can increase the efficiency of biogas production, and the crop itself forms a powerful vegetative mass, which is quite favourable for anaerobic digestion in biogas plants by its chemical composition.

Maize cultivation has two main focuses: grain and silage. The area under grain maize cultivation has been steadily increasing, reaching 5.5 million hectares in 2021, and in 2022 it decreased by 17% to 4.267 million hectares, due to a range of factors, including Russia's military aggression against Ukraine, problems with fuel and lubricants (especially for drying grain), logistics, etc. In 2021, silage maize occupied only 214 thousand hectares, although it is effectively used as a substrate for a biogas plant (Statistical Yearbook, 2021). M. Doyeni *et al.* (2021) point out that in European countries, growing silage maize for biogas plants is a widespread practice and has proven to be effective. Ukraine, which has significant agricultural resources, also has the potential to successfully develop a biogas sector. The cultivation of silage maize for biogas production has been supported in developed European countries (Germany, the UK, Poland, Switzerland, Denmark, the Netherlands, France, etc.) due to favourable legislative policies (IEA, 2019).

V. Smutný *et al.* (2018), S. Theuerl *et al.* (2019) note that the use of green mass of maize for biogas fermentation in biogas production is based on an anaerobic fermentation process under the influence of microorganisms. The use of green mass of maize has the following advantages over other crops: high yields, high organic matter content, high starch content, and the well-established technology of growing silage maize. N. Boltianska and O. Boltianskyi (2019) point out that as a result of green mass fermentation, apart from biogas, digestate is obtained, which is a very valuable bioorganic fertiliser for increasing soil fertility, replenishing macro- and microelements and carbon accumulation in the soil (carbonisation), increasing crop yields

and quality. According to S. Corden *et al.* (2019), up to 180 million tonnes of digestate are produced annually in the EU countries, a considerable share of which is digestate from maize silage. J. Urrea *et al.* (2019) and B. Karimi *et al.* (2022) note that loading 100 thousand tonnes of silage into a biogas plant produces the same amount of bio-organic fertiliser (digestate), which contains calcium, phosphorus, and nitrogen in liquid and solid fractions.

V. Popović *et al.* (2020) note that the use of digestate will reduce the need for mineral fertilisers, which are industrially produced with significant use of fossil fuels and increased CO₂ emissions. S. Kudria *et al.* (2022) note that for Ukraine, as an agricultural country in the world, the prospects for using green mass of maize for biogas production and digestate production are determined by the availability of substantial acreage of this crop, a large assortment of hybrid composition of various maturity groups, favourable soil and climatic conditions and chemical composition of the green mass. V. Palamarchuk *et al.* (2023) notes that the efficiency of the biological process of anaerobic digestion and the yield of biogas and digestate from maize silage depends on the chemical composition of green mass and the amount of organic matter. Furthermore, the chemical composition of organic matter is influenced by climatic conditions, growing area, hybrid composition, growing and silage technology, and the duration of the growing season.

A review of the literature showed that the chosen subject is understudied. That is why the purpose of this study was to investigate the efficiency of using green mass of maize as a substrate for biogas plants, depending on the dynamics of changes in the chemical composition during certain periods of vegetation of hybrids of various maturity groups.

MATERIALS AND METHODS

Research on the quality of silage maize and elements of its cultivation technology has been conducted since 2020 (as of 18.10.2023, research is ongoing) at "Organic-D" LLC in Sutysky village, Vinnytsia region, Ukraine. The findings presented in this paper were collected in 2020. The researchers used maize hybrids of two maturity groups: mid-early Amaros (FAO 230) and P9071 (FAO 280) and mid-season P9170 (FAO 320) and Burito (FAO 390).

The soil of the experimental plot is grey forest medium loamy by mechanical composition, with a humus content determined according to the Tyurin method (DSTU 7828:2015, 2016) of 1.5%; hydrolytic acidity – 1.3 mg/eq. per 100 g of soil; the amount of absorbed bases – 28 mg/eq. per 100 g of soil; nitrogen content was determined according to the Cornfield method (DSTU 7863:2015, 2016) – 9.6 mg per 100 g of soil, mobile phosphorus – 8.5 mg and exchangeable potassium – 11.4 mg per 100 g of soil determined according to the Chirikov method (DSTU 4115:2002, 2003). The technology is generally accepted for this soil and climate zone. The predecessor was winter wheat, sown in the third decade of April using a wide-row method with a John Deere precision seeder (United States of America), with a row spacing of 70 cm and a plant density of 75 thous. pcs./ha. The maize hybrids were sown in the third decade of April at a soil temperature of 10-12°C at the depth of seed placement. The main tillage system included disking and ploughing at a depth of 22-25 cm. The fertilisation system involved the application of 60 t/ha of Effluent bio-organic fertiliser, produced through anaerobic digestion of pig manure in a biogas plant.

Replication of the experiment was fourfold; variants were placed in the experiment in randomised blocks. The size of the variant plot is 25 m², and the accounting plot is 10.5 m², i.e., four adjacent rows of 5 m in length. Harvesting and accounting of maize silage

yield was carried out in the phase of milky wax and wax ripeness of grain manually from each experimental plot by cutting 10 typical plants from two adjacent rows of each variant (Ushkarenko *et al.*, 2014). The quality and chemical composition of the green (silage) mass of maize hybrids was determined in the laboratory of the Institute of Feed and Agriculture of Podillia, National Academy of Agrarian Sciences of Ukraine, accredited to perform measurements in the field of quality control and safety of feed, raw materials, and food products in the "UkrAgroStandart" system.

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

RESULTS AND DISCUSSION

The study found a link between the quality of maize silage and soil and climatic conditions, elements of cultivation technology, genetic characteristics of a particular hybrid, its maturity group, harvesting time, etc. Thus, for the harvesting of green mass on 10-14 August 2020 in the phase of milky wax ripeness, the chemical composition, carbohydrate-lignin complex in natural and absolutely dry matter are presented in Tables 1-3.

Table 1. Chemical composition of green mass of maize hybrids, %

Sample name (green mass of maize)	Dry matter	Crude protein		Crude fibre	
		in a natural substance	in an absolutely dry substance	in a natural substance	in an absolutely dry substance
Amaros (FAO 230)	26.61	1.75	6.58	6.52	24.51
P9071 (FAO 280)	22.21	1.65	7.43	5.50	24.77
P9170 (FAO 320)	23.52	2.01	8.55	6.32	26.86
Burrito (FAO 390)	22.87	1.88	8.22	6.56	28.68

Source: compiled by the authors

Table 1 shows that the dry matter content in the green mass of maize as of 10–14 August was as follows in the group of medium early hybrids: Amaros (FAO 230) – 26.61%, P9071 (FAO 280) – 22.21%, and in the group of mid-season hybrids: P9170 (FAO 320) – 23.52% and Burrito (FAO 390) – 22.87%. Proceeding from the data on dry matter content (Table 1), the genetic characteristics of the hybrid had the greatest influence on this indicator, specifically, the mid-early hybrid Amaros (26.61%) stood out in terms of dry matter content on 10-14 August 2020 compared to other hybrids under study.

The content of crude protein in natural and absolutely dry matter was the highest in the hybrids of the mid-season group P9170 – 2.01% and 8.55% and Burrito – 1.88% and 8.22%, respectively, while in the group of mid-early hybrids it was for Amaros – 1.75%

and 6.58%, and for the P9071 hybrid – 1.65% and 7.43%, respectively. The biogas yield and the quality of the silage feed value are substantially influenced by the crude fibre content, which was also found in natural and dry matter. The highest crude fibre content was observed in the hybrid P9170 – 6.32% and 26.86%, respectively, in natural and absolutely dry matter. This hybrid belongs to the mid-season ripeness group. The crude fibre content of other hybrids was as follows: Amaros (FAO 230) – 6.52% and 24.51%, P9071 (FAO 280) – 5.50% and 24.77%, and Burrito (FAO 390) – 6.56% and 28.68%, respectively. Therewith, the findings of the study revealed a difference in the carbohydrate-lignin complex of the green mass of the studied maize hybrids of different maturity groups depending on the genetic characteristics of the hybrid itself (Table 2).

Table 2. Carbohydrate-lignin complex of green mass of maize hybrids, % in natural matter

Sample name (green mass of maize)	Starch	Sugar	Sum of easily soluble carbohydrates	Hemicellulose	Cellulose	Lignin	Sum of structural carbohydrates
Amaros (FAO 230)	0.63	5.04	12.69	6.66	6.33	1.35	14.34
P9071 (FAO 280)	0.44	3.39	9.40	5.29	4.47	1.09	10.85
P9170 (FAO 320)	0.37	2.15	9.71	6.83	6.61	1.25	14.69
Burrito (FAO 390)	0.18	6.91	13.44	6.03	5.31	1.60	12.94

Source: compiled by the authors

The content of readily soluble carbohydrates and sugars ensures high quality silage. Table 2 shows that the highest starch content as of 10-14 August 2020 was observed in the Amaros hybrid – 0.63%, while in other hybrids under study it was: for P9071 – 0.44%, for P9170 – 0.37%, and for Burito – 0.18%; sugar content was as follows: Burito – 6.91%, Amaros – 5.04%, P9071 – 3.39% and P9170 – 2.15%. The amount of easily soluble carbohydrates was the highest in the mid-season hybrid Burito – 13.44% and the mid-early hybrid Amaros – 12.69%, while in other hybrids it was 9.40% and 9.71% in P9071 and P9170, respectively.

Hemicellulose, cellulose, and lignin have rather poor solubility, and their high content in plant biomass can reduce biogas yields. The content of these substances in the

hybrids under study ranged within 5.29-6.83% for hemicellulose, 4.47-6.61% for cellulose, and 1.09-1.60% for lignin. The highest content of hemicellulose and cellulose was found in hybrids P9170 (FAO 320) – 6.83% and 6.61% and Amaros (FAO 230) – 6.66% and 6.33%, respectively. The lignin content was highest in the mid-season maize hybrid Burrito at 1.60% and the mid-early hybrid Amaros at 1.35%. The amount of structural carbohydrates was the highest in hybrids P9170 (FAO 320) – 14.69% and Amaros (FAO 230) – 14.34%, while in the Burito hybrid (FAO 390) it was 12.94%, and in the P9071 hybrid (FAO 280) it was the lowest and amounted to 10.85%. An analogous analysis can be carried out for the carbohydrate-lignin complex of the green mass of maize hybrids in terms of absolutely dry matter (Table 3).

Table 3. Carbohydrate-lignin complex of green mass of maize hybrids, % in absolutely dry matter

Sample name (green mass of maize)	Starch	Sugar	Sum of easily soluble carbohydrates	Hemicellulose	Cellulose	Lignin	Sum of structural carbohydrates
Amaros (FAO 230)	2.38	18.95	47.68	25.04	23.78	5.08	53.90
P9071 (FAO 280)	1.98	15.26	42.31	23.81	20.12	4.89	48.82
P9170 (FAO 320)	1.58	9.16	41.31	29.04	28.09	5.32	62.45
Burrito (FAO 390)	0.79	30.21	58.77	26.38	23.22	7.00	56.60

Source: compiled by the authors

Table 3 shows that the patterns of accumulation of chemicals in terms of absolutely dry matter are analogous to those in natural matter. Thus, the highest starch content was in Amaros (FAO 230) – 2.38% and P9071 (FAO 280) – 1.98%, while in other hybrids it was P9170 (FAO 320) – 1.58% and Burito (FAO 390) – 0.79%. The highest sugar content was found in the Burito hybrid (FAO 390) – 30.21%, while in other hybrids it was Amaros (FAO 230) – 18.95%, P9071 (FAO 280) – 15.26% and P9170 (FAO 320) – 9.16%. The amount of easily soluble carbohydrates in the hybrids under study ranged within 41.31-58.77%, with the highest value in the mid-season maize hybrid Burito – 58.77% and the lowest value in the mid-season hybrid P9170 – 41.31%, while among the

mid-early hybrids this indicator in Amaros was 47.68% and in P9071 – 42.31%. The amount of structural carbohydrates (hemicellulose, cellulose, and lignin) was the highest in mid-season maize hybrids P9170 – 62.45% and Burito – 56.60%. In the group of mid-early hybrids, this figure was 53.90% for Amaros and 48.82% for P9071.

The shift in the harvesting time of the green mass of the maize hybrids under study also affected the chemical composition and carbohydrate-lignin complex. Thus, when harvesting green mass on 20-24 September at the beginning of the waxy ripeness phase, the chemical composition of green mass and the carbohydrate-lignin complex in absolutely dry matter are presented in Tables 4-5.

Table 4. Chemical composition of green mass of maize hybrids, % in absolutely dry matter

Sample name (green mass of maize)	Crude protein	Crude fibre
Amaros (FAO 230)	6.45	19.63
P9071 (FAO 280)	7.22	14.72
P9170 (FAO 320)	7.19	15.67

Table 4. Continued

Sample name (green mass of maize)	Crude protein	Crude fibre
Burrito (FAO 390)	9.49	14.04

Source: compiled by the authors

Table 4 shows that when harvesting green mass on 20-24 September 2020, the mid-season maize hybrid Burito had the highest crude protein content of 9.49%. Whereas in the other hybrids under study, it was 7.22% for P9071 (FAO 280), 7.19% for P9170 (FAO 320), and 6.45% for Amaros (FAO 230). The crude fibre

content was highest in maize hybrids Amaros (FAO 230) – 19.63% and P9170 (FAO 320) – 15.67%. The characteristics of the carbohydrate-lignin complex of the green mass of maize hybrids harvested on 20-24 September in absolutely dry matter are presented in Table 5.

Table 5. Carbohydrate-lignin complex of green mass of maize hybrids, % in absolutely dry matter

Sample name (green mass of maize)	Starch	Sugar	Sum of easily soluble carbohydrates	Hemicellulose	Cellulose	Lignin	Sum of structural carbohydrates
Amaros (FAO 230)	27.76	5.05	48.94	15.47	14.14	3.53	33.14
P9071 (FAO 280)	39.70	4.49	57.19	13.02	13.02	3.63	29.67
P9170 (FAO 320)	38.10	4.09	54.97	12.78	10.44	3.56	26.78
Burrito (FAO 390)	33.25	4.81	59.29	16.23	15.48	3.88	35.59

Source: compiled by the authors

During this period, the starch content increased by 25.38-27.72% compared to the content on 10-14 August 2020, and amounted to 39.70% for P9071 hybrid (FAO 280), 38.10% for P9170 hybrid (FAO 320), 33.25% for Burrito hybrid (FAO 390) and 27.76% for Amaros hybrid (FAO 230). The sugar content in the green mass decreased by 5.07-25.40% compared to the period of 10-14 August and amounted to Amaros (FAO 230) – 5.05%, Burrito (FAO 390) – 4.81%, P9071 (FAO 280) – 4.49%, and P9170 (FAO 320) – 4.09%. Therewith, the amount of easily soluble carbohydrates was the highest in the mid-season

maize hybrid Burito – 59.29% and the mid-early hybrid P9071 – 57.19%. The sum of structural carbohydrates (hemicellulose, cellulose, and lignin) was the highest in the mid-season maize hybrid Burito – 35.59% and the mid-early hybrid Amaros – 33.14%. To determine the biogas yield, it is essential not only to know the chemical composition of the green mass of maize hybrids and the dry matter content, but also the yield of the dry mass of maize per unit area. The characteristics of the green mass yield of the maize hybrids under study as of 14 August 2020 are presented in Table 6.

Table 6. Green mass yield of maize hybrids of different ripeness groups (for 2020)

Sample name (green mass of maize)	Dry matter, %	Yield, t/ha	Dry matter yield per 1 ha, t
Amaros (FAO 230)	26.61	55.3	14.72
P9071 (FAO 280)	22.21	68.9	15.30
P9170 (FAO 320)	23.52	73.1	17.19
Burrito (FAO 390)	22.87	78.1	17.86
LSD ₀₅ , t/ha		4.82	1.30

Source: compiled by the authors

Table 6 shows that the yield of green mass of the maize hybrids under study significantly depended on the maturity group of maize hybrids and was highest in the mid-season maize hybrids Burito – 78.1 t/ha and P9170 – 73.1 t/ha, while in the group of mid-early hybrids it was Amaros – 55.3 t/ha and P9071 – 68.9 t/ha. A comparable trend can be observed for the dry matter yield per unit area. The highest yields were recorded for mid-season maize hybrids Burito – 17.86 t/ha and P9170 – 17.19 t/ha. Thus, lengthening the growing season in the maize hybrids under study not only increases the yield of green mass but also increases the dry matter yield per unit area. The findings are confirmed by the

studies of other scientists who have worked in this area and investigated the chemical composition of maize silage and the specifics of using silage and green mass for anaerobic digestion in biogas plants to increase the efficiency of biogas and digestate production.

R. Skliar (2020) notes that the addition of lignin-containing organic plant material to the biogas reactor helps to reduce nitrogen losses during the fermentation process. S. Shvorov and Ye. Antypov (2019) argue that silage as a technological operation improves biogas production and methane yield from maize plant material compared to the use of green mass without silage. O. Skliar and R. Skliar (2020) indicate that the high

content of organic matter, cellulose, starch and lignin in maize silage allows for, after fermentation and obtaining digestate to be applied to the soil, the increase of the humus content and accumulate carbon in the soil and ensure the cultivation of environmentally friendly products. According to H. Heletukha *et al.* (2022), the use of special energy maize hybrids with a dry weight yield of 18-25 t/ha as a substrate for biogas production will yield 5,300-9,000 m³/ha of methane, depending on the hybrid, growing conditions, and the harvesting phase of maize.

A. Ehmann *et al.* (2018) point out that the quality and structure of organic matter in biogas digestates depends on the feedstock, its chemical composition and the technology used. M. Grabovsky (2019) notes that due to the higher dry matter content, the specific biogas yield per unit of silage mass applied to maize was 33.7-50.6% higher than that of sugar sorghum and 9.2-13.0% higher than that of a mixture of these crops. With a dry matter content of 30-42%, the average methane yield was 0.40 m³/kg of substrate. M. Szymanska *et al.* (2018) point out the difficulty of using plant biomass from crops other than maize as a substrate for biogas plants due to unfavourable physical and mechanical (dry matter content, moisture content, organic dry matter content) and chemical composition (protein, carbohydrate, and fat content). For instance, the use of plant biomass, which contains a branched capillary structure covered with hydrophobic components (waxes, lignin), contributes to the formation of dense floating layers in the bioreactor.

M. Grabovsky (2020) found that an increase in the maturity group of maize hybrids contributes to a decrease in the specific methane yield. The optimum specific methane yield was observed at a dry matter content of 30-35%. If the dry matter content is less than 20%, the silage properties deteriorate, a significant amount of filtrate is produced and the potential for biogas production and yield is substantially reduced. It was found that the highest yield of green mass (73.1 t/ha and 78.1 t/ha) was formed by hybrids of the mid-season ripeness group P9170 (FAO 320) and Burito (FAO 390) with a dry matter yield of 17.19 t/ha and 17.86 t/ha, which is characterised by a favourable carbohydrate-lignin complex, crude protein and fibre content, and therefore the use of green mass of these hybrids as a substrate for biogas plants to increase biogas yield will be most appropriate.

CONCLUSIONS

The chemical composition and carbohydrate-lignin complex of the green mass of the maize hybrids under study can vary depending on the phenological phase of plant growth and development. The highest content

of dry matter was recorded in the mid-early Amaros hybrid – 26.61%, crude protein in natural and absolute dry matter – in the hybrids of the mid-season group P9170 – 2.01% and 8.55%, and Burito – 1.88% and 8.22%, respectively. The starch content in Amaros was 0.63%, in P9071 – 0.44%, P9170 – 0.37% and in Burrito – 0.18%, while the sugar content in Burrito was 6.91%, in Amaros – 5.04%, in P9071 – 3.39% and in P9170 – 2.15%. Hemicellulose, cellulose, and lignin have rather poor solubility, and their high content in plant biomass can reduce biogas yield, the highest content of hemicellulose and cellulose was found in hybrids P9170 (FAO 320) – 6.83% and 6.61% and Amaros (FAO 230) – 6.66% and 6.33%, respectively. The lignin content was highest in the mid-season maize hybrid Burrito at 1.60% and the mid-early hybrid Amaros at 1.35%.

Shifting the harvesting dates of the green mass of the maize hybrids under study to 20-24.09.2020 also affected the characteristics of the chemical composition and carbohydrate-lignin complex. Therewith, the starch content increased by 25.38-27.72% during this period, while the sugar content in the green mass decreased by 5.07-25.40% compared to the content on 10-14 August. The amount of structural carbohydrates (hemicellulose, cellulose, and lignin) in the period 20-24.09.2020 was the highest in the mid-season maize hybrid Burito – 35.59% and the mid-early hybrid Amaros – 33.14%.

The yield of green mass and dry matter yield of the maize hybrids under study significantly depended on the maturity group of maize hybrids and was the highest in the mid-season maize hybrids Burito – 78.1 t/ha and 17.86 t/ha and P9170 – 73.1 t/ha and 17.19 t/ha, while in the group of medium-early hybrids it was Amaros – 55.3 t/ha and 14.72 t/ha and P9071 – 68.9 t/ha and 15.30 t/ha, respectively. Therefore, the most suitable for biogas production as a substrate for biogas plants are silage maize hybrids of the medium-ripening maturity group P9170 (FAO 320) and Burito (FAO 390), which provide the highest yield and organic matter yield per unit area and have a favourable chemical composition and carbohydrate-lignin complex of green mass. In the future, it is planned to confirm the obtained patterns by long-term research and investigate the efficiency of using maize silage with different chemical composition in the biogas reactor of "Organic-D" LLC.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Дослідження ефективності вирощування кукурудзи на силос для переробки на біогаз та дигестат

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Анотація. Дослідження, в умовах зростання площ вирощування кукурудзи в Україні та світі, можливості використання силосної маси як компонента біогазових станцій для отримання альтернативного виду палива – біогазу та біоорганічного добрива – дигестату мають високу актуальність та виробничу необхідність. Метою роботи було вивчення хімічного складу зеленої маси кукурудзи та зміни його залежно від періоду вегетації для використання її на біогаз. В роботі використовували польові, лабораторні, лабораторно-польові та статистичні методи досліджень. Результатами проведених досліджень встановлено, що якість силосної маси кукурудзи залежить від ґрунтово-кліматичних умов, елементів технології вирощування, генетичних особливостей конкретного гібриду, його групи стиглості, строків проведення збиральних робіт та ін. Найбільший вплив на вміст сухої речовини здійснювали генетичні особливості гібриду, зокрема за вмістом сухої речовини на 10-14 серпня 2020 року виділився середньоранній гібрид Амарос (26,61 %). Найвищий вміст сирої клітковини відмічений у гібриду П9170 – 6,32 та 26,86 %, відповідно у натуральній та абсолютно сухій речовині. Даний гібрид відноситься до середньостиглої групи стиглості. Зміщення строків збирання зеленої маси досліджуваних гібридів кукурудзи вплинуло і на характеристику хімічного складу та на вуглеводно-лігніновий комплекс зеленої маси досліджуваних гібридів кукурудзи. Урожайність зеленої маси гібридів кукурудзи істотно залежала від групи стиглості гібридів кукурудзи і найвищою вона була у середньостиглих гібридів кукурудзи Буріто – 78,1 т/га та П9170 – 73,1 т/га, тоді як у групі середньоранніх гібридів вона склала Амарос – 55,3 т/га та П9071 – 68,9 т/га. Гібриди із тривалим вегетаційним періодом мають вищу урожайність зеленої маси та вихід сухої речовини із одиниці площі. Отримані результати можуть використовуватись господарствами, які займаються вирощуванням силосної кукурудзи та мають можливість використовувати вегетативну масу в якості компоненту біогазових станцій для виробництва біогазу та отримання дигестату.

Ключові слова: біоорганічні добрива; силосна кукурудза; суха речовина; крохмаль; лігнін; структурні вуглеводи; сирий протеїн



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Organic cultivation of carrot in the right-bank Forest-Steppe of Ukraine

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Abstract. The carrot (*Daucus carota* var. *sativus* L.) is one of the most widely grown vegetable crops in the world. It is characterised by prominent taste qualities and a rich chemical composition, which makes it valuable for fresh and processed consumption. Considering the population's need for high-quality, environmentally friendly products, it is vital to investigate the organic cultivation of the carrot. The purpose of this study was to investigate the organic cultivation of carrots in the right-bank Forest-Steppe of Ukraine. The following research methods were used: field, measuring and weighing, mathematical, and statistical. The paper presents findings for 2020-2022, where the

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growth, development, and formation of the crop under organic cultivation technology were investigated. The phenological phases and their duration, biometric parameters of carrot plants, and their yield were studied. The organic technology of growing carrots helped to reduce the interphase periods. The end of the vegetation period of carrots was observed earlier under organic cultivation technology by 2 and 3 days compared to the control variant. The height of the carrot plants was the highest in the Bolivar F₁ hybrid under organic cultivation technology – 37 cm, which is 7 cm more than the control variant. The highest leafiness was observed in the studied variety and hybrid under organic cultivation technology. In the Olympus variety, the increase relative to the control was 3 pcs./plant, in the Bolivar F₁ hybrid – 2 pcs./plant. The regularity of increasing the parameters of carrot roots under organic cultivation technology was noted. The increase in the diameter and length of the root crop under organic cultivation technology was 1.0-2.0 cm and 3 cm, respectively. The largest weight of root crops was under organic cultivation technology, where the increase relative to the control variant was 5 g for the Olympus variety and 7 g for the Bolivar F₁ hybrid. When using organic technology, which involves biological products, the total yield of root crops increased by 3.1 t/ha compared to the control variant

Keywords: organic technology; variety; hybrid; biometric parameters; interphase periods; yield

INTRODUCTION

Organic production has a much lower impact on nature and contributes to its preservation. Modern agricultural production methods require intensification of production to achieve maximum results, which has an adverse environmental impact and, subsequently, affects people who consume low-quality products. I. Bender (2021) notes that organic production is one of the fastest growing sectors of agriculture, as demand for organic products is increasing. Organic agriculture is based on the use of new crop varieties, the introduction of precise and more efficient technologies, crop rotations, and biological products to improve soil fertility. Thus, organic technologies involve the use of renewable resources, preservation of soil, water, environment, and growing plants without artificial and synthetic fertilisers and chemical plant protection products.

According to V. Radojević *et al.* (2021), M. Rajković *et al.* (2021), organic production of carrots can be profitable, but it is essential to consider the risks of this production. Organic farming is a production system that is classified as alternative agriculture. Furthermore, scientists believe that organic production is a system of managing ecological and biological processes to obtain a sufficiently high yield and the possibility of obtaining high-quality products. B. Nasyev *et al.* (2022) believe that organic technology of growing agricultural plants involves the use of biological products. The use of biological products and organic biofertilisers of the latest generation as a biological technology on barley and safflower crops. According to the experimental data, under the biologically based technology in the conditions of the area under study, barley yield increased by 27.02% compared to conventional technologies, and protein yield increased by 0.02 t/ha.

According to S. Pyda *et al.* (2022), microbial preparations contribute to the increase in the number and weight of nodules on bean roots. It was found that plants grown from inoculated seeds had a higher survival rate during the growing season compared to the

control variant by 9.2% when using Rhizobophyte and 13.4% when using Rhizohumine. There was an increase in bean yields by 4.4 c/ha (or 15.0%) (Rhizobophyte) and 6.2 c/ha (or 21.2%) (Rhizobophyte). V. Silva *et al.* (2019) noted that pesticide use underlies agricultural intensification, which has been observed over the past few decades. As a result, soil contamination by pesticide residues has become a growing concern due to the high persistence of some pesticides in the soil and toxicity to non-target species. The reason for this is that pesticides used in plant cultivation can accumulate in the crops and in the soil.

According to J. Trávníček *et al.* (2022), market research suggests that the number of organic products is increasing every year, but their cost is also higher. To produce high-quality, environmentally friendly vegetable products, research is underway to develop and implement organic technologies in open and closed ground and to improve the quality of the products themselves. I. Pariasa and A. Hardana (2023) point out that carrots are one of the main vegetables grown in the world due to respective alternative technologies and are in high demand due to their nutritional and chemical composition. The use of considerable amounts of organic fertilisers leads to intensive growth of the vegetative mass of plants. Organic fertiliser can be beneficial to the yield and quality of carrots if applied appropriately.

P. Atta Poku Snr *et al.* (2020) state that the formation of carrot yields is significantly influenced by soil type and its porosity. When growing carrots, the mechanical composition of the soil is essential, as carrots form branched root crops on heavy, compacted clay soils. Properly prepared soil increases yields and product quality. Studies have shown that fresh manure should be applied under the predecessor, as its direct application leads to a deterioration in the quality of root crops. A. Bjarnadottir (2023) notes that organic farming uses natural methods of growing crops, including vegetables. There is insufficient research in the world

comparing conventional and organic carrot cultivation. According to the results of such technologies, no carotenoids were found, while carrot products obtained by conventional technology contained pesticide residues. The long-term health consequences of consuming low-quality pesticides are unknown, and a range of scientists have expressed their concern.

The variety, sowing scheme, and timing are essential in the technology of growing carrots. Studies found that in the conditions of the Forest-Steppe of Ukraine, it is recommended to sow carrots at an earlier date, which causes long interphase periods in the plants under study. The specified seed sowing time ensures the use of sufficient moisture, friendly germination, higher yields, with excellent product quality, and greater marketability of root crops. The yield of carrots sown in the third decade of March was 41.4–47.1 t/ha. The marketability index for this sowing period was 86.4–88% (Palamarchuk, 2022).

Organic cultivation technology involves the use of the best varieties and hybrids that are adapted to the relevant soil and climatic conditions. Thus, the Brilliant F₁ carrot hybrid provided the largest root crop weight. The yield of the Shantane CL variety was the highest and provided an increase in yield compared to the control variant by 1.6 t/ha. In hybrids, this indicator was highest in the Brilliant F₁ hybrid, which outperformed the control by 6.1 t/ha. The use of mulching materials is also positive, as their application increases the yield of vegetable crops relative to non-mulching options (Vdovenko *et al.*, 2018; Semenov *et al.*, 2019).

Considering the above analysis of literature sources, the purpose of this study was to investigate the benefits of organic carrot cultivation in the right-bank Forest-Steppe of Ukraine.

MATERIALS AND METHODS

Research on organic technology of carrot culture was conducted in the right-bank Forest-Steppe of Ukraine in 2020–2022. The right-bank Forest-Steppe zone is suitable for growing cold-resistant vegetable crops, including carrots. Weather conditions during the years of research were characterised by lower precipitation and higher temperatures compared to the average long-term data for the growing season but were generally favourable for growing carrots. The highest temperature and precipitation were in 2022, at 16.8°C and 345 mm, respectively. The lowest average temperature and precipitation for the growing season was recorded in 2020, at 14.5°C and 294 mm.

According to the guidelines, an experimental design was developed, which included 4 variants. The study involved observing the phases of plant growth and development, measuring biometric parameters, and calculating the yield (Rozhkov *et al.*, 2016). The predecessor of carrots was cucumbers. The technologies for growing carrots were studied with the Olympus variety

and the Bolivar F₁ hybrid. The variants in the experiment were placed using the randomised block method. To record the data, all measurements and calculations were carried out according to the methodology of the experimental case on 10 accounting plants of each variant of the experiment. The experiment included the use of both organic and recommended cultivation technologies with a three-fold replication. The research used the Olympus variety and the Bolivar F₁ hybrid. The control variant was the recommended cultivation technology, which is basic for the Forest-Steppe zone.

The organic technology of carrot cultivation included the use of preparations from the Ukrainian manufacturer BTU-Centre, depending on the phenological development of plants, and was characterised by the following indicators:

Fertigation: Groundfix 5.0 l/ha in combination with Mycohelp preparation 2.0 l/ha;

Phase of 6th–9th true leaves: Phytohelp 1.0 l/ha in combination with preparations Azotophyt 0.3 l/ha and HelpRost 2.0 l/ha; Organic Balance 0.5 l/ha; Actoverm Formula 8.0 l/ha; Lepidocide 7.0 l/ha; Humifriend 0.6 l/ha; Liposam 0.3 l/ha.

Root formation phase: Mycohelp 3.0 l/ha in combination with preparations HelpRost Boron 2.0 l/ha; Azotophyt 0.3 l/ha; Organic Balance 0.5 l/ha; Humifriend 0.6 l/ha; Liposam 0.3 l/ha.

According to the methodological recommendations, the phases of plant growth and development and the duration of the periods between the phases were noted during the study. Specifically, single and mass shoots of plants, the formation of true leaves, the beginning of root growth, and technical maturity were noted. Root mass in dynamics and biometric parameters of plants were measured (Rozhkov *et al.*, 2016).

At the onset of technical ripeness, the carrot yield was calculated based on the current standard (DSTU 7035:2009, 2010). For each variant, the average weight of root crops was determined separately, based on which the average indicator was subsequently calculated separately for the variants. The diameter of the carrot fruit was measured with a caliper and its length with a measuring ruler. The marketability of carrots was defined according to the current standard as the ratio of marketable roots in total weight to non-standard products expressed in %. The coefficient of phenotypic stability of Levis (SF_n) was calculated using the formula, which was determined according to the following formula: $S.F. = X_{max}/X_{min}$, where X_{max} is the highest yield; X_{min} is the lowest yield. The resulting value, if it is close to 1, indicates more stable traits of the variety's productivity (Rozhkov *et al.*, 2016).

The indicators obtained as a result of the research were processed statistically using the method of analysis of variance. Experimental plant studies (both cultivated and wild), including the collection of plant material, were conducted following institutional, national, or

international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS AND DISCUSSION

During the research on carrot plants in the open field, the interphase periods of the plant were determined. Studies showed that varietal characteristics and cultivation technology affect the duration of the interphase periods of carrot plants. The emergence of mass shoots

of carrot plants in all variants was noted on the 8th day after sowing carrots. The emergence of the 1st pair of true leaves was noted earlier than in the recommended cultivation technology – on Day 14, which was 1 day earlier than in the organic technology. Phenological observations of the phases of growth and development of carrot plants revealed that the second pair of true leaves appeared earlier in the Bolivar F₁ hybrid under organic cultivation technology, namely on Day 27, while in the control this phase was noted 1 day later, and in the Olympus variety – 2 days later (Table 1).

Table 1. Duration of the interphase periods of carrots depending on the variety and cultivation technology. Average value for 2020-2022

Variety	Cultivation technologies	Days from sowing seeds					
		emergence of mass shoots	emergence of the 1st pair of leaves	emergence of the 2nd pair of leaves	shedding of the root crop	root crop formation	end of the growing season
Olympus	Recommended (control)	8	14	29	46	64	162
	Organic	8	15	29	43	61	164
Bolivar F ₁	Recommended (control)	8	14	28	41	49	141
	Organic	8	15	27	39	47	144

Source: compiled by the authors

An essential phase in root crops is the shedding of the root crop, which was observed in different periods in the experimental variants. In the early period, this phase was observed in the Bolivar F₁ hybrid – 39 days after the emergence of mass shoots, which is 2 days earlier compared to the recommended technology (control), and 4 and 7 days earlier than in the Olympus variety under organic and recommended technology. Root formation is essential in assessing the growth and development phases of carrot plants. In the experiment, the formation of the product organ was not observed simultaneously due to the technological nature of cultivation. A certain positive effect was found under organic cultivation technology: much earlier root crop formation was observed in the Bolivar F₁ hybrid – on Days 47 and 49, which is 15 and 14 days earlier than in the Olympus variety. Notably, the organic technology of growing carrots helped to accelerate the formation of root crops due to the work of soil microorganisms and the prompt provision of nutrients as a result of foliar feeding and the passage of photosynthesis. Thus,

under this technology, the phase of root crop formation was observed by 2 days in the Bolivar F₁ hybrid and by 3 days in the Olympus variety earlier compared to the control variants.

The end of the growing season of carrots was noted at Days 141-164 days mass shoots. Earlier this interphase period was observed in the Bolivar F₁ hybrid at Days 141 and 144, depending on the cultivation technology used, which is 11 and 10 days earlier compared to the Olympus variety. Notably, this interphase period was influenced by the cultivation technology. Thus, under organic cultivation technology, this phase was observed in the variety and hybrid under study 2 and 3 days earlier than in the control variant. Thus, the variety under study and cultivation technology had an impact on the duration of the interphase periods of carrots. Studies showed that the duration of the phases of growth and development of carrot plants varied depending on the varietal characteristics of the plant and the activity of microorganisms that formed the basis of organic technology (Table 2).

Table 2. Duration of growth and development phases of carrot plants depending on varieties and cultivation technology, days, average for 2020-2022

Cultivar	Cultivation technology	Emergence of sprouts	Emergence of the 1st pair of leaves	Root crop formation	The phase of technical ripeness
Olympus	Recommended (control)	3	3	85	17
	Organic	3	3	83	15
Bolivar F ₁	Recommended (control)	3	3	73	16
	Organic	3	2	70	14

Source: compiled by the authors

As a result of the conducted study, it was found that the emergence of carrot plants lasted 3 days. The emergence of the first pair of leaves was less long compared to other variants of the Bolivar F_1 hybrid and was 2 days under organic cultivation technology. The longest was the phase of root crop formation and was 70-85 days in the variants under study. The rapid formation of the root crop occurred in the Bolivar F_1 hybrid relative to the Olympus variety. This phase was shorter by 12 and 13 days. Under the organic technology of growing the variety Olympus and the Bolivar F_1 hybrid, it was shorter by 2 and 3 days compared to the control variant. The technical ripeness phase lasted 14-17 days. The longest period was observed in the Olympus variety under the recommended technology and amounted to 17 days. In

addition, the shortest period was in the Bolivar F_1 hybrid and was only 14 days.

The cultivation technology used in the open field also had a considerable impact on the biometric parameters of carrots. The unconditional factor was usually the plant's nutritional regime, prompt control of pests, proper soil condition, and favourable climatic conditions. Notably, it was essential to control the beneficial microflora of the soil and to prevent the growth of pathogenic microorganisms, especially the genera *Fusarium*, *Rhizoctonia*, and *Alternaria*. Thus, the height of carrot plants was the highest in the Bolivar F_1 hybrid under organic cultivation – 37 cm, which was 7 cm higher than the control. The same pattern was also observed in the variety Olympus, where the growth relative to the control was 7 cm when using organic technology (Table 3).

Table 3. Biometric parameters of carrot plants depending on varieties and cultivation technology. Average for 2020-2022

Cultivar	Cultivation technology	Height, cm	Number of leaves in the rosette, pcs.	Root diameter, cm	Root length, cm	Root crop weight, g	Length to diameter ratio of the root crop
Olympus	Recommended (control)	29	13	3.5	10	107	2.9
	Organic	36	16	4.5	13	112	2.9
Bolivar F_1	Recommended (control)	30	14	3.5	11	102	3.1
	Organic	37	16	5.5	14	109	2.5

Source: compiled by the authors

The highest leafiness was observed in the variety and hybrid under study under organic cultivation technology. Thus, in the variety Olympus, the growth of leaf mass relative to the control was 3 pcs./plant, in the hybrid Bolivar F_1 – 2 pcs./plant. The regularity of increasing the parameters of root crops in carrots was noted under organic cultivation technology. Comparing the biometric parameters of root crops obtained in the experiment, they were higher in the Bolivar F_1 hybrid. The increase in the diameter of the root crop under organic cultivation technology was 1.0-2.0 cm, respectively. The increase in the length of the root crop was at the level of 3 cm relative to the control variant.

The key research data that affects the yield of root crops is their weight. As a result of the unfavourable microclimate in autumn, especially with uneven precipitation during the root crop formation period, its weight was considerably lower than the technical characteristics of the variety. However, the use of organic cultivation technology resulted in obtaining this indicator at the level of 112 g for the variety Olympus and 109 g for the hybrid Bolivar F_1 , and the increase was 5 g for the variety Olympus and 7 g for the hybrid Bolivar F_1 , compared to the control variant. The use of organic cultivation technology and proper soil maintenance resulted in a decrease in the ratio of root length to diameter only in the Bolivar F_1 hybrid. In this variant, the studied value of the condition

was 2.5 units, which was 0.6 units less than the control. As a result of growing the Olympus variety, the ratio of the length of the root crop to its diameter did not decrease under this growing system but was similar to the control and amounted to 2.9 units.

The analysis of the data obtained on the biometrics of carrot plants showed the advantage of organic cultivation technology. The use of organic cultivation technology increased the height of the carrot plant, which indicates a positive effect of bacteria on the processes of photosynthesis, accumulation of dry matter and root mass. In the open field, in the absence of an irrigation system, Bolivar F_1 plants can form only 14 leaves, while the Bolivar F_1 hybrid can form up to 16 leaves per plant when using organic cultivation technology. The prompt supply of nutrients in organic cultivation helps to increase the diameter and length of root crops.

The yield of carrot roots varied over the years of cultivation and depended on climatic conditions. Thus, 2021 was a more favourable year for carrot cultivation, with an even supply of moisture and nutrients to the plants. However, the analysis of the obtained yield indicator revealed its dependence on the varietal characteristics of carrots and the technology of growing in open ground conditions. The average yield of carrots over the years of cultivation can be assessed as quite high, as it ranged within 44.6-48.6 t/ha (Table 4).

Table 4. Yields of carrot plants depending on the variety and cultivation technology, t/ha

Cultivar	Cultivation technology	Years of experiment			Mean	± to control		Marketability, %	Levis stability coefficient (K_{st})
		2020	2021	2022		t/ha	%		
Olympus	Recommended (control)	43	48	49	46.6	–	–	82	1.1
	Organic	50	51	45	48.6	+1.8	+3	89	1.1
Bolivar F ₁	Recommended (control)	43	46	45	44.6	–	–	84	1.1
	Organic	45	49	49	47.6	+3.1	+7	91	1.0
Least significant difference ₀₅		0.9	1.3	1.5					
(A)		1.5	1.7	1.9					
(B)		2.0	2.0	2.2					
(AB)									

Source: compiled by the authors

This value is quite high for the varieties under study, and the value of root crop yields of 47.6–48.6 t/ha indicates that organic technology fully meets the biological requirements of the plant, and the elements of the technology are adapted to the conditions of Ukraine. The resulting root crops were typical, met the requirements of the standard, were harvested in technical ripeness and were quite suitable for further use. On average, the highest yield of root crops was characterised by the late-ripening carrot variety Olympus, which had a total root yield of 47.6 t/ha. The resulting root crops corresponded to the general characteristics of the variety and were not damaged by pests and diseases. The Bolivar F₁ hybrid was characterised by lower, but also quite high yields, with an average yield of 46.1 t/ha, which was only 3% lower than that of the Olympus variety. Root crops were characterised by typical varietal colour and shape, and fully met the requirements of the standard.

The positive impact of organic cultivation technology on the recommended technology was determined only for the Bolivar F₁ hybrid. Upon using biological products and with a prompt provision of plants with essential nutrients, the total yield of root crops increased and averaged 47.6 t/ha, which substantially exceeded the yield of the control variant by 3.1 t/ha. As a result of the analysis of the values, it was found that the yield of the Bolivar F₁ hybrid can increase by 7% from the use of organic cultivation technology. During the cultivation of the Olympus carrot variety, there was no significant increase in yield from the use of organic cultivation technology. In this variant, the increase in yield was caused exclusively by varietal characteristics and prompt plant care.

The Levis stability coefficient is considered to be an indicator that determines the compliance of the cultivation technology. The closer its value is to 1, the more suitable the cultivation technology is considered to be. In the experiment, the Levis coefficient was at the level of 1.0–1.1, and therefore it should be assumed that the cultivation technologies in Ukraine are optimal for carrots. However, it was found that when growing the Bolivar F₁ hybrid and using organic cultivation technology,

the coefficient was 1.0 and was lower than the control variant. The calculations showed that growing the Olympus variety is profitable, as the Levis coefficient, regardless of technology, was 1.1.

The social role of organic farming is twofold: it provides the market with organic products and, through biological protection, contributes to the preservation of the environment. The production of organic vegetable products on an industrial scale is far superior to conventional cultivation. From an environmental standpoint, organic plant cultivation preserves the natural state of the environment; contributes to the preservation of biodiversity in agricultural landscapes; preserves or restores soil fertility; prevents pollution of various water bodies; and, most importantly, supplies the population with high-quality vegetable products. Unfortunately, most Ukrainian enterprises are not profitable, and the products they produce do not meet international standards, which means that they cannot enter the world market, leading to loss-making farms. Considering the global trends in production, most producers prefer to produce organic products, which contributes to the preservation of the environment (Wierzbowska *et al.*, 2017; Milić & Simin, 2023).

According to I. Bender *et al.* (2020), organic carrot cultivation technology has a range of advantages over conventional technology. The yield of plants and their quality increase, namely lower nitrate content and higher vitamin C content. Thereby, the yield of marketable products increases. A 14.5% higher marketable yield and a 10.0% lower yield of non-standard organic products was obtained compared to the average conventional treatment. Furthermore, organic carrots had 14.1% less nitrates and 10.0% more vitamin C than conventionally grown carrots.

Analysing the study by S. Kalenska *et al.* (2023), it should be noted that the organic technology of carrot cultivation was slightly inferior to the integrated cultivation system in terms of chemical composition of products. Despite this, the marketability index was at the same level for both technologies. A. Wickham and J. Davis (2023) note that the use of cyanobacterial fertilisers in the cultivation of organic

carrots resulted in an increase in the length of root crops and their yield. According to the results of the experiment by M. Kumar *et al.* (2022), it was found that the use of organic fertilizers FYM and vermicompost in combination with biofertilizers PSB with the treatment (T8) FYM (10 t/ha) + VC (2.5 t/ha) + PSB (2.5 kg/ha) gives the highest level of growth, yield, and quality characteristics, including plant height, number of leaves per plant, leaf area per plant, root length, root diameter, yield.

As a result of the application of organic cultivation technology, carrot yields can increase up to 51 t/ha for the Olympus variety, which is somewhat contrary to the data from I. Palamarchuk (2022) for total yields. However, such a value can be achieved with the proper use of bacterial preparations, which are manufactured by the Ukrainian enterprise "BTU-Tsentr". The Bolivar F₁ carrot hybrid can also produce high root crop yields, provided that the recommendations for the use of the Forest-Steppe cultivation technology of the right-bank Ukraine are followed.

The studies conducted did not reveal an increase in harmful elements in root crops, and therefore the opinion of T. Kundilovska *et al.* (2019) fully meets the needs of consumers in obtaining environmentally friendly products and ensures the protection and development of rural areas in the future. In earlier studies, I. Palamarchuk (2022) obtained a carrot yield of 41.4-47.1 t/ha and a marketability of 86.4-88%. As a result of the application of organic technology and control of the main parameters of nutrition and protection against harmful objects through the integrated use of bacterial preparations from "BTU-Tsentr", the yield of carrot roots can increase up to 51 t/ha, which was proved by the research conducted in this paper.

Thus, according to the findings of the conducted study and the data of other scientists, the effectiveness of the use of organic technology for growing carrots was established. The positive impact of organic cultivation technology on the main phases of carrot growth and development, biometric parameters of plants and the formation of overall productivity was revealed.

CONCLUSIONS

The findings showed the impact of organic cultivation technology on the growth, development, and yield of

carrots. According to the study, it was found that the variety under study and organic cultivation technology had an impact on the duration of the interphase periods of carrots. Thus, under this technology, the phase of root crop formation was observed 2 days earlier in the Bolivar F₁ hybrid and 3 days earlier in the Olympus variety compared to the control variant. The end of the vegetation period of carrots was observed earlier under organic cultivation technology by 2 and 3 days earlier than in the control, respectively.

The biometric parameters of carrots were influenced by the growing technology. The height of the carrot plants was the highest in the Bolivar F₁ hybrid under organic cultivation technology – 37 cm, which is 7 cm higher than the control. The highest leafiness was observed in the varieties and hybrids under study under organic cultivation technology. The regularity of increasing the parameters of carrot roots under organic cultivation technology was noted. The increase in the diameter of the root crop under organic cultivation technology was 1.0-2.0 cm, respectively. The increase in root length was 3 cm relative to the control variants. The weight gain of the root crop relative to the control variant was 5 g in the Olympus variety and 7 g in the Bolivar F₁ hybrid.

The positive impact of organic cultivation technology on the recommended technology was determined only for the Bolivar F₁ hybrid. Upon the use of biological products and prompt provision of plants with essential nutrients, the total yield of root crops increases and can average 47.6 t/ha, which is significantly higher than the yield of the control variant by 3.1 t/ha. Considering the value of carrots as a vegetable plant, it is worth exploring other elements of technology in combination with organic cultivation, which will allow for improved cultivation technology, environmentally friendly quality products and increased yields. In the future, it is planned to investigate the impact of biological products on the yield and quality of green vegetable crops in open and closed ground.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Органічне вирощування моркви столової в правобережному Лісостепу України**Сергій Анатолійович Вдовенко**

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Анотація. Морква столова (*Daucus carota* var. *sativus* L.) відноситься до найбільш вирощуваних овочевих культур у світі. Вона характеризується високими смаковими якостями та багатим хімічним складом, а тому є цінною для споживання у свіжому та переробленому вигляді. Враховуючи потребу населення у якісній екологічно чистій продукції важливим є вивчення органічного вирощування моркви столової. Метою дослідження було вивчення органічного вирощування моркви столової в правобережному Лісостепу України. Використовувалися такі методи дослідження: польовий, вимірювально-ваговий, математичний та статистичні. Представлено результати досліджень за 2020-2022 рр., де досліджено ріст, розвиток та формування врожаю за органічної технології вирощування. Вивчено фенологічні фази та їх тривалість, біометричні параметри рослин моркви столової та їх врожайність. Органічна технологія вирощування моркви столової сприяла скороченню міжфазних періодів. Кінець вегетації моркви столової відмічали раніше за органічної технології вирощування на 2 та 3 порівняно з контрольним варіантом. Висота рослин моркви столової найбільшою була у гібриду Болівар F₁ за органічної технології вирощування – 37 см, а це більше контрольного варіанту на 7 см. Найбільшу облиствленість відмічали у досліджуваних сорту та гібриду за органічної технології вирощування. У сорту Олімпус приріст відносно контролю становив – 3 шт./рослину, у гібриду Болівар F₁ – 2 шт./рослину. Відмічено закономірність збільшення параметрів коренеплодів моркви за органічної технології вирощування. Приріст діаметру та довжини коренеплоду за органічної технології вирощування склав 1,0-2,0 см та 3 см відповідно. Найбільша маса коренеплодів була за органічної технології вирощування де приріст відносно контрольного варіант склав по сорту Олімпус – 5 г, а по гібриду Болівар F₁ – 7 г. За використання органічної технології, яка передбачає використання біологічних препаратів загальна урожайність коренеплодів підвищується на 3,1 т/га порівняно з контрольним варіантом

Ключові слова: органічна технологія; сорт; гібрид; біометричні параметри; міжфазні періоди; врожайність

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Ecological safety of sunflower seeds in the conditions of agricultural intensification

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Abstract. Sunflower cultivation using intensive technologies, which manifests itself in the disruption of crop rotation, high rates of mineral fertilisers and pesticides, leads to an increased risk of products contaminated with heavy metals, nitrates, and pesticide residues. The purpose of this study was to identify the risks of toxic substances accumulation in the soil and sunflower seeds. In this study, the atomic absorption spectrophotometric method was used to determine the content of toxic substances in soil and sunflower seeds. The content of alkaline-hydrolysed nitrogen, mobile phosphorus, exchangeable potassium, the reaction of the soil solution pH, as

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well as the content of mobile forms of heavy metals: lead, cadmium, copper, zinc, mercury, cobalt, and molybdenum; boron, and radioactive caesium were determined in the soil after sunflower cultivation. The factual content of soil contaminants was compared with their maximum permissible concentrations. The study investigated the content of heavy metals, namely, lead, cadmium, copper, and zinc, as well as nitrates in sunflower seeds. Their content was compared with the values of maximum permissible concentrations. The coefficients of accumulation of the heavy metals under study by sunflower seeds were calculated. Thus, copper and zinc are most intensively absorbed from the soil and accumulate in sunflower seeds, while lead is the least absorbed. The findings suggest that since no excess of the permissible levels of heavy metals and nitrates in sunflower seeds was found, there is a higher probability of their increased accumulation in sunflower by-products: seed husks, stems, leaves, petioles, and roots. The study results can be used in organic sunflower cultivation

Keywords: heavy metals; nitrates; accumulation; cultivation; soil fertility

INTRODUCTION

The widespread use of sunflower oil in the food industry and its by-products, such as meal and fuselage, in animal feeding requires constant monitoring of the quality and environmental safety of the raw material, sunflower seeds. The absence of mandatory control over the environmental safety of sunflower seeds in Ukraine raises the risk of growing seeds with excessive use of mineral fertilisers and pesticides. And this, according to A. Zimarioieva *et al.* (2021), can cause the accumulation of heavy metals, pesticides, nitrates, and radionuclides in sunflower seeds, which can accumulate in vegetable fats and adversely affect human health and contaminate livestock products.

O. Andriienko *et al.* (2020) proved that the considerable demand for oilseeds in the world substantially affects the dynamics of their acreage. Among all oilseeds, sunflower is the most significant, with its oilseeds having favourable nutritional characteristics. The high selling price of sunflower seeds and their unpretentiousness to growing conditions are driving the growth of sunflower acreage. The economic attractiveness of sunflower cultivation leads to an oversaturation of crop rotations with this crop. V. Mazur *et al.* (2021) and S. Razanov *et al.* (2020) argue that, while it is scientifically sound to return sunflower crops to their previous location after 7 years, they are, in fact, grown in the same place much more often. This leads to a range of agro-ecological issues caused by the impaired resilience of such an agro-ecosystem to the effects of pests, diseases, weeds, and soil overdrying and nutrient depletion. Under such management conditions, the yield of this profitable crop is declining.

I. Didur *et al.* (2020) and A. Melnyk (2018) show that in case of violation of the terms of sunflower return to the previous place in the crop rotation and its cultivation after unfavourable predecessors, the soil is depleted of the main nutrients: alkaline hydrolysed nitrogen, mobile phosphorus, and exchangeable potassium. It also accelerates the acidification of the soil solution, destruction of the soil structure, and increases water erosion. M. Sadak *et al.* (2023) and I. Tkalic *et al.* (2018) note that under such conditions, the sustainability of

the sunflower agroecosystem is also affected. Its crops are becoming increasingly infested with annual late weeds: white quinoa, common ragweed, and especially the quarantine specialised species – sunflower broomrape. Fungal diseases of sunflower, such as white and grey rot, phomopsis, etc., cause irreparable damage, which can lead to a 70% loss of yield.

According to S. Aloud *et al.* (2022) and I. Savchuk *et al.* (2021), maintaining and increasing sunflower seed production under such conditions is possible with the improvement of cultivation techniques, the main of which are increasing rates of mineral fertilisers and increasing the frequency of pesticide application, primarily for the control of weeds, diseases, and pests. Studies by L. Romanchuk *et al.* (2019), D. Knoell and T. Wyatt (2021) have shown that with increasing rates of mineral fertilisation, the fertility and agro-ecological condition of the soil changes, which can affect the quality and environmental safety of the grown seeds. The risk of accumulation of heavy metals, especially lead, cadmium, copper, zinc, and mercury, nitrates and radionuclides in the soil and sunflower seeds is growing.

According to M. Ahmed *et al.* (2021), J. Han *et al.* (2022), the scientifically sound rates of mineral fertiliser application for sunflower crops are $N_{60}P_{60}K_{60}$. However, if the period for returning its crops to the previous place of cultivation is shortened, the factual fertiliser rates can increase by 1.5-2 times. Under such conditions, the volume of low-grade mineral fertilisers produced using imperfect technologies is growing, which increases the risk of toxicity from their use. This, according to B. Shafiq *et al.* (2021), this leads to a deterioration in seed quality and environmental safety. Nitrates, heavy metals (lead, cadmium, zinc, copper, mercury, cobalt), and pesticide residues can be the main environmental factors of seed contamination. Conventionally, sunflower is a crop that can accumulate cadmium in high concentrations, and therefore the issue of quality control and environmental safety of sunflower seeds in intensive farming is becoming increasingly significant.

Therefore, the purpose of this study was to identify the content of toxic substances in the soil at factual soil

fertility levels and to trace the accumulation of toxicants in sunflower seeds and determine their hazard in terms of maximum permissible concentrations.

MATERIALS AND METHODS

Field experiments were conducted in 2021-2022 at the Zorya Vasylivka Farm in the village of Vasylivka, Vinnytsia district, Vinnytsia region (Ukraine). The farm grew sunflowers using intensive technology on grey, podzolised medium loamy soils. The predecessor of sunflower was maize. Fertilization of sunflower crops included the introduction of complex ammonium nitrate phosphate fertiliser under pre-sowing cultivation by spreading in the norm of $N_{90}P_{90}K_{90}$. Mechanical care of the crops included two inter-row mechanical cultivation, which was carried out by a row cultivator KRN-5.6 in combination with a tractor MTZ-82 in the phase of the first pair of true leaves and in the phase of the third pair of true leaves of sunflower. Chemical crop care included the application of the herbicide Oreol-Maxi (active ingredient quizalofop-P-ethyl, 125 g/l) at a rate of 0.8 l/ha with a working solution consumption of 200 l/ha by a tractor sprayer in the phase of two to three leaves of annual cereals and at a height of 10-15 cm of perennial cereal weeds. The study also used the fungicide Amistar Gold 250 SC (active ingredient azoxystrobin, difenoconazole) at a rate of 1.0 l/ha with a working fluid consumption of 200 l/ha against diseases such as *Alternaria*, *Phomopsis*, *Puccinia helianthi*, *Septoria*, *Sclerotinia*, and *Phoma oleracea* during the sunflower budding phase by tractor spraying. Desiccation was performed to stimulate plant drying in the pre-harvest period in the second decade of September. The Roundup Max desiccant (active ingredient potassium salt of glyphosate) was used at a rate of 2.4 l/ha in the waxy ripeness phase of seeds with a tractor sprayer using a working fluid of 200 l/ha. Sunflower was threshed by direct combining at the stage of full seed maturity with a John Deere combine.

Therefore, the first step in the study was to determine the parameters of contamination of soil and sunflower seeds, the main type of agricultural product, with toxic substances. This helped to establish the probability of toxicants accumulating in sunflower seeds used for food or technical purposes. The second step of this study was to determine the level of accumulation of toxicants in sunflower by-products: seed husks, stems, leaves, petioles, capitulum pulp, and other parts of the plant. Soil samples were taken before harvesting at ten points along the diagonal of the field to the

depth of the topsoil (25 cm). Laboratory soil analysis was conducted in a certified and accredited laboratory of the Zhytomyr branch of the State Institution "DerzhHruntOkhorona" of the Institute of Soil Protection of Ukraine, using standard methods (DSTU ISO 10381-1:2004, 2006). The following soil fertility indicators were determined: humus content, alkaline-hydrolysed nitrogen, mobile phosphorus, exchangeable potassium, and soil solution pH. The content of toxic substances in the soil was also investigated: mobile forms of heavy metals: lead, cadmium, copper, zinc, mercury, iron, cobalt, molybdenum, as well as boron and radioactive caesium according to atomic absorption spectrophotometric method (DSTU ISO 10381-1:2004, 2006). The determined indicators of soil toxicants were compared with their maximum permissible concentrations (MPC).

Sunflower seeds were sampled from one batch using a grain probe. The seeds were analysed for heavy metals: lead, cadmium, copper, and zinc, as well as nitrates. Laboratory studies of sunflower seeds were conducted at the Zhytomyr branch of the State Institution "DerzhHruntOkhorona" (n.d.) of the Institute of Soil Protection of Ukraine according to the atomic absorption spectrophotometric method (DSTU 4117:2007, 2007). The obtained values were compared with the standards of maximum permissible concentrations of these toxicants in sunflower seeds. The coefficient of accumulation of heavy metals by sunflower seeds was calculated as the ratio of their determined content in seeds to the content of mobile forms of these substances in the soil. Experimental studies of plants (both cultivated and wild), including the collection of plant material, were following the institutional, national, or international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS AND DISCUSSION

With the intensification of farming, sunflower, as one of the most profitable crops, is growing in terms of mineral fertiliser application rates, which can primarily affect the agrochemical composition of the soil. Thus, the humus content in the experimental plot during sunflower cultivation was 1.70 ± 0.03 (%), which corresponds to a "low" value on a six-point scale. With the intensification of farming and an emphasis on fertilising the soil with mineral fertilisers, there will be a further decline in humus content, and thus a reduction in macronutrients readily available to plants in the soil (Table 1).

Table 1. Agrochemical composition of soil under sunflower cultivation, $M \pm m$

Agrochemical parameters	Value	Content
Humus, %	1.70 ± 0.03	Low content
Alkaline hydrolysed nitrogen, mg/kg	107.3 ± 2.4	Low content
Mobile phosphorus, mg/kg	100.0 ± 1.8	Average content

Table 1. Continued

Agrochemical parameters	Value	Content
Exchangeable potassium, mg/kg	158.0±2.5	High pH of
the soil solution, units	5.37±0.1	Subacidic reaction

Source: compiled by the authors

The supply of alkaline hydrolysed nitrogen to the soil was low and amounted to (107.3±2.4) mg/kg. Alkaline hydrolysed nitrogen in the soil is an indicator of potentially available nitrogen for plants. This form of nitrogen does not factor in nitrate nitrogen, which is extremely mobile in the soil and changes very frequently, especially when applying nitrogen-containing fertilisers, after heavy rainfall or during intensive plant growth. The content of alkaline hydrolysed nitrogen in the soil is stabilised in autumn or early spring before fertilisation, especially with nitrogen-containing fertilisers. Low humus content in the soil will result in a decrease in the content of alkaline hydrolysed nitrogen, and therefore in intensive farming, the reserves of this form of nitrogen in the soil are replenished by applying high rates of nitrogen mineral fertilisers, which briefly contributes to increase the content of alkaline hydrolysed nitrogen in the soil, and then decrease when plants use its reserves for their growth and development. However, together with the mineral nitrogen from fertilisers, soils become excessively acidic, which reduces the effectiveness of all types of mineral fertilisers and increases the mobility of toxic substances that can be present in the soil and accumulate in crop products. It is nitrogen fertilisers that have the most substantial impact on sunflower seed yields, which is why these fertilisers are a priority for growing the crop.

The availability of mobile phosphorus in the soil of the experimental plot was average with its content of (100.0±1.8) mg/kg. The rapid transition of phosphorus minerals that entered the soil with mineral fertilisers to unavailable compounds, as well as the limited absorption of mineral phosphorus by plant roots, leads to the accumulation of phosphorus compounds in soils, an increase in their content, soil overphosphating, and a decrease in the effectiveness of phosphate fertilisers. Since intensive farming uses recommended rates of mineral phosphorus and does not consider its factual content in the soil, this can lead not only to overphosphating the soil, but also to the accumulation of increased concentrations of heavy metals, which phosphate mineral fertilisers are rich in.

The content of exchangeable potassium in the soil was (158.0±2.5) mg/kg, which corresponded to a high

content. This form of potassium is readily available to plants. Compared to nitrogen and phosphorus, sunflower crops consume potassium the most, but this element does not have a decisive impact on the yield of sunflower seeds, and therefore moderate rates of mineral potassium are applied to sunflower crops. It is the sufficient content of exchangeable potassium and mobile phosphorus in the soil that decelerates the movement of toxic substances in the soil, such as heavy metals and radionuclides. The reaction of the soil solution pH of the area where sunflower was grown corresponded to a subacidic value with (5.37±0.1) pH. This indicator is formed depending on the nature of the soil type and is also substantially affected by the application of nitrogen fertilisers, which are predominantly acidic. Soil acidity increases especially with the intensification of farming. Acidic soils increase the vertical migration of toxicants in soils and their transfer to plants.

The large and powerful above-ground mass of sunflower plants can substantially dry out the soil and deplete it of nutrients. Toxic substances such as heavy metals, radionuclides, etc., are absorbed from the soil along with the main macroelements. Among the heavy metals, the most dangerous toxicants are lead, cadmium, zinc, mercury, and copper. At the same time, this group includes iron, cobalt, and molybdenum. The main sources of heavy metals in the soil can be mineral fertilisers, especially in high application rates, which is typical for intensive farming, as well as emissions from industrial enterprises and vehicles.

The factual content of mobile forms of lead in the sunflower soil was (1.55±0.1) mg/kg. With a maximum permissible concentration of lead in the soil of 6.0 mg/kg, its content was 0.3 MPC and was safe. The content of mobile forms of cadmium was also below the maximum permissible concentration of 0.14 MPC. A comparable dependence was observed for other toxicants that occurred in the soil during sunflower cultivation, specifically, the content of copper was 0.25 MPC, zinc – 0.15 MPC, mercury – 0.002 MPC, cobalt – 0.2 MPC, molybdenum – 0.01 MPC, boron – 0.05 MPC, and radioactive caesium – 0.3 MPC, which can enter the soil from the earth's interior, as a result of the Chernobyl disaster, as well as with mineral fertilisers (Table 2).

Table 2. The content of mobile forms of toxicants in the soil during sunflower cultivation, M±m

Toxicant	Factual content	Maximum permissible concentration	Content in relation to MPC
Lead, mg/kg	1.55±0.1	6.0	smaller
Cadmium, mg/kg	0.10±0.03	0.7	smaller
Copper, mg/kg	0.75±0.02	3.0	smaller
Zinc, mg/kg	3.35±0.4	23.0	smaller

Table 2. Continued

Toxicant	Factual content	Maximum permissible concentration	Content in relation to MPC
Mercury, mg/kg	0.005±0.001	2.1	smaller
Iron, mg/kg	57.20±2.0	–	–
Cobalt, mg/kg	1.00±0.1	5.0	smaller
Molybdenum, mg/kg	0.10±0.3	10	smaller
Boron, mg/kg	1.64±0.3	30	smaller
Caesium, Ci/km ²	0.30±0.04	1.0	smaller

Source: compiled by the authors of this study based on personal findings

If there are residues of toxicants in the soil during sunflower cultivation, the probability of their accumulation in the seeds increases.

The actual content of lead in sunflower seeds was below the maximum permissible concentration and amounted to 0.18 MPC, cadmium – 0.10 MPC,

copper – 0.13 MPC, zinc – 0.12 MPC, nitrates – 0.11 MPC (Table 3).

The coefficients of toxicant accumulation in sunflower seeds were calculated. Specifically, the coefficient of accumulation of lead by sunflower seeds was 0.06, cadmium – 0.1, copper – 1.7, zinc – 1.8 (Fig. 1).

Table 3. Toxicant content in sunflower seeds, $M \pm m$

Toxicant	Factual content	Maximum permissible concentration	Content in relation to MPC
Lead, mg/kg	0.09±0.02	0.5	smaller
Cadmium, mg/kg	0.010±0.02	0.1	smaller
Copper, mg/kg	1.27±0.35	10.0	smaller
Zinc, mg/kg	6.12±1.10	50.0	smaller
Nitrates, mg/kg	32.5±2.60	300.0	smaller

Source: compiled by the authors

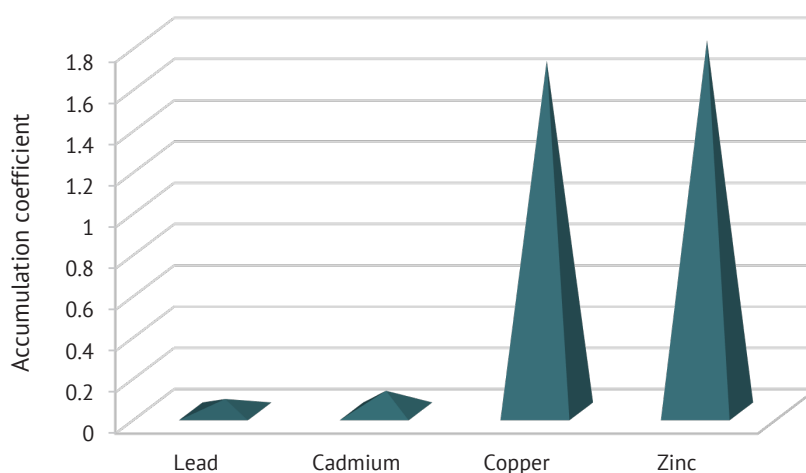


Figure 1. Coefficient of heavy metal accumulation in sunflower seeds

Source: compiled by the authors

S. Kalenska et al. (2020), G. Cecoli et al. (2022) agree that if crop rotation is observed during sunflower cultivation, the period of return to the previous place of cultivation and the recommended rates of mineral fertilisers are applied, the environmental safety of the sunflower seeds produced will be satisfactory. However, in case of violation of these indicators, the risk of accumulation of toxic substances in sunflower seeds will increase. Today, in Ukraine, under conditions of intensified farming, sunflower cultivation is characterised by a considerable disruption of crop rotations and a reduction in the time required to return to the previous

place. According to V. Almashova and S. Skok (2022), the greatest danger is posed by heavy metals: lead, cadmium, copper, zinc, which can be contained in mineral fertilisers and are applied at increased rates, especially phosphorus-potassium fertilisers, the production process of which involves the use of raw materials and production components containing toxic heavy metals.

According to A. Melnyk et al. (2020), there are various methods for cleaning contaminated soils. One of the most promising and inexpensive of these methods is the use of phytoremediation, which is lower in cost, compatible with natural processes and requires fewer

resources than other cleaning methods. Sunflower can be an effective phytoremediation for toxic contamination, as it is known for its intensive absorption of both nutrients and toxic substances from the soil. According to O. Bielashov *et al.* (2022), the use of phytoremediation properties of sunflower should be balanced, since the absorption of pollutants by sunflower plants, their accumulation and entry into the food chain can cause various human diseases.

Y. Domaratskyi (2021) found that with an increase in the concentration of toxic substances in the soil, their accumulation in sunflower plants increases, and the toxicity effect increases, which is manifested in a decrease in the efficiency of nutrient absorption from the soil. Laboratory experiments conducted by O. Degani *et al.* (2022) with sunflower plants grown on samples of soil contaminated with heavy metals and on toxicant-free soils showed that the concentration of heavy metals in sunflower plants grown on contaminated soils was several times higher. Yu. Cheng *et al.* (2023) found that the concentration of heavy metals in the roots and leaves of sunflower plants increased substantially. This dependence is confirmed by the present study, which did not reveal high concentrations of heavy metals in sunflower seeds. Therefore, it is probable that all toxicants accumulate in the leaf and stem mass of sunflower plants.

According to M. Hejna *et al.* (2019), a physiological feature of sunflower plants is the intensive accumulation of cadmium in their seeds in high concentrations. Cadmium replaces calcium in the soil, contributing to the acidification of the soil solution. Therefore, control over this element should be mandatory. However, the present study did not find its high content in sunflower seeds, which may be caused by the low content of mobile forms of cadmium in the soil. I. Tkalič *et al.* (2018) found that the intensive uptake of nutrients from the soil by the sunflower root system leads to a strong depletion of nutrients from the soil. Together with them, plants intensively absorb toxic elements contained in the soil. The findings of the present study confirm this thesis only partially, as it found intensive absorption of copper and zinc from the soil, and much less of lead and cadmium.

I. Didur *et al.* (2020) note that under such conditions, there is a risk of not only a substantial decrease in the content of nutrients and humus in the soil when growing sunflower, but also a low content of toxic substances, specifically heavy metals, which entered the soil with mineral fertilisers. This is the reason why laboratory analysis of the soil after sunflower cultivation often fails to detect elevated concentrations of heavy metals. This is confirmed by the present study, as it did not find any excess of mobile forms of heavy metals in the soil during sunflower cultivation.

As noted by H. Pinkovsky (2019), N. Novytska *et al.* (2020), all heavy metals that migrated from the soil accumulate in sunflower plants. According to this

manifestation, sunflower plants, as those capable of absorbing and retaining toxic substances in increased concentrations through the formation of a powerful vegetative mass, are phytoremediants, i.e., plants that can cleanse the soil of excess toxic substances (heavy metals, pesticide residues, radionuclides), accumulating them in their tissues in increased concentrations. This is fully confirmed by the findings of the present study.

There were also differences in the uptake of nitrate by sunflower plants. According to D. Hilwa *et al.* (2019), with a sufficient content of nitrate nitrogen in the soil, it is intensively absorbed by sunflower plants and accumulates in their tissues, preventing the supply of other forms of nitrogen to sunflower plants. Research by O. Tymchyshyn *et al.* (2021) shows that these processes are also directly affected by the reaction of the soil solution pH. According to M. Ghaffari *et al.* (2021), nitrate nitrogen plays a much greater role in the growth and development of sunflower plants than ammonium nitrogen. However, this factor contributes to the accumulation of nitrates in the tissues of sunflower plants. According to E. Shahini *et al.* (2022), A. Sher *et al.* (2022), nitrate accumulates most in the petioles of sunflower leaves compared to other parts of the leaves.

Thus, research has shown a low level of soil fertility after sunflower cultivation. This is explained by the strong depletion of nutrients from the soil by this crop. At the same time, current studies have not revealed any excess of mobile forms of heavy metals in the soil. They were also not found in significant amounts in sunflower seeds. This may indicate that this culture has remedial properties.

CONCLUSIONS

Therefore, the study established that for growing sunflowers using intensive technology, which involved the application of complex ammonium nitrate phosphate fertiliser in the norm of $N_{90}P_{90}K_{90}$ and triple application of pesticides (herbicide, fungicide, and desiccant) during the growing season on grey podzolized medium loamy soils with low content of humus (1.7%) and alkaline hydrolysed nitrogen (107.3 mg/kg), medium content of mobile phosphorus (100.0 mg/kg) and high content of exchangeable potassium (158.0 mg/kg), as well as subacidic reaction of the soil solution (5.37 pH) at the end of its growing season, the content of the main toxic substances in the soil was much lower than their maximum permissible concentrations (MPC): lead – 0.3 MPC, cadmium – 0.1 MPC, copper – 0.3 MPC, zinc – 0.1 MPC, mercury – 0.002 MPC, cobalt – 0.2 MPC, molybdenum – 0.01 MPC, boron – 0.05 MPC, caesium – 0.3 MPC.

In addition, no exceedance of the maximum permissible concentrations of heavy metals (lead – 0.2 MPC, cadmium – 0.1 MPC, copper – 0.1 MPC, and zinc – 0.1 MPC) and nitrates (0.1 MPC) was detected in sunflower seeds. The calculated coefficient of accumulation of heavy metals by sunflower seeds showed

that a significant level of transfer of toxicants from soil to seeds was observed for copper, which is 1.7 times higher than its content in the soil, and for zinc, which is 1.8 times higher than its content in the soil. At the same time, the level of transfer of lead and cadmium from the soil to sunflower seeds is much lower than their content in the soil and was 0.06 and 0.1 less than in the soil, respectively. According to the research objective, no accumulation of toxic substances (heavy metals, radionuclides, nitrates) in the soil and its seeds was observed on grey podzolised medium loamy soils with average fertility indicators during sunflower cultivation. This may indicate the powerful phytoremediation

properties of sunflower plants with the simultaneous production of environmentally safe seeds in terms of heavy metals and nitrates. Proceeding from the data obtained, further research should be conducted to determine the concentrations of toxic substances in sunflower by-products: seed husks, stems, leaves, petioles, and roots.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Екологічна безпечність насіння соняшнику в умовах інтенсифікації землеробства

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Анотація. Вирощування соняшнику за інтенсивних технологій, що проявляється у порушенні чергування культури у сівозміні, внесенні високих норм мінеральних добрив і пестицидів, призводить до зростання ризику одержання продукції забрудненої важкими металами, нітратами, залишками пестицидів. Метою роботи було визначити ризики накопичення токсичних речовин у ґрунті та насінні соняшнику. В роботі використовували атомно-адсорбційний спектрофотометричний метод для визначення вмісту токсичних речовин у ґрунті та насінні соняшнику. Визначено у ґрунті після вирощування соняшнику вміст азоту лужногідролізованого, фосфору рухомого, калію обмінного, реакцію ґрунтового розчину рН, а також вміст рухомих форм важких металів: свинцю, кадмію, міді, цинку, ртуті, кобальту та молібдену; бору, а також радіоактивного цезію. Фактичний вміст забрудників ґрунту був порівняний із їх гранично-допустимими концентраціями. Досліджено вміст важких металів: свинцю, кадмію, міді та цинку, а також нітратів у насінні соняшнику. Проведено порівняння їх вмісту із значеннями гранично-допустимих концентрацій. Розраховано коефіцієнти накопичення досліджуваних важких металів насінням соняшнику. Таким чином з ґрунту найбільш інтенсивно поглинаються і акумулюються у насінні соняшнику мідь та цинк, а найменше – свинець. Проведені дослідження дозволили зробити висновок, що оскільки не виявлено перевищення допустимих рівнів вмісту важких металів і нітратів насінні соняшнику, то зростає ймовірність їх підвищеного накопичення у побічній продукції соняшнику: лушпинні насіння, стеблах, листках, черешках та коренях. Результати досліджень можна використовувати при органічному вирощуванні соняшнику

Ключові слова: важкі метали; нітрати; накопичення; вирощування; родючість ґрунту



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Heritable variability in winter wheat at the interaction of genotype with factors of high genetic activity

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Abstract. Identification of new opportunities in the use of certain ecogenetic factors for the genetic improvement of winter wheat is a vital component of improving existing cereal agroecosystems within the framework of the food security strategy. The purpose of this study was to demonstrate the potential of substances with prominent genetic activity in inducing potentially beneficial changes and to establish the possibility of obtaining optimised schemes for obtaining new forms and more controlled trait variability. Mutations were detected by visual observation and biometric and biochemical analysis from the second generation to the sixth, with the inheritance of the detected traits in subsequent generations being checked. The study presented the possibilities of the mutation process for certain genetic and breeding-value forms, the specific features of induction of such traits, which makes it possible to increase the predictability of obtaining the necessary mutant lines and partially controlled high variability in certain traits. It was found that the most promising for use is the complex application of moderate doses of sodium azide and dimethyl sulphate as ecogenetic factors. It was shown that in combination with three varieties among the ones under study (Kalanča, Polyanka, Pochayna), it is possible to create highly efficient genotype-mutagenic systems with increased yield of valuable forms. The increase in mutagenic depression using more harmful substances can be substantially offset by an increase in the proportion of beneficial changes. The negative side is their complex nature. The nature of the ecogenetic factor is no less significant than the concentration of the

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mutagen or the genotype of the original form, which is sometimes overlooked. It was confirmed that the use of these factors is quite promising in terms of obtaining small biochemical changes (micromutations). Several promising mutant lines were identified that have a comprehensive improvement in grain yield and quality without additional negative changes. The findings of this study can be used in breeding programmes to create new varieties of winter wheat

Keywords: mutations; chemical mutagenesis; supermutagens; yield; grain quality

INTRODUCTION

Changes in the environment require agricultural crops to adapt. The relevance of genetic improvement methods, such as experimental mutagenesis, is to create more resistant and adapted plant varieties that can survive in the face of climate change. Chemical mutagens have shown particular effectiveness in this regard. The effect of chemical mutagenesis on cultivated plants can cause mutations in their genome (Lal *et al.*, 2020), which can lead to heritable changes. According to J. Chaudhary *et al.* (2019), these mutations may be random and not always beneficial, but in some cases, they can lead to beneficial changes. The mutation process is random, and most mutations will not be beneficial or may be harmful to the plant. Therefore, after mutagenesis, the resulting plants are selected and tested to identify those with desirable properties, such as increased yields, disease resistance (Bezie *et al.*, 2020) or other useful characteristics. Thus, W. Yali and T. Mitiku (2022) found that the effect of chemical mutagenesis on cultivated plants can change their development and heredity, but the results will depend on the particular mutations that occurred and the characteristics they affected.

Chemical supermutagens with prominent damage capacity are one of the most popular factors for creating new mutant forms, especially with complex changes. They are used not only for genetic improvement of cultivated plants but also for research on the effect of mutagens on plant organisms in general to identify genetic control of traits. M. Spencer-Lopes *et al.* (2018) found that most of the successful production of complex economically valuable forms of major crops is conditioned by the effect of chemical mutagenesis, which, given the level of relevant biological research, is almost widely used to enhance the crossing components of parental forms, to investigate genetic systems for individual valuable key traits, to obtain fundamentally new biochemical compositions of traits (Kartseva *et al.*, 2023) in combination with other positive changes.

The site-specificity of chemical factors determines their increased affinity for certain structural features of cultivated plant genotypes, which can lead to a substantial increase in the yield of valuable forms. The researcher can identify and exploit these site-specific interactions, which can improve the efficiency of the breeding process by several orders of magnitude. G. Abaza *et al.* (2020) found that two key components must be considered: the variety as a subject for mutagenic action and the nature

of the chemical mutagenic factor. For factors of another nature, such variants are not possible. The resulting systems can generate 60-80% more moulds with positive changes. This approach requires a large volume of experiments with numerous variants of chemical supermutagens and concentrations, a large set of different varieties, preferably considering the ecological and geographical diversity (Ram *et al.*, 2019; Abdel-Hamed *et al.*, 2021) in origin. Changes caused by prominent concentrations of mutagens can be difficult to predict and control (Ahumada-Flores *et al.*, 2021). Different plants can react differently to the same mutagen, and this can complicate the breeding process.

The results of global statistics may vary depending on the plant species, particular mutagen, research conditions, and other factors. Therefore, it is vital to conduct research and consider all aspects before using high concentrations and highly damaging mutagens in genetic improvement. A more careful and controlled approach may be safer and more effective in achieving the desired results in crop plant breeding (Cann *et al.*, 2022). Winter soft wheat is a crucial crop in Ukraine and other countries with comparable climatic conditions. It is essential for the production of bread and other food products and is the main source of nutrition for millions of people. The problem of global warming and climate change (Sala & Herbei, 2023) may affect the cultivation of this crop. One of the major issues is the increase in winter temperatures, which can cause disruptions in plant development and a substantial correction in the timing of certain phases. As a result, yields may decrease.

To overcome these challenges, new genotypes of winter wheat are being developed based on either forms of different ecological and geographical origin or the creation of new forms from local material (Nazarenko *et al.*, 2021). The development of more efficient crop improvement methods could also be useful for ensuring sustainable winter wheat production under climate change (Jalal *et al.*, 2021). Managing climate change and adapting the agricultural sector to new conditions is a vital task for ensuring food security.

The purpose of this study was to identify the specific features of heritable variability of winter wheat varieties based on the frequency and range of positive changes arising from the interaction between the nature of the factor and the characteristics of the genotype.

MATERIALS AND METHODS

The field experiments were conducted in the experimental fields of the Educational and Research Centre of Dnipro State Agrarian and Economic University (48°51'10"N, 35°25'31"E) in 2018-2022 (second to sixth generation of mutants). The experimental plots have a homogeneous cover consisting of ordinary low-humus, leached, medium loamy chernozem on loam. The experimental field is located in the Dnipro district of the Dnipro region of Ukraine, which belongs to the Northern subzone of the Steppe with insufficient moisture. Its climatic resources are characterised by the following indicators: hydrothermal coefficient >0.9, rare precipitation during the growing season – 250-280 mm, annual precipitation – 450-490 mm, and the sum of temperatures during the period with temperatures above 10°C – about 2,900°C.

Winter wheat seeds (1,000 grains per each concentration and water) of eight varieties: Balaton (Germany), Borovytsia, Zeleny Gai, Zoloto Ukrainy, Kalancha, Niva Odeska, Polyanka, Pochayna (Ukrainian selection) were treated with EMS (ethyl methane sulphonate) 0.025%, 0.05%, 0.1%; SA (sodium azide) 0.01%, 0.025%, 0.05%, 0.1%; DMS (dimethyl sulphate) 0.0125%, 0.025%, 0.05% (Sigma-Aldrich, Germany). The exposure to chemical supermutagen treatment was 24 hours following the generally accepted protocols. These concentrations are trivial for mutagens (chemical supermutagens) of this group. Controls were soaked in water.

In the second and third generations, possible mutations were examined visually and by manual reseeded by families (1-2-row plots, row spacing – 0.15 m, row spacing length – 1.5 m), and the inheritance of the newly identified traits was studied. The mutation frequency was calculated as the ratio of mutation cases to the total number of families investigated in the variant in percentage terms. Mutant lines were analysed to measure yield and quality in the fourth to sixth generation. The area of the plots was 5-10 m², depending on the year of the experiment and generation, repetition was 1-2 times, standard (original variety and national variety Podolianka for determining productive forms) – every 20 numbers. The protein content of wheat grain was measured using an Infratec 1241 (FOSS, Denmark), and the content of glutenins and gliadins was measured by liquid chromatography using an RP-HPLS (INRA, France). The content of trace elements was determined using an Agilent 5110 inductively coupled plasma atomic emission spectrometer (Agilent, Germany) with light intensity based on wavelength characteristics. A multi-element test solution (Mg, Mn, Zn, Mo, Co, Cu) was used as Agilent standards.

Statistical analysis of data was performed according to ANOVA, the data was grouped and evaluated using discriminant and cluster analysis (Euclidean distance, single link) (Statistica 10.0, multivariate statistics module, TIBCO, Palo Alto, USA). The normality of the

data distribution was verified using the Shapiro-Wilk W-test. The differences between the individual variants were assessed using the Tukey test (pairwise comparison). The study of the plant material obtained by the treatment took place in two stages. During the first one, the second and third generations were used to identify changes in the original forms and to detect their inheritance by visual and biometric analysis. The resulting lines were again tested for heritability and their yield parameters were determined in a trial during the fourth to sixth generation. In parallel, small, hidden micromutations were analysed for biochemical components (protein, gluten, certain components of wheat grain reserve proteins significant for the baking industry, and the presence of some valuable trace elements). A total of 48,800 families were investigated for the three substances, of which 4,171 mutant lines were identified.

The following types of changes can be used as positive mutations: thick stem, short stem, semi-dwarfism, dwarfism, intensive wax coating (associated with high drought tolerance, in the variety model for the Northern Steppe), coarse grain, long ear, large ear, early maturity, disease tolerance (evaluation and selection for these traits started in generations M₂-M₃), productivity, ability to tillering (preliminary evaluation and selection in generations M₂-M₃, environmental testing in generations M₄-M₆), increased protein content, positive changes in protein components (content of high and low molecular weight glutenins and gliadins), positive changes in the content of trace elements (Mg, Mn, Zn, Mo, Co, Cu) (evaluation and selection in environmental testing of generations M₄-M₆).

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

RESULTS AND DISCUSSION

Generally, all varieties belong to stable genotypes and the level of spontaneous variability is low. The results of the first mutagen, EMS, are presented in Table 1. A total of 15,800 families were investigated for controls and variants, including 1,043 mutant lines in total. The number of useful changes (possible complex changes, two or more per line) was 267, including negative aspects (additional changes) of 159 lines, of which 68 forms were finally tested as promising forms, which were of great interest for direct use but mainly as donors of traits (genetically valuable). The number of families in each treatment was 500, except for Balaton, EMS 0.1%, and Niva Odeska, EMS 0.1%, for other genotypes and concentrations, the depressant effect did not affect the amount of material for evaluation.

Table 1. Frequency of positive mutations in the fourth to sixth generation, EMS, ($\bar{x} \pm SD$, $n=400-500$)

Variant	Frequency of positive changes, %	Share of total frequency	Number of lines, pcs.	Number of promising lines, pcs.	Frequency of promising lines, %
Balaton	0.20±0.04 ^a	0.50	1	0	0.00±0.00 ^a
Balaton, EMS 0.025%	1.40±0.11 ^b	0.21	5	3	0.60±0.07 ^b
Balaton, EMS 0.05%	2.20±0.17 ^c	0.24	6	2	0.40±0.06 ^c
Balaton, EMS 0.1%	3.25 ±0.20 ^d	0.22	7	2	0.50±0.09 ^{bc}
Zoloto Ukrainy	0.40±0.08 ^a	0.33	1	0	0.00±0.00 ^a
Zoloto Ukrainy, EMS 0.025%	2.00±0.17 ^b	0.26	6	3	0.60±0.07 ^b
Zoloto Ukrainy, EMS 0.05%	3.40±0.22 ^c	0.31	11	4	0.80±0.09 ^b
Zoloto Ukrainy, EMS 0.1%	1.80±0.16 ^b	0.13	6	3	0.60±0.07 ^b
Zeleny Gai	0.20±0.04 ^a	0.33	1	0	0.00±0.00 ^a
Zeleny Gai, EMS 0.025%	2.40±0.18 ^b	0.44	7	3	0.60±0.06 ^b
Zeleny Gai, EMS 0.05%	2.60±0.19 ^b	0.36	6	3	0.60±0.06 ^b
Zeleny Gai, EMS 0.1%	2.00±0.17 ^b	0.21	5	2	0.40±0.04 ^c
Niva Odeska	0.00±0.00 ^a	0.00	0	0	0.00±0.00 ^a
Niva Odeska, EMS 0.025%	1.80±0.13 ^b	0.26	7	3	0.60±0.08 ^b
Niva Odeska, EMS 0.05%	2.40±0.18 ^c	0.25	6	3	0.60±0.08 ^b
Niva Odeska, EMS 0.1%	3.50±0.29 ^d	0.22	8	3	0.75±0.11 ^b
Borovytsia	0.20±0.06 ^a	0.25	0	0	0.00±0.00 ^a
Borovytsia, EMS 0.025%	1.20±0.11 ^b	0.21	5	3	0.60±0.07 ^b
Borovytsia, EMS 0.05%	2.60±0.16 ^b	0.35	8	4	0.80±0.11 ^c
Borovytsia, EMS 0.1%	3.00±0.21 ^b	0.29	6	2	0.40±0.06 ^{ab}
Kalancha	0.40±0.01 ^a	0.40	1	0	0.00±0.00 ^a
Kalancha, EMS 0.025%	1.40±0.12 ^b	0.23	5	3	0.60±0.07 ^b
Kalancha, EMS 0.05%	2.80±0.19 ^c	0.34	9	4	0.80±0.09 ^b
Kalancha, EMS 0.1%	3.00±0.27 ^c	0.23	8	3	0.60±0.07 ^b
Polyanka	0.20±0.04 ^a	0.50	1	0	0.00±0.00 ^a
Polyanka, EMS 0.025%	1.60±0.10 ^b	0.35	6	3	0.60±0.07 ^b
Polyanka, EMS 0.05%	1.60±0.11 ^b	0.24	5	2	0.40±0.05 ^b
Polyanka, EMS 0.1%	2.20±0.16 ^c	0.25	6	3	0.60±0.07 ^b
Pochayna	0.20±0.04 ^a	0.50	1	0	0.00±0.00 ^a
Pochayna, EMS 0.025%	1.40±0.10 ^b	0.29	5	3	0.60±0.07 ^b
Pochayna, EMS 0.05%	1.60±0.11 ^b	0.25	5	2	0.40±0.05 ^b
Pochayna, EMS 0.1%	1.80±0.12 ^{bc}	0.20	5	2	0.40±0.05 ^b

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

Source: compiled by the authors

Only when compared to the standard is the number of beneficial changes in all varieties statistically significantly higher. Only in Balaton and Niva Odeska varieties a significant increase in the frequency of beneficial changes was observed with each increase in EMS concentration, in Zoloto Ukrainy the frequency first increased and then decreased at the third concentration to the level of the first one ($F=2.26$; $F_{0.05}=4.99$; $P=0.08$). In Borovytsia and Zeleny Gai varieties, the variability under the influence of all concentrations was at the same level, in Kalancha there was no significant difference under the influence of the second and third concentrations ($F=4.06$; $F_{0.05}=4.99$; $P=0.06$); for the Polyanka genotype – the first and second ($F=3.22$; $F_{0.05}=4.99$; $P=0.07$); for Pochayna – the third differed from the first ($F=7.11$;

$F_{0.05}=4.99$; $P=0.02$), but did not differ from the second ($F=4.01$; $F_{0.05}=4.99$; $P=0.06$), which did not have a significant difference between them ($F=3.55$; $F_{0.05}=4.99$; $P=0.07$). As for the general characteristic of the parameter, the variability for the mutagen concentration was generally significant ($F=17.23$, $F_{0.05}=3.86$, $P=0.001$), and the difference in the response for varieties was also significant ($F=18.17$, $F_{0.05}=4.11$, $P=0.001$). As for the genotype-mutagenic interaction, it was significant only for Borovytsia and Niva Odeska ($F=8.33$; $F_{0.05}=3.10$; $P=0.01$).

The proportion of useful changes in the total frequency reached 0.2-0.4 for different variants but was generally slightly higher for the first to third concentration. No statistically significant relationships were found. The same applies to the number of valuable

lines, except for the genotypes Kalancha, Polyanka, and Pochayna. The large number of additional negative changes and their complex nature led to a slight decrease in lines at the highest concentration, despite the higher frequency of useful forms in general (mainly semi-dwarfs and dwarfs).

The last parameter, the frequency of selected promising lines as a percentage of the total amount of material, is quite important. The indicator is much less variable than the frequency of valuable forms in general, but it can be said that half of the varieties (Zoloto Ukrainy, Borovytsia, Kalancha, Polyanka) have more or less the same level of the trait at any concentration, but a significant difference with the control. For Pochayna and Zeleny Gai, there was a slight gradual decrease, Niva Odeska showed an increase, while Balaton was unstable. In any case, the effect of different concentrations was significant ($F=5.01$, $F_{0.05}=3.86$, $P=0.04$), the difference by genotype was not ($F=3.98$, $F_{0.05}=4.11$, $P=0.06$), as was the genotype-mutagenic interaction ($F=2.03$, $F_{0.05}=3.10$, $P=0.07$). Thus, it was difficult to find promising compositions among the available varieties

under the influence of EMS to create a system with an increased yield of useful forms.

Under the action of sodium azide, considering the greater variety of concentrations (which is mainly due to the wide range of agents in inducing changes and substantially less general knowledge of the ecogenetic factor), the largest amount of source material of 19,400 families in the second and third generations was investigated, of which 1,692 mutant lines were obtained and 301 lines were selected as potentially useful, 178 forms were proposed for further genetic improvement as donors of valuable traits and direct use (Table 2). The number of families in the variants for higher concentrations was reduced to 400, except for the more resistant Pochayna and Polyanka varieties. Thus, the negative effects had a greater impact on the viability of the samples than the previous factor. The same range of beneficial changes was observed for the mutagen, but the variability for most traits was substantially higher, primarily in the induction of shorter stemmed forms ($F=21.17$; $F_{0.05}=3.05$; $P=1.94 \cdot 10^{-5}$) and positive biochemical changes ($F=52.63$; $F_{0.05}=3.01$; $P=3.47 \cdot 10^{-8}$).

Table 2. Frequency of positive mutations in the fourth to sixth generation, SA, ($\bar{x} \pm SD$, $n=400-500$)

Variant	Frequency of positive changes, %	Share of total frequency	Number of lines, pcs.	Number of promising lines, pcs.	Frequency of promising lines, %
Balaton	0.20±0.03 ^a	0.50	0	0	0.00±0.00 ^a
Balaton, SA 0.01%	2.00±0.21 ^b	0.29	7	5	1.00±0.17 ^b
Balaton, SA 0.025%	3.20±0.25 ^c	0.33	11	7	1.40±0.25 ^b
Balaton, SA 0.05%	3.60±0.28 ^c	0.26	12	7	1.40±0.26 ^b
Balaton, SA 0.1%	3.50±0.28 ^c	0.17	6	3	0.75±0.12 ^{bc}
Zoloto Ukrainy	0.40±0.08 ^a	0.33	1	0	0.00±0.00 ^a
Zoloto Ukrainy, SA 0.01%	2.20±0.21 ^b	0.35	6	5	1.00±0.12 ^b
Zoloto Ukrainy, SA 0.025%	2.60±0.22 ^b	0.33	9	6	1.20±0.15 ^b
Zoloto Ukrainy, SA 0.05%	4.00±0.29 ^c	0.31	11	7	1.40±0.16 ^{bc}
Zoloto Ukrainy, SA 0.1%	4.25±0.34 ^c	0.22	12	5	1.25±0.15 ^b
Zeleny Gai	0.40±0.08 ^a	0.67	0	0	0.00±0.00 ^a
Zeleny Gai, SA 0.01%	1.80±0.17 ^b	0.33	5	4	0.80±0.11 ^b
Zeleny Gai, SA 0.025%	2.60±0.21 ^c	0.32	9	5	1.00±0.12 ^b
Zeleny Gai, SA 0.05%	3.00±0.25 ^c	0.26	9	5	1.00±0.12 ^b
Zeleny Gai, SA 0.1%	4.00±0.30 ^d	0.21	10	5	1.25±0.15 ^{bc}
Niva Odeska	0.00±0.11 ^a	0.00	0	0	0.00±0.00 ^a
Niva Odeska, SA 0.01%	2.20±0.25 ^b	0.28	7	4	0.80±0.11 ^b
Niva Odeska, SA 0.025%	3.00±0.29 ^c	0.28	9	5	1.80±0.21 ^c
Niva Odeska, SA 0.05%	4.60±0.35 ^d	0.32	12	7	1.40±0.16 ^d
Niva Odeska, SA 0.1%	4.00±0.30 ^d	0.20	10	6	1.50±0.17 ^{dc}
Borovytsia	0.20±0.03 ^a	0.25	0	0	0.00±0.00 ^a
Borovytsia, SA 0.01%	2.20±0.21 ^b	0.38	7	4	0.80±0.11 ^b
Borovytsia, SA 0.025%	2.60±0.22 ^b	0.33	8	5	1.00±0.12 ^b
Borovytsia, SA 0.05%	3.60±0.31 ^c	0.30	11	6	1.20±0.14 ^{bc}
Borovytsia, SA 0.1%	3.50±0.30 ^c	0.18	9	5	1.25±0.14 ^{bc}
Kalancha	0.40±0.07 ^a	0.40	1	0	0.00±0.00 ^a
Kalancha, SA 0.01%	3.60±0.28 ^b	0.58	9	6	1.20±0.14 ^b

Table 2. Continued

Variant	Frequency of positive changes, %	Share of total frequency	Number of lines, pcs.	Number of promising lines, pcs.	Frequency of promising lines, %
Kalancho, SA 0.025%	3.40±0.31 ^b	0.44	11	5	1.00±0.11 ^b
Kalancho, SA 0.05%	4.40±0.34 ^c	0.34	11	7	1.40±0.16 ^{bc}
Kalancho, SA 0.1%	5.50±0.36 ^d	0.26	14	8	2.00±0.19 ^d
Polyanka	0.00±0.00 ^a	0.00	0	0	0.00±0.00 ^a
Polyanka, SA 0.01%	2.60±0.23 ^b	0.59	6	5	1.00±0.11 ^b
Polyanka, SA 0.025%	4.00±0.32 ^c	0.61	11	7	1.40±0.15 ^c
Polyanka, SA 0.05%	2.80±0.25 ^b	0.26	9	5	1.00±0.11 ^b
Polyanka, SA 0.1%	3.40±0.27 ^d	0.27	10	5	1.00±0.11 ^b
Pochayna	0.00±0.00 ^a	0.00	0	0	0.00±0.00 ^a
Pochayna, SA 0.01%	2.00±0.21 ^b	0.43	6	5	1.00±0.11 ^b
Pochayna, SA 0.025%	3.00±0.26 ^c	0.44	11	6	1.20±0.14 ^b
Pochayna, SA 0.05%	3.20±0.27 ^d	0.31	11	6	1.20±0.14 ^b
Pochayna, SA 0.1%	4.00±0.32 ^e	0.33	10	7	1.40±0.16 ^{bc}

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

Source: compiled by the authors

The index of frequency of variability in the spectrum of useful changes shows that for all variants the difference with the control was statistically significant, and the variability by genotype ($F=18.21$; $F_{0.05}=3.55$; $P=3.56 \cdot 10^{-4}$) and increasing concentration was significant ($F=34.82$; $F_{0.05}=3.11$; $P=4.17 \cdot 10^{-7}$). The genotype-mutagen interaction was significant ($F=24.17$; $F_{0.05}=3.01$; $P=5.52 \cdot 10^{-6}$). Only in Pochayna did the frequency increase statistically significantly with each concentration. In the varieties Zoloto Ukrainy ($F=4.82$, $F_{0.05}=5.17$, $P=0.06$), Kalancho ($F=4.44$, $F_{0.05}=5.17$, $P=0.07$), and Borovytsia ($F=4.76$, $F_{0.05}=5.17$, $P=0.06$) there was no difference between the effect of the first and second concentrations. There was no difference between the second and third concentration in Balaton ($F=3.11$, $F_{0.05}=5.17$, $P=0.09$) and Zeleny Gai ($F=3.82$, $F_{0.05}=5.17$, $P=0.08$), in the Polyanka variety there was a difference ($F=7.92$; $F_{0.05}=5.17$; $P=0.02$), but the frequency decreased before the first concentration ($F=4.12$; $F_{0.05}=5.17$; $P=0.08$). In terms of the activity of the third and highest concentration, there was no difference in Balaton ($F=4.82$; $F_{0.05}=5.05$; $P=0.07$), Zoloto Ukrainy ($F=4.22$; $F_{0.05}=5.05$; $P=0.07$), Niva Odeska ($F=3.81$; $F_{0.05}=5.05$; $P=0.08$), and Borovytsia ($F=3.42$; $F_{0.05}=5.05$; $P=0.09$), while in all other varieties it increased significantly. Thus, in this case, the use of an SA concentration of 0.1% is justified for most cases.

In the total frequency, the proportion of valuable changes varied from 0.2 to 0.6, with a gradual decrease with increasing concentration for most genotypes upon reaching higher concentrations, with a possible gradual increase of SA within 0.01-0.05%. No significant trends were observed in the number of promising lines, except for the increase in complex changes at higher concentrations. According to the analysis of the frequency of selected lines in any variant, there was a difference

with the control, only in the varieties Niva Odeska and Kalancho there was some significant variability, which was expressed in a significant increase under the influence of higher concentrations. Generally, the frequency gradually increased moderately, or was not lower than the previous one, except for Balaton and Zoloto Ukrainy, where it decreased at 0.1% SA. For all varieties, the genotype-mutagen interaction was positive for Balaton ($F=9.17$; $F_{0.05}=4.22$; $P=0.002$), Kalancho ($F=9.98$; $F_{0.05}=4.22$; $P=0.001$), Polyanka ($F=10.10$; $F_{0.05}=4.22$; $P=0.001$), and Pochayna varieties ($F=13.16$; $F_{0.05}=4.22$; $P=4.13 \cdot 10^{-5}$). The effect of sodium azide as a mutagen differed significantly from EMS in that it has a much higher genetic activity both in terms of the frequency of individual changes in traits and the total number of genetically and breeding-value changes.

The effect of DMS led to a substantial decrease in the amount of plant material in the first generation, specifically, at a concentration of 0.05%, the number of families in some cases (Balaton, Niva Odeska) was reduced to 200. In total, 13,600 families in the second and third generation were studied as source material, from which 1,436 mutant lines were obtained (very high genetic activity of the factor influenced, and therefore with a substantial reduction in the amount of material, variability in some cases (mainly negative) exceeded the effect of SA, in some cases (mainly negative) exceeded the effect of SA and was much higher than the effect of EMS) and 226 lines were selected as potentially useful, 89 forms were proposed for further genetic improvement as donors of valuable traits and direct use (Table 2). The presence of numerous sterile forms and undesirable variability in ear shape had an impact. On the other hand, the ecogenetic factor was effective in mutations in stem structure and disease resistance and often induced rare types of mutations.

Table 3. Frequency of positive mutations in the fourth to sixth generation, DMS, ($\bar{x} \pm SD$, $n=200-500$)

Variant	Frequency of positive changes, %	Share of total frequency	Number of lines, pcs.	Number of promising lines, pcs.	Frequency of promising lines, %
Balaton	0.20±0.05 ^a	0.50	0	0	0.00±0.00 ^a
Balaton, DMS 0.0125%	4.40±0.38 ^b	0.42	12	5	1.00±0.12 ^b
Balaton, DMS 0.025%	5.97±0.45 ^c	0.27	11	4	1.33±0.14 ^c
Balaton, DMS 0.05%	5.75±0.43 ^c	0.27	5	2	1.00±0.12 ^b
Zoloto Ukrainy	0.40±0.10 ^a	0.33	0	0	0.00±0.00 ^a
Zoloto Ukrainy, DMS 0.0125%	3.60±0.31 ^b	0.39	11	4	0.80±0.10 ^b
Zoloto Ukrainy, DMS 0.025%	4.50±0.39 ^c	0.23	12	3	1.00±0.12 ^b
Zoloto Ukrainy, DMS 0.05%	3.20±0.27 ^b	0.17	5	1	0.29±0.06 ^c
Zeleny Gai	0.40±0.10 ^a	0.67	0	0	0.00±0.00 ^a
Zeleny Gai, DMS 0.0125%	3.80±0.33 ^b	0.37	7	5	1.00±0.13 ^b
Zeleny Gai, DMS 0.025%	4.55±0.41 ^b	0.26	9	4	1.00±0.12 ^b
Zeleny Gai, DMS 0.05%	4.00±0.36 ^b	0.19	6	2	0.67±0.07 ^c
Niva Odeska	0.00±0.16 ^a	0.00	0	0	0.00±0.00 ^a
Niva Odeska, DMS 0.0125%	4.40±0.40 ^b	0.43	11	4	0.80±0.21 ^b
Niva Odeska, DMS 0.025%	5.67±0.49 ^c	0.28	10	4	1.33±0.23 ^b
Niva Odeska, DMS 0.05%	4.00±0.43 ^b	0.23	9	3	1.50±0.24 ^{bc}
Borovytsia	0.60±0.08 ^a	0.75	1	1	0.20±0.09 ^a
Borovytsia, DMS 0.0125%	4.60±0.34 ^b	0.48	12	4	0.80±0.17 ^b
Borovytsia, DMS 0.025%	4.90±0.38 ^b	0.29	13	5	1.25±0.22 ^b
Borovytsia, DMS 0.05%	3.47±0.36 ^c	0.19	6	1	0.29±0.11 ^a
Kalancha	0.20±0.04 ^a	0.20	0	0	0.00±0.00 ^a
Kalancha, DMS 0.0125%	4.40±0.38 ^b	0.47	12	5	1.00±0.17 ^b
Kalancha, DMS 0.025%	6.60±0.43 ^c	0.31	13	6	1.50±0.21 ^b
Kalancha, DMS 0.05%	8.50±0.43 ^d	0.34	11	3	1.00±0.17 ^b
Polyanka	1.00±0.11 ^a	0.50	1	1	0.20±0.00 ^a
Polyanka, DMS 0.0125%	3.20±0.29 ^b	0.40	11	5	1.00±0.11 ^b
Polyanka, DMS 0.025%	3.97±0.31 ^c	0.31	11	6	1.33±0.14 ^b
Polanka, DMS 0.05%	4.60±0.40 ^c	0.26	9	3	0.75±0.09 ^{bc}
Pochayna	0.00±0.00 ^a	0.00	0	0	0.00±0.00 ^a
Pochayna, DMS 0.0125%	2.60±0.26 ^b	0.31	7	4	0.80±0.10 ^b
Pochayna, DMS 0.025%	3.13±0.30 ^b	0.24	6	3	0.60±0.08 ^b
Pochayna, DMS 0.05%	2.60±0.26 ^b	0.14	5	1	0.60±0.18 ^b

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

Source: compiled by the authors

In terms of the frequency of beneficial changes, it was statistically significantly different from the control at all concentrations. The variability of concentrations in the effect by variety was much lower, mainly due to the rapid increase in negative mutations at higher concentrations. Thus, Zeleny Gai and Pochayna varieties had no variability in concentration. Among the other varieties, the Borovytsia genotype ($F=3.17$, $F_{0.05}=4.99$, $P=0.08$) had no significant difference between the first and second concentrations. In Balaton ($F=5.06$; $F_{0.05}=5.15$; $P=0.06$) and Polyanka varieties ($F=4.76$; $F_{0.05}=5.15$; $P=0.06$), there was no difference in mutagen activity under the influence of the second and

third concentrations; there was a difference in Zoloto Ukrainy ($F=6.76$; $F_{0.05}=5.15$; $P=0.02$) and Niva Odeska ($F=7.11$; $F_{0.05}=5.15$; $P=0.008$), but the frequency of beneficial changes falls to the level of the first concentration ($F=4.16$; $F_{0.05}=5.15$; $P=0.07$ and $F=4.92$; $F_{0.05}=5.15$; $P=0.06$). Again, only in the Kalancha variety did each subsequent concentration lead to a significant increase in the number of beneficial changes. The index of frequency of variability in the spectrum of useful changes showed that for all variants the difference with the control was statistically significant, and the variability according to genotype ($F=19.77$; $F_{0.05}=3.65$; $P=2.26 \cdot 10^{-5}$) and increasing concentration was significant ($F=14.82$;

$F_{0.05}=3.22$; $P=5.09 \cdot 10^{-5}$). The genotype-mutagen interaction was also significant ($F=5.17$; $F_{0.05}=3.11$; $P=0.02$).

The proportion of valuable changes in the total mutation frequency was 0.2-0.5, which was slightly lower than the SA, but generally quite high. No significant statistical patterns were found, except for a predominantly gradual decrease in the share of valuable changes in most varieties with increasing concentration and a relatively high level at 0.0125% DMS. Many additional negative changes resulted in a rather low number of forms selected for testing, which was significantly inferior to the effect of the previous mutagen and more comparable to EMS.

In terms of the frequency of such lines, it was found that this parameter is more variable than in the SA. Thus, there was no variability with the change in concentration

in the Niva Odeska, Kalancha, Polyanka, and Pochayna varieties, and it dropped to the level of the control under the influence of 0.05% DMS in Borovytsia. Balaton, Zoloto Ukrainy, and Zeleny Gai varieties had a gradual increase in the moderate range with a further decrease for higher concentrations. The genotype-mutagenic interaction was significant in a positive sense for the varieties Niva Odeska ($F=7.08$, $F_{0.05}=4.30$, $P=0.007$), Kalancha ($F=6.90$, $F_{0.05}=4.30$, $P=0.008$), and Polyanka ($F=5.07$, $F_{0.05}=4.30$, $P=0.02$). According to the effect of different mutagens by nature, a cluster analysis was performed to consider the site-specific response of all genotypes depending on the group of substances and each substance separately (notably, there was no significant difference in the grouping by EMS and DMS, as further demonstrated by the discriminant analysis (Fig. 1).

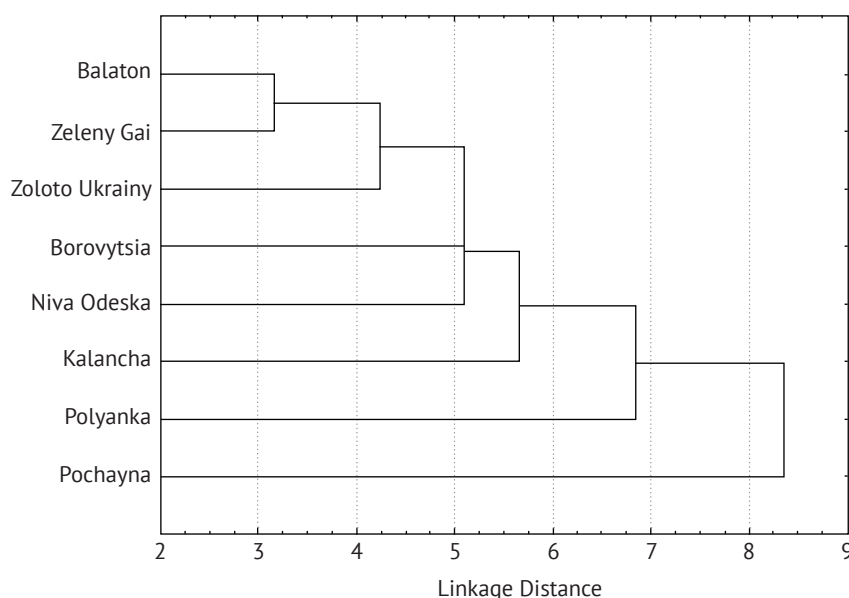


Figure 1. Results of cluster analysis by frequency of positive changes according to genotype

Source: compiled by the authors

In total, four groups were found, three of which were minor. The first group included Balaton, Zeleny Gai, Zoloto Ukrainy, Borovytsia, and Niva Odeska varieties, for which the genotype-mutagenic interaction was not factually significant despite the preliminary results of factor analysis for individual substances. The study found that the use of these genotypes to create a high-performance mutagen-initial form system is not possible for substances with high damaging properties. This does not mean that the use of this group will not lead to the emergence of promising forms, but the effectiveness of this process will be relatively mediocre. In the second group, one variety, Kalancha, showed a specific response to EMS and SA; due to the specific features of the genotype structure, it can be used as a component for enhancing mutational activity. The third group included the Polyanka variety, the fourth –

the Pochayna variety, which showed increased affinity for the action of SA. Considering the specific features of this factor, one should expect a relatively sizeable number of forms with biochemical and physiological microchanges.

The findings of the discriminant analysis performed according to mutagen (Fig. 2) suggested that EMS and DMS were actually more equally effective in terms of their effect on the entire group of genotypes. The effect of SA was the most variable and, clearly, SA as a mutagen has a more site-specific nature of action than other factors and is more dependent on the range of the used starting material on the particular subject of action. That is, systems with this factor are more likely to be selected and should be given further attention for the selection of new initial forms. A more thorough screening would also be warranted.

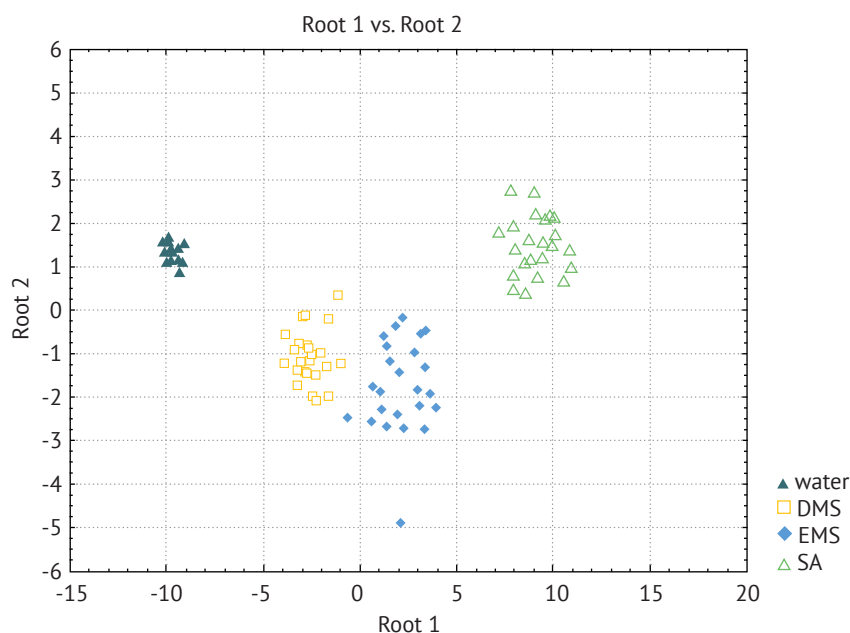


Figure 2. Identification of individual mutagenic factors according to genotype-mutagenic interaction

Source: compiled by the authors

In the spectrum of the obtained promising changes, the discriminant analysis identified signs that reliably show significant variability under the influence of certain substances (Table 4). An analysis was also carried out according to the individual characteristics of the mutation process. Thus, it was found that SA is more potent as a mutagen in terms of the range of induced traits (almost all traits except thick stem and yield), followed by DMS, which was not significantly effective in inducing mutations for intense waxy coat, large grain, large ear, high-yielding bushy forms, and higher protein content in wheat grain. The least successful in terms of the spectrum was the effect on the presented varietal set of EMS, which significantly affected the mutation frequencies for short stemmedness, induction of semi-dwarfs, forms with long ear, with positive changes in the content of trace elements. Analysing the traits, the

forms with a thick stem (resistant to lodging) should use only DMS, forms with intensive wax coating (potentially resistant to drought) and with large grain and ear – SA, bushy forms and those with a higher protein content – also SA. Thus, considering the higher efficiency of DMS in inducing dwarfs and disease-resistant forms, the use of SA and DMS is effective for this set of genotypes. The use of EMS is not advisable. Therewith, the emergence of yielding forms is not a model and cannot be predicted from the data obtained. Therefore, the data obtained need to be supplemented by expanding the variety of source forms.

Based on the results of a three-year small-plot environmental trial, the following mutant lines were obtained that were the most promising in terms of stable high yields (Table 5), and their characteristics in terms of grain quality are presented in Table 6.

Table 4. Identification of variability-significant features

Feature in the model	Wilks Lambda λ	Mutagen in the model	$F_{0.05}$	P-level
Thick stem	0.234	DMS	3.91	0.06
Short-stemmed	0.077	EMS, SA, DMS	18.12	0.001
Semi-dwarfs	0.089	EMS, SA, DMS	9.36	0.001
Dwarfs	0.157	SA, DMS	6.14	0.005
Intensive wax coating	0.212	SA	3.77	0.076
Large grain	0.213	SA	3.58	0.079
Long ear	0.084	EMS, SA, DMS	10.14	0.001
Large ear	0.286	SA	2.14	0.099
Early ripening	0.093	SA, DMS	8.04	0.004
Disease resistance	0.092	SA, DMS	7.77	0.003
Productive	0.229	absent	2.77	0.087

Table 4. Continued

Feature in the model	Wilks Lambda λ	Mutagen in the model	F _{0.05}	P-level
Bushy	0.214	SA	4.80	0.046
Higher protein content	0.230	SA	4.43	0.039
Positive changes in protein components	0.104	SA, DMS	8.67	0.003
Positive changes in the content of trace elements	0.103	EMS, SA, DMS	8.98	0.003

Source: compiled by the authors

Table 5. Yield characteristics of the obtained mutant lines
(Dnipro State Agrarian and Economic University, 2021-2023), t·ha⁻¹

Variant	Origin	2021	2022	2023	Average
1	Podolianka, st.	5.38±0.09 ^a	5.56±0.08 ^a	6.22±0.12 ^a	5.72±0.10 ^a
26	Balaton, SA 0.025%	6.93±0.17 ^b	7.12±0.19 ^b	6.98±0.17 ^b	7.01±0.16 ^b
45	Zoloto Ukrainy, SA 0.05%	5.67±0.12 ^c	6.05±0.13 ^c	6.17±0.13 ^a	5.96±0.10 ^c
234	Zeleny Gai, EMS 0.05%	6.45±0.14 ^d	6.57±0.12 ^d	6.24±0.15 ^a	6.42±0.14 ^d
256	Niva Odeska, DMS 0.0125%	6.11±0.12 ^e	6.18±0.10 ^c	6.26±0.13 ^a	6.18±0.11 ^c
278	Borovytsia, EMS 0.025%	5.98±0.14 ^e	6.55±0.10 ^d	5.99±0.11 ^a	6.17±0.11 ^c
334	Kalancha, DMS 0.0125%	6.78±0.15 ^{bd}	6.99±0.11 ^b	6.34±0.12 ^a	6.70±0.12 ^{bd}
356	Kalancha, SA 0.05%	6.43±0.11 ^d	7.02±0.15 ^b	7.00±0.15 ^b	6.82±0.14 ^{bd}
391	Polyanka, SA 0.025%	6.24±0.11 ^{ed}	6.54±0.12 ^d	6.43±0.13 ^{ac}	6.40±0.12 ^d
412	Polyanka, DMS 0.0125%	6.79±0.14 ^{bd}	7.03±0.16 ^b	6.74±0.14 ^b	6.85±0.14 ^{bd}
423	Pochayna, SA 0.025%	7.01±0.15 ^b	7.12±0.16 ^b	7.07±0.14 ^b	7.07±0.15 ^b
446	Pochayna, SA 0.05%	6.77±0.10 ^{bd}	6.91±0.10 ^b	6.84±0.12 ^b	6.84±0.10 ^{bd}

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

Source: compiled by the authors

Among the productive lines, all the original forms can be found in the origin, but the varieties Balaton, Zoloto Ukrainy, Zeleny Gai, Niva Odeska, and Borovytsia are represented by only one line each. The varieties Kalancha, Polyanka, and Pochayna each produced two lines. The use of SA in moderate concentrations yielded 6 lines, DMS – 3 lines, and EMS – 2 lines. The use of high concentrations that were at or above the LD₅₀ and RD₅₀ in the first generation did not produce any form for direct use (many genetically valuable forms, primarily dwarfs, semi-dwarfs, early maturing, and disease-resistant).

A total of 11 lines were identified for yielding qualities; the years of testing did not differ significantly in terms of soil and climatic conditions, but 2023 was the best year in terms of standard yields. Thus, in 2021, the productive lines were factually divided into 6 groups: the first was the Podolianka standard, the second line 45, which exceeded the standard but was inferior to other lines, the third lines 256 and 278, which exceeded the first and second groups but were inferior to others, the fourth line 234, 356, which exceeded the previous three groups, the fifth lines 26 and 423, which were record-breaking in 2021, and the intermediate position was occupied by the minor groups 334 and 412 (in terms of yield between the record-breaking and fourth

groups) and line 391 (in terms of yield between the third and fourth groups). Thus, lines 26, 334, 412, 423, mainly varieties with high genotype-mutagenic interaction, deserve more attention.

In 2022, the diversity was substantially lower, there were no transitional groups, and there was a clear differentiation into four groups, the first being the standard, the second lines 45, 256, which exceeded the standard but were inferior to other lines, the third 234, 278, 391, which exceeded the standard and the second group, and the fourth with the highest productivity lines 26, 356, 412, 423, 446. In 2023, the diversity was the lowest, with only three groups: the first standard and lines 45, 234, 256, 278, 334; the second transitional line 391, which did not differ from some lines in the first group but differed from others and the standard; and the third lines 26, 356, 412, 423, 446, which significantly exceeded the first and second groups. According to the results of three years, five groups were found, of which the cluster of lines 26, 423 ($F=7.99$; $F_{0.05}=3.89$; $P=0.01$) was the most productive and the transitional 334, 356, 412, 446 ($F=7.14$; $F_{0.05}=3.71$; $P=0.01$) were also interesting.

In terms of the technological qualities, 278 ($F=5.09$; $F_{0.05}=4.01$; $P=0.03$), 234 ($F=5.69$; $F_{0.05}=4.01$; $P=0.02$), 446

($F=7.29$; $F_{0.05}=4.01$; $P=0.008$), 234 ($F=5.00$; $F_{0.05}=4.01$; $P=0.03$), 391 ($F=7.99$; $F_{0.05}=5.98$; $P=0.01$) were negatively affected, all the rest formed sufficient quality for

strong wheat. The traits are quite low in variation, unlike the following (content of reserve protein components) (Table 6).

Table 6. Technological qualities of wheat grain (Dnipro State Agrarian and Economic University, 2022)

Variety/line	Protein, %	Gluten, %	Glutenins		Gliadins
			HMW	LMW	
Podolianka	13.98 ^a	25.18 ^a	0.15999 ^a	0.46346 ^a	0.4574 ^a
26	14.07 ^a	25.99 ^a	0.17423 ^b	0.48435 ^b	0.4545 ^a
45	14.02 ^a	26.03 ^a	0.15245 ^a	0.48477 ^b	0.4553 ^a
234	13.51 ^b	22.19 ^b	0.16313 ^a	0.46569 ^a	0.4435 ^b
256	13.78 ^{ab}	24.56 ^a	0.15356 ^a	0.46223 ^a	0.4101 ^c
278	14.27 ^c	27.74 ^c	0.16405 ^a	0.42100 ^c	0.4124 ^c
334	14.27 ^c	26.97 ^{ac}	0.17341 ^b	0.45404 ^a	0.4557 ^a
356	14.01 ^a	25.78 ^a	0.18513 ^b	0.45313 ^a	0.4416 ^b
391	13.53 ^b	22.23 ^b	0.18022 ^b	0.40719 ^d	0.4512 ^a
412	14.02 ^a	26.10 ^a	0.19403 ^c	0.46300 ^a	0.4548 ^a
423	14.15 ^{ac}	26.78 ^c	0.18478 ^b	0.41999 ^d	0.4618 ^a
446	14.52 ^d	28.17 ^d	0.19357 ^c	0.40976 ^d	0.4639 ^{ad}

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

Source: compiled by the authors

For durum wheat, a high content of high-molecular weight glutenins, a lower content of low molecular weight glutenins, and a high content of gliadins are desirable. According to the first variant, lines 26, 334, 356, 391, 412, 423, 446 had the best results. The low-molecular-weight glutenin content was lower in mutant forms 278, 391, 423, 446, and negative in 26 and 45. The gliadin content was higher only in line 446, and lower negative in lines 234, 256, 278, and 356. This combines high productivity and the best quality of lines 26 (negative for high low molecular weight glutenin), 423, 334, 356, 412, and separately, form 446 (combining high yield and best quality in all parameters).

Thus, for the material studied, it is advisable to use mainly SA in concentrations of 0.025-0.05% and DMS in concentrations of 0.0125% in the complex as mutagens, and while the effectiveness of SA has already been discussed, DMS is substantially understudied. EMS as a mutagen is limited in its effectiveness both in terms of characteristics and material yield. It is possible to obtain forms with a high damaging effect under the influence of moderate concentrations of mutagens that combine improvements in a complex of economically valuable traits. Based on the eight varieties studied and three mutagens at 10 concentrations, six systems with increased yields of valuable forms and an increase in efficiency of up to half of the baseline have already been identified, which indicates the prospects of searching in this area.

The analysis of the data obtained so far (M. Nazarenko *et al.*, 2022) showed a decrease in site

specificity with an increase in the activity and damage capacity of the mutagen. However, when using DMS and SA (as opposed to EMS), the trend was quite different (OlaOlorun *et al.*, 2021), and the response of a particular source material to the action was significant, as the pairwise comparison of genotypes suggests, which can be partially reproduced in the number of traits with a significant effect of a single factor, indicating the contrast of individual genotypes (Kalancho, Pochayna, Pol-yanka) and the possibility of creating high-performance systems based on them. The described regularity of the mutation process allows using this type of variability to obtain new material with the required potential in a more manageable, reliable, and predictable manner, which was previously considered impossible. Mutations in research have proven to be a reliable and constant source for local genetic resources, as indicated by previous studies by A. Anter (2021), for traits such as long grained ear, early maturity, with a set of valuable biochemical changes and disease resistance.

Previously obtained data by M. Hussain *et al.* (2021) show that the use of SA and DMS as mutagenic factors is advisable primarily to obtain forms for use as components (Mangi *et al.*, 2021) for further improvement of existing forms through recombinant breeding. It is less likely to create forms that can be used directly as commercial varieties in the future. This variant again stays outside the forecast (Ergün *et al.*, 2023a), despite a fairly large sample size. To increase the reliability of the forecast, as previously pointed out by M. Hassine *et al.* (2023), it is necessary to expand the set of genotypes used in the

study substantially. First of all, SA and DMS (additionally as mutagenic factors show a more significant dependence on the specific features of genotype-mutagenic interaction, i.e., they depend on the DNA structure of the source material of the site variety, as was mentioned earlier by H. Shimelis *et al.* (2019), with a clear differentiation from genetically determined responsiveness to mutagenic effects.

The regularities established during the study also demonstrate that a fairly significant number of traits that are definitely economically valuable are highly variable under the influence of the factors under study (Ergün *et al.*, 2023b) (with the exception of productive mutations). This once again indicates that in the case of chemical supermutagens, a great deal depends on a well-chosen starting material, depending on the particular factor. As the concentration of mutagenic substances increases, the risk of negative and positive changes in the complex continues to increase substantially (Shabani *et al.*, 2022). The number of such cases is quite considerable even for moderate concentrations, which is why up to half of all promising mutant forms obtained are ignored. This is a general trend (le Roux *et al.*, 2021). Varieties that demonstrate high variability both in the first generation in terms of the effects of mutagenic depression and in the second or third generation in terms of the frequency and spectrum of heritable changes are not always more suitable for use, but this trend is not absolute. In our research, two of the three promising varieties, Polyanka and Pochayna, are less vulnerable.

A close relationship was found between economically valuable traits and the frequency of valuable mutations obtained for these genotypes, the overall frequency and spectra of visually identifiable mutations. Although this pattern has already been identified (Mamenko & Yakymchuk, 2019), it was not for chemical mutagenesis. Thus, for the genetic improvement of winter wheat varieties, the six identified combinations of three varieties of Ukrainian breeding and moderate concentrations of sodium azide and dimethyl sulphate can be used, which guarantee a substantial increase in the yield of forms with economically valuable traits by about 20-50%.

CONCLUSIONS

The genetic material of Ukrainian breeding (varieties Kalancha, Pochayna, Polyanka) is a successful component as an initial form for the creation, together with

the action of chemical supermutagens of high damage capacity (SA and DMS), of systems with a prominent stable yield of mutants with positive changes in economically valuable traits. That is, six systems based on three varieties and two mutagens (SA and DMS) were identified, the use of which led to guaranteed production of lines with positive complex shifts in valuable biochemical components, increased yield of useful forms (up to 50% compared to other variants). The forms obtained by applying the proposed systems can have additional valuable qualities, but the effectiveness of the variety-mutagen complex begins to decline with increasing concentration, primarily due to the increased risk of additional negative qualities. When obtaining such systems, it is justified to invest in early continuous screening of forms by biometric and biochemical analysis in the second or third generation after mutagenic exposure, which guarantees the availability of additional valuable material for genetic improvement of the crop. Thus, during the genetic improvement, it is worth using a moderate amount of sodium azide (0.025-0.05%) and, as an additional source of variability, moderate concentrations of DMS (0.0125%), while the use of EMS is less justified. Based on the results of research on the identification of beneficial changes and their correlations in the system of interaction between nature, factor concentrations, and characteristics of the source material, promising complexes of these components were identified, the combination of which leads to a substantial increase in the efficiency of the genetic improvement of winter wheat. Further research will focus on such valuable traits as drought and winter hardness, specific features of mineral use and accumulation by the studied lines to confirm the parameters that provide advantages in yield and grain quality, and it is also planned to investigate the induction of new forms by highly active mutagens based on new Ukrainian and international forms with the same traits.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Спадкова мінливість у пшениці озимої при взаємодії генотипа з чинниками високої генетичної активності

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Анотація. Виявлення нових можливостей в застосуванні окремих екогенетичних чинників для генетичного поліпшення пшениці озимої є важливим компонентом вдосконалення існуючих агроценозів зернових культур в рамках стратегії продовольчої безпеки. Метою дослідження було показати можливості речовин з високою генетичною активністю в індукції потенційно-корисних змін та встановити можливість отримання оптимізованих схем для отримання нових форм та більш керованої мінливості за ознаками. Мутації виявляли візуальним спостереженням та біометричним, біохімічним аналізом починаючи з другого покоління до шостого з перевіркою успадкування виявлених ознак у наступних поколіннях. Показано можливості мутаційного процесу за окремими генетично- та селекційно-цінними формами, особливості індукції таких ознак, що дає змогу підвищити прогнозованість отримання необхідних мутантних ліній та частково-керованої високої варіативності за окремими ознаками. Встановлено, що найбільш перспективним для використання є комплексне застосування помірних доз азиду натрію та диметилсульфату як екогенетичних чинників. Показано, що в комплексі з використанням трьох сортів серед досліджуваних (Каланча, Полянка, Почайна) можливе створення високоефективних генотип-мутагенних систем з підвищеним виходом цінних форм. Зростання мутагенної депресії при використанні більш шкочинних речовин може бути суттєво скомпенсованим підвищенням частки корисних змін. Негативним є їх комплексний характер дії. Природа екогенетичного фактору як чинник не менш суттєва ніж концентрація мутагену або генотип вихідної форми, що іноді ураховується недостатньо. Підтверджено, що використання наведених факторів є доволі обіцяючим з огляду отримання малих біохімічних змін (мікромутацій). Виділено декілька перспективних мутантних ліній, що мають комплексне поліпшення врожайності та якості зерна, без додаткових негативних змін. Результати дослідження можна використати в селекційних програмах для створення нових сортів пшениці озимої

Ключові слова: мутації; хімічний мутагенез; пшениця озима; супермутагени; врожайність; якість зерна



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Productivity of brown mustard plants depending on pre-sowing application of mineral fertilisers and foliar feeding

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Abstract. Brown mustard has a prominent genetic potential for seed productivity, which can be fulfilled by optimising the elements of cultivation technology, namely the nutrition system, which makes research relevant. The purpose of this study was to determine the complex effect of the combination of different variants for pre-sowing mineral feeding and foliar feeding with water-soluble fertilisers on the formation of productivity elements and biological yield of brown mustard seeds. Field, laboratory

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(for accounting for structural elements of the crop), and statistical (for mathematical processing of materials) research methods were used in the study. The study was conducted in 2020, 2021, and 2023 on the basis of the farm "KYRYCHENKO M" located in the central part of Borivskiy district of Kharkiv region. The experiment compared five variants of pre-sowing mineral feeding and five variants of foliar feeding with various combinations of water-soluble fertilisers. The largest number of seeds on the plant and the largest mass were in the variants of the combination of pre-sowing application of $N_{45}P_{45}K_{45}$ and carrying out two foliar feedings during microphases 21-23 and 50-53 with a mixture of carbamide and complex fertiliser Quantum technical – 627.3 pcs. and 1.97 g, respectively. However, these indicators did not significantly differ from the variant with a lower dose of pre-sowing fertilisers – $N_{45}P_{30}K_{30}$. Generally, according to the experiment, the highest biological yield of brown mustard seeds in an average of three years was in the variants of the combination of pre-sowing application of $N_{45}P_{45}K_{45}$ and $N_{45}P_{30}K_{30}$ with two foliar top dressings with different mixtures of fertilisers – 2.72-2.79 t/ha. Proceeding from this, the best variant in the experiment was the pre-sowing application of $N_{45}P_{30}K_{30}$ in combination with two foliar feedings during microphases 21-23 and 50-53 with a mixture of carbamide and the complex water-soluble fertiliser under study in single doses of 10 kg/ha and 2.0 l/ha, respectively. It was established that the highest biological yield of mustard seeds in all years was on the variants of pre-sowing application – $N_{45}P_{45}K_{45}$ and $N_{45}P_{30}K_{30}$. There was no significant difference between these variants by year. The findings of this study can be used by agricultural enterprises to adjust the nutrition system (pre-sowing application of complex fertilisers and foliar feeding), which will ensure a more complete fulfilment of the genetic potential of brown mustard productivity

Keywords: cultivation technology; brown mustard variety; nutrition system; mineral fertilisers; crop structure; biological seed yield; productivity; weather conditions

INTRODUCTION

The uncontrolled increase in sunflower acreage, the associated negative trends in soil degradation, and global warming are forcing us to reconsider the structure of our acreage and expand the acreage of crops that are, on the one hand, alternatives to sunflower and, on the other hand, adapted to modern climate change. Brown mustard is perhaps the best in this regard. An essential aspect of expanding the area under this crop is improving technological approaches to its cultivation. O. Zhuykov and T. Hodos (2021), along with the ability to form prominent seed yields, note the ability of mustard to tolerate prolonged droughts and hot temperatures more easily. According to A.N. Sivak and T.K. Kostyukovich (2021), these advantages are also added to by the growing demand for Ukrainian mustard abroad, especially in the EU. The average yield of mustard seeds in Ukraine is lower than in the leading EU countries, due to the insufficient fulfilment of the crop's genetic potential as a result of insufficient attention to the plant nutrition system. S.K. Verma *et al.* (2022) noted that only by optimising the nutrition system can the yield of mustard seeds be increased by 40% or more. This can be achieved by meeting the nutrient needs of plants during not only the initial stages, but the entire life cycle of plants. In this regard, S.S. Rathore *et al.* (2019) and R. Mondal *et al.* (2020) emphasise the expediency of foliar feeding, which makes it possible to promptly eliminate nutrient deficiencies and to offset the effects of abiotic and biotic stresses.

In Ukraine, research is constantly being conducted to improve the elements of mustard agronomy, including the nutrition system. I.J. Irin *et al.* (2020) and S.J. Butenko and J. PeiPei (2022) consider the nutrition

system to be the main source of improving the fulfilment of the genetic potential of plant productivity and yield of mustard seeds. The development of best possible solutions based on a harmonious combination of various types of application of the entire line of mineral nutrients required for plants, from the main application to foliar feeding, will help obtain the actual possible yield, which will be limited solely by the weather conditions of the growing season. Among producers, there is a widespread erroneous practice of introducing the so-called "statistical average" norm of mineral fertilisers, which for most oil crops is $N_{40}P_{60}$. S.V. Zherdetska (2017) notes that such a primitive approach is the reason for the underutilisation of agricultural resources and insufficient fulfilment of the genetic potential of brown mustard crops.

Specialised scientific literature provides various suggestions for the use of mineral fertilisers in mustard cultivation, which usually differ fundamentally, making it difficult for producers to decide which variant is best for them. To a large extent, the advantage of a particular fertiliser application variant is related to its cost. Therefore, given the rapid rise in the price of fertilisers in recent years, the recommendations developed earlier are often outdated and do not reflect current economic realities. N. Nehal *et al.* (2017) and S.S. Dhaliwal *et al.* (2021) substantiate the feasibility of conducting research on optimising the nutrition system through the introduction of new varieties of brown mustard, which, differing in morpho-biological type, can respond differently to the use of nutrition elements and stimulant substances.

To increase the efficiency of nitrogen use, I.A. Shevchenko *et al.* (2017) recommend applying it

in parts, including the application of foliar feeding. In their opinion, this method of nitrogen application is better utilised by plants and the return on investment will be higher than with a single application. Along with determining the best doses of pre-sowing feeding, A. Shahid (2018), R. Banerjee *et al.* (2019) emphasise the significance of finding ways to increase the yield of mustard seeds through foliar feeding, noting that it is the harmonious combination of various types of feeding that will help solve the problem of low yields of mustard seeds, increase the competitiveness of its cultivation, and therefore make it more attractive for production.

A wide range of both complex and mono-element water-soluble fertilisers makes it possible to provide nutrient mixtures that are suitable for plants based on their needs in a particular period. Furthermore, along with nutrients, if necessary, one can apply stimulant, growth-regulating, or anti-stress agents. Given the leading role of optimising the plant nutrition system, including the combination of its various components, including pre-sowing fertilisation and foliar feeding of crops during the growing season, research in this area is relevant today. Therefore, the purpose of this study was to find the best possible combinations of pre-sowing mineral fertilisation and foliar feeding with water-soluble complexes that ensure the formation of

higher indicators of individual plant productivity and biological yield of brown mustard seeds.

MATERIALS AND METHODS

The study was conducted in 2020, 2021, and 2023 on the basis of the farm "KYRYCHENKO M" located in the southern part of Kharkiv region. The predecessor of mustard was winter wheat. After harvesting, the field was disked in two tracks. At the end of September, the field was ploughed to a depth of 22-25 cm. Brown mustard was sown after the soil was warmed up to 5-6°C at a depth of 6-8 cm with a seed drill SZD-360 V (Ukraine) to a depth of 2-3 cm.

After sowing, the field was sprayed with Tizer herbicide at a dose of 3.0 l/ha and rolled. At the phase of 4-5 leaves, the crops were sprayed with Galera herbicide at a dose of 0.3 l/ha against annual and perennial cereal weeds. The crops were harvested with a US-made combine harvester, the Case IH Axial-Flow 6130, which ensures high-quality harvesting of small-seeded crops. The plots of the first order (factor A) in the conducted two-factor experiment were five variants of pre-sowing application of mineral fertilisers (ammonium nitrate phosphate fertiliser and ammonium nitrate): 1 – control (without fertilisers); 2 – $N_{15}P_{15}K_{15}$; 3 – $N_{30}P_{30}K_{30}$; 4 – $N_{45}P_{45}K_{45}$; 5 – $N_{45}P_{30}K_{30}$. The second-order plots were five variants of foliar feeding (Table 1).

Table 1. Scheme of the variants for foliar feeding under study (factor B)

Variant	Phases of growth and development according to the international BBCH scale	
	21-23	50-53
1 (control)	–	–
2	Carbamide (10 kg/ha) + Quantum technical (2.0 l/ha)	–
3	Carbamide (10 kg/ha) + Quantum technical (2.0 l/ha)	Carbamide (10 kg/ha) + Quantum technical (2.0 l/ha)
4	Carbamide (10 kg/ha) + Quantum technical (2.0 l/ha) + Quantum BOR Active (1.0 l/ha)	Carbamide (10 kg/ha) + Quantum technical (2.0 l/ha)
5	Carbamide (10 kg/ha) + Quantum technical (2.0 l/ha)	Carbamide (10 kg/ha) + Quantum technical (2.0 l/ha) + Quantum BOR Active (1.0 l/ha)

Source: compiled by the authors of this study

The experiment was set up, observations, records, and analyses were carried out according to the generally accepted methodology (Ushkarenko *et al.*, 2019). Statistical processing of the findings was carried out using the Statistica 6.0 and Excel 2003 software. Highly concentrated complex chelated fertiliser Quantum Technical from the Research and Production Company Kvadrat is designed for foliar feeding and pre-sowing treatment of technical crops. It has a high content of copper (11 g/l), zinc (12 g/l), and manganese (11.3 g/l). Additionally, it contains a complex of biologically active substances, has a slightly alkaline reaction (pH – 7.5-8.5).

The mono-element fertiliser Quantum Boron Active is made based on readily available biologically active forms of boron with a content of 140 g/l (14.0%). The product also contains amino acids (1.5%), which

improve the absorption of boron by plant leaves, activate metabolism, and increase plant stress resistance. The study was conducted with a new variety of brown mustard, Felicia, created at the Carpathian State Agricultural Station of the National Academy of Agrarian Sciences. The variety was included in the State Register in 2018 and is recommended for cultivation in all agro-climatic zones of Ukraine.

The research area is characterised by unstable moisture conditions and a significant temperature difference. During the years of research, the amount of precipitation during the growing season significantly deviated from the long-term average. In March and April 2020, the amount of precipitation was significantly lower than the climate norm. In May, on the contrary, the amount of precipitation was more than twice the long-term average.

Due to significant precipitation in May, as well as in the first and second decades of June, there was sufficient moisture in the second half of the 2020 mustard growing season. The air temperature in May was significantly lower than the long-term average, which complicated the growth processes of brown mustard.

In 2021, the supply of moisture to mustard plants during the growing season was sufficient. The distribution of precipitation was more balanced than in the previous year. The temperature conditions were similar to 2020. In the third decade of June, the air temperature reached 32°C, which worsened the course of growth processes at the end of the mustard growing season. The most favourable weather conditions for the growth and development of mustard plants were in 2023, which was reflected in the seed yields. In April, May, June, and the first ten days of July, there was more precipitation than the long-term average, and its distribution was even. The air temperature during the growing season met the biological needs of the crop and was virtually on par with the long-term average. On the warmest days of the summer period, it did not exceed 28.0°C. Generally, different temperature conditions and divergence in the amount of precipitation and its distribution during the spring and summer vegetation of mustard crops significantly affected the ontogeny of

plants, which considerably affected their productivity. At the same time, this helped to investigate the effect of the variants of the nutrition system under study on the elements of plant productivity and biological yield of brown mustard seeds in greater depth.

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

RESULTS AND DISCUSSION

Elements of mustard plant productivity underwent greater changes under the influence of pre-sowing mineral fertilisation. The number of pods on a plant, the number of seeds in a pod and on one plant were the largest in the variants of pre-sowing application of ammonium nitrate phosphate fertiliser in the dose of $N_{45}P_{45}K_{45}$ – 55.6 pcs., 11.0 pcs., and 614.1 pcs., respectively (Table 2). Compared to the control, the increase in performance was 11.2%, 15.8%, and 28.3%, respectively. The number of seeds per plant changed significantly, which is logical since pre-sowing fertilisation contributed to the formation of more pods and the number of seeds in them.

Table 2. Elements of mustard plant productivity under different variants of pre-sowing application of complex fertilisers and foliar feeding (average for 2020, 2021, 2023)

Pre-sowing application (factor A)	Variants of fertilisers (factor B)	Quantity			Seed weight per plant, g
		pods per plant, pcs.	seeds per pod, pcs.	seeds per plant, pcs.	
Control (without fertilisers)	1	49.2	9.0	442.5	1.24
	2	50.2	9.5	478.9	1.37
	3	50.2	9.8	494.4	1.46
	4	50.4	9.7	487.6	1.43
	5	50.2	9.7	489.7	1.45
$N_{15}P_{15}K_{15}$	1	52.5	9.7	510.2	1.50
	2	53.4	10.1	540.1	1.61
	3	53.9	10.2	549.3	1.66
	4	53.9	10.1	550.0	1.67
	5	54.0	10.2	551.4	1.68
$N_{30}P_{30}K_{30}$	1	53.9	10.2	552.7	1.66
	2	54.4	10.6	578.8	1.76
	3	54.3	10.7	580.9	1.80
	4	54.4	10.6	576.1	1.79
	5	54.6	10.7	587.0	1.82
$N_{45}P_{45}K_{45}$	1	55.2	10.7	591.2	1.80
	2	55.5	11.0	611.4	1.89
	3	55.9	11.2	627.3	1.97
	4	55.7	11.1	620.6	1.95
	5	55.9	11.1	619.9	1.94
$N_{45}P_{30}K_{30}$	1	55.1	10.5	581.1	1.77
	2	55.7	10.9	609.4	1.87
	3	55.6	11.0	615.2	1.91
	4	55.6	11.1	619.0	1.92
	5	55.7	11.0	615.8	1.92

Pre-sowing application (factor A)	Variants of fertilisers (factor B)	Quantity			Seed weight per plant, g
		Pods per plant, pcs.	Seeds per pod, pcs.	Seeds per plant, pcs.	
Average by factor A	control	50.0	9.5	478.6	1.39
	$N_{15}P_{15}K_{15}$	53.5	10.1	540.2	1.62
	$N_{30}P_{30}K_{30}$	54.3	10.6	575.1	1.77
	$N_{45}P_{45}K_{45}$	55.6	11.0	614.1	1.91
	$N_{45}P_{30}K_{30}$	55.5	10.9	608.1	1.88
Average by factor B	1	53.2	10.0	535.5	1.59
	2	53.8	10.4	563.7	1.70
	3	54.0	10.6	573.4	1.76
	4	54.0	10.5	570.7	1.75
	5	54.1	10.5	572.8	1.76
LSD ₀₅ of the effect A*		1.7-2.7	0.3-0.5	16.2-24.2	0.04-0.07
LSD ₀₅ of the effect B		1.9-2.7	0.3-0.6	17.5-26.5	0.06-0.07
LSD ₀₅ of partial comparisons A		2.4-3.0	0.5-0.7	19.1-27.3	0.07-0.09
LSD ₀₅ of partial comparisons B		2.5-3.1	0.6-0.8	19.3-27.7	0.08-0.11

Note: *LSD is the least significant difference

Source: compiled by the authors

The analysis of the quantitative elements of the productivity of mustard plants did not show a significant difference between the variants of pre-sowing application of $N_{45}P_{45}K_{45}$ and $N_{45}P_{30}K_{30}$. With the same dose of nitrogen, but a lower dose of phosphorus and potassium, the number of seeds per plant decreased by only 6 pcs., which is significantly less than the LSD₀₅ indicator, which over the years was 16.2-24.2 pcs. Among the variants of foliar feeding under study, the formation of the largest number of seeds per plant was ensured by the variant of two foliar feedings during microphases 21-23 and 50-53 on the international BBCH scale with a mixture of carbamide (10 kg/ha) and complex fertiliser Quantum Technical (2.0 l/ha). On average, the number of seeds in this variant was 573.4 pcs., which is 37.9 pcs. or 7.1% more than in the control. The addition of mono-elemental fertiliser Quantum Boron Active to the working mixtures did not have a positive effect on increasing the number of seeds per plant.

The formation of a greater number of seeds per plant in the variants of foliar feeding was conditioned by the greater number of seeds in the pod. The number of pods per plant did not change significantly under their influence. Under the influence of pre-sowing fertilisation, other patterns were observed, namely, they contributed to a significant increase in both the number of pods per plant and the number of seeds per pod. Therewith, their impact on these structural indicators was comparable. For instance, the number of pods and seeds in a pod increased by 10-12% in the $N_{45}P_{45}K_{45}$ applications compared to the control.

By analogy with the number of seeds on one plant, their mass was the largest in the variants of pre-sowing application of $N_{45}P_{45}K_{45}$ and $N_{45}P_{30}K_{30}$. On average, the weight of seeds on these variants of foliar feeding was 1.91 g and 1.88 g, which is 0.52 g (37.4%) and 0.49 g (35.3%) more than in the control. The difference

between the weight of seeds of one plant under the influence of foliar feeding was much smaller. As well as the number of seeds per plant, the weight of seeds per plant was highest in the variant with two foliar applications of a mixture of carbamide and Quantum Technical fertiliser. In this variant, it was 1.76 g, which is 0.17 g (10.7%) more than in the control. The addition of Quantum Boron Active fertiliser (fourth and fifth variants of factor B) to the working mixtures did not increase the weight of seeds per plant. Generally, according to the experiment, the largest mass of seeds of one plant was in the variant of pre-sowing application of $N_{45}P_{45}K_{45}$ and carrying out two foliar fertilising with a mixture of carbamide and Quantum Technical fertilisers – 1.97 g. However, according to the statistical analysis, it did not significantly differ from the variant with a lower dose of pre-sowing fertilisers – $N_{45}P_{30}K_{30}$ in combination with the same variant of foliar feeding. The factors under study caused significant changes in the biological yield of mustard seeds, and their influence on this indicator was higher than on the analysed elements of plant productivity, due to the positive effect of pre-sowing fertilisation and foliar feeding on the preservation of more plants to the full ripeness phase.

Factor A had a much greater influence on the change in the biological yield of mustard seeds. The highest biological yield of seeds on average by year and variant of foliar feeding was in the variant of pre-sowing application of $N_{45}P_{45}K_{45}$ – 2.71 t/ha, which is 0.86 t/ha or 46.5% more than in the control (Table 3). Therewith, it did not significantly differ from the variant of pre-sowing application of $N_{45}P_{30}K_{30}$. The difference between these variants was only 0.05 t/ha, and according to the statistical analysis using the ranking of the results, the biological yield of seeds on these variants was within the same rank group. The effect of foliar feeding on the biological yield of seeds was much less. The largest difference

between the indicators under their influence was 0.27 t/ha or 12.3%. All variants of foliar feeding provided a significant increase in the biological yield of seeds compared to the control. Furthermore, high efficiency of

feeding during microphases 50-53 was proved, since the biological yield in variants with two instances of foliar feeding was higher compared to the variant where only one feeding was carried out during microphases 21-23.

Table 3. Biological yield of mustard seeds depending on pre-sowing application of complex fertilisers and foliar feeding, t/ha (average for 2020, 2021, 2023)

Pre-sowing application (factor A)	Foliar fertilisation (factor B)	Biological yield, t/ha		± to control factor A	± to control factor B
		indicator	RG*		
Control (without fertilisers)	1	1.61	I	–	–
	2	1.80	II	–	+0.19
	3	1.95	III	–	+0.34
	4	1.93	II	–	+0.32
	5	1.96	III	–	+0.35
N ₁₅ P ₁₅ K ₁₅	1	2.01	I	+0.40	–
	2	2.20	II	+0.40	+0.19
	3	2.29	III	+0.34	+0.28
	4	2.30	III	+0.37	+0.29
	5	2.31	III	+0.35	+0.30
N ₃₀ P ₃₀ K ₃₀	1	2.30	I	+0.69	–
	2	2.46	II	+0.66	+0.16
	3	2.52	III	+0.57	+0.22
	4	2.51	III	+0.58	+0.21
	5	2.54	III	+0.58	+0.24
N ₄₅ P ₄₅ K ₄₅	1	2.53	I	+0.92	–
	2	2.68	I	+0.88	+0.15
	3	2.79	II	+0.84	+0.26
	4	2.77	II	+0.84	+0.24
	5	2.76	II	+0.80	+0.23
N ₄₅ P ₃₀ K ₃₀	1	2.49	I	+0.88	–
	2	2.64	I	+0.84	+0.15
	3	2.73	II	+0.78	+0.24
	4	2.73	II	+0.80	+0.24
	5	2.73	II	+0.77	+0.24
Average by factor A	control	1.85	I	–	–
	N ₁₅ P ₁₅ K ₁₅	2.22	II	+0.37	–
	N ₃₀ P ₃₀ K ₃₀	2.47	III	+0.62	–
	N ₄₅ P ₄₅ K ₄₅	2.71	IV	+0.86	–
	N ₄₅ P ₃₀ K ₃₀	2.66	IV	+0.81	–
Average by factor B	1	2.19	I	–	–
	2	2.36	II	–	+0.17
	3	2.45	III	–	+0.26
	4	2.45	III	–	+0.26
	5	2.46	III	–	+0.27

Note: *RG – rank groups

Source: compiled by the authors

There was virtually no difference between the variants for carrying out two instances of foliar feeding with different mixtures of water-soluble fertilisers, which indicates that it is inexpedient to incur additional costs for the purchase and application of the mono-elemental fertiliser Quantum Boron Active. Generally, according to the experiment, the highest biological

yield of brown mustard seeds in an average of three years (2.72-2.79 t/ha) was formed in the variants of the combination of pre-sowing application of N₄₅P₄₅K₄₅ and N₄₅P₃₀K₃₀ with two instances of foliar feeding with different mixtures of fertilisers. Proceeding from this, the variant of pre-sowing application of N₄₅P₃₀K₃₀ in combination with two instances of foliar feeding is best in the

experiment. Increasing the dose of pre-sowing mineral fertilisers, as well as including the mono-elemental fertiliser Quantum Boron Active in tank mixtures, did not provide a significant increase in the biological yield of seeds, while growing costs increased significantly. The biological yield of mustard seeds underwent significant changes under the influence of weather conditions.

Favourable temperature conditions, sufficient precipitation, and their uniform distribution during the vegetation of mustard plants in 2023 contributed to a higher biological yield of seeds. Specifically, on average, the biological yield of mustard seeds in 2020, 2021, and 2023 was 1.99 t/ha, 2.43 t/ha, and 2.71 t/ha, respectively (Table 4).

Table 4. Biological yield of brown mustard seeds under different variants of pre-sowing application of complex fertilisers and foliar feeding, t/ha

Pre-sowing application (factor A)	Foliar fertilisation (factor B)	Year					
		2020		2021		2023	
		BY*	RG**	BY	RG	BY	RG
Control (without fertilisers)	1	1.36	I	1.65	I	1.81	I
	2	1.48	II	1.90	II	2.03	II
	3	1.60	III	2.06	III	2.20	III
	4	1.60	III	1.99	II	2.19	III
	5	1.59	III	2.05	III	2.24	III
N ₁₅ P ₁₅ K ₁₅	1	1.71	I	2.04	I	2.29	I
	2	1.82	I	2.23	II	2.56	II
	3	1.91	II	2.34	III	2.61	II
	4	1.90	II	2.30	II	2.70	III
	5	1.92	II	2.38	III	2.63	II
N ₃₀ P ₃₀ K ₃₀	1	1.92	I	2.36	I	2.61	I
	2	2.09	II	2.53	II	2.76	II
	3	2.13	II	2.54	II	2.89	III
	4	2.13	II	2.55	II	2.84	III
	5	2.14	II	2.55	II	2.93	III
N ₄₅ P ₄₅ K ₄₅	1	2.17	I	2.56	I	2.86	I
	2	2.28	I	2.69	I	3.06	II
	3	2.33	II	2.82	II	3.23	III
	4	2.36	II	2.82	II	3.13	II
	5	2.33	II	2.76	II	3.17	III
N ₄₅ P ₃₀ K ₃₀	1	2.14	I	2.56	I	2.76	I
	2	2.24	I	2.75	II	2.93	II
	3	2.25	I	2.75	II	3.15	III
	4	2.23	I	2.76	II	3.20	III
	5	2.30	II	2.74	II	3.14	III
Average by factor A	control	1.53	I	1.93	I	2.09	I
	N ₁₅ P ₁₅ K ₁₅	1.85	II	2.26	II	2.56	II
	N ₃₀ P ₃₀ K ₃₀	2.08	III	2.51	III	2.81	III
	N ₄₅ P ₄₅ K ₄₅	2.29	IV	2.73	IV	3.09	IV
	N ₄₅ P ₃₀ K ₃₀	2.23	IV	2.71	IV	3.04	IV
Average by factor B	1	1.86	I	2.23	I	2.47	I
	2	1.98	II	2.42	II	2.67	II
	3	2.04	II	2.50	II	2.82	III
	4	2.04	II	2.48	II	2.81	III
	5	2.06	II	2.50	II	2.82	III
Mean		1.99		2.43		2.71	
LSD ₀₅ of the main effect A		0.07		0.08		0.09	
LSD ₀₅ of the main effect B		0.09		0.08		0.11	
LSD ₀₅ of the partial comparisons of the effect of A		0.11		0.12		0.14	
LSD ₀₅ of the partial comparisons of the effect of B		0.13		0.13		0.16	

Notes: * BY – biological yield, t/ha; **RG – rank group

Source: compiled by the authors

In all years, the biological yield of mustard seeds was more affected by the influence of pre-sowing fertilisation. Their impact was slightly higher in the less favourable weather conditions of 2020. Specifically, this year's pre-sowing application of mineral fertilisers ensured a maximum increase in the biological yield of mustard seeds by 48%, compared to 41.5% and 47.8% in 2021 and 2023, respectively. Thus, weather conditions made certain adjustments to the effectiveness of pre-sowing fertilisation, while the general pattern of effectiveness of the compared variants was maintained. The highest biological yield of mustard seeds in all years was in the fourth and fifth variants of pre-sowing application of mineral fertilisers – $N_{45}P_{45}K_{45}$ and $N_{45}P_{30}K_{30}$. There was no significant difference established between these variants by year.

In contrast to pre-sowing mineral feeding, the effect of foliar feeding on changes in the biological yield of mustard seeds varied over the years. In less favourable weather conditions of the 2020 and 2021 growing seasons, all variants of foliar feeding contributed to a significant increase in the biological yield of seeds compared to the control variant, with no difference between them. That is, the second foliar feeding during 50-53 microphases with different mixtures of water-soluble fertilisers did not increase the biological yield of seeds compared to the variant of a single foliar feeding during microphases 21-23. Under more favourable conditions in 2023, the second foliar application provided a significant increase in the biological yield of seeds. According to the authors, this is logical, as this year's weather conditions "enabled" plants to fulfil their potential to a greater extent, and in such conditions the role of optimising the plant nutrition system is increasing, which is why the additional introduction of a complex of micro- and macroelements at the beginning of budding (microphases 50-53) enables plants to fulfil their potential significantly better.

The findings of the conducted study show that the productivity of brown mustard plants and their biological seed yield largely depend on the nutrition system. Among the components of the plant nutrition system, the key factor was the pre-sowing application of mineral fertilisers. While noting the crucial role of the main, pre-sowing, and seedbed fertiliser application in mustard cultivation, the opinions of scientists differ significantly on the issue of fertiliser application rates. This is logical, because the fertiliser application rate is adjusted by a range of factors, the main one being the agro-climatic potential of the growing area. In this regard, M.I. Blaschuk and N.M. Tetereshenko (2017) recommend determining the doses of their application based on the planned yield, soil fertility, and predecessor. Depending on these components, they outline the range of application rates of mineral fertilisers from $N_{40}P_{40}K_{40}$ to $N_{80}P_{60}K_{80}$. Therewith, there are recommendations that go beyond this range. Most

fertiliser application rate recommendations fall within this range, but there are recommendations that go beyond it. Specifically, the pre-sowing application of $N_{45}P_{30}K_{30}$ was the best variant in the experiment. Therefore, the dose of phosphorus and potassium was outside this range. An analogous recommendation is provided by A.V. Melnyk *et al.* (2019), who prefer pre-sowing application of $N_{30}P_{30}K_{30}$ when growing brown mustard of Prima and Retro varieties. Therewith, there is a rationale for pre-sowing fertilisation that, on the contrary, exceeds this range. Specifically, in the experiments conducted by A. Sarkar *et al.* (2021) on the Ganges soils of West Bengal, the highest increases in mustard seed yields were formed against the background of pre-sowing N_{100} . While investigating different doses of pre-sowing fertiliser in Indonesia, B. Utomo (2022) found that the highest indicators of plant productivity and yield of brown mustard seeds are formed with doses of fertilisers $N_{115}P_{115}K_{115}$.

No less controversial are the issues of foliar feeding. Varying opinions are expressed regarding the phases of application, types of fertilisers, and their combinations. K. Rana *et al.* (2020) note the higher efficiency of foliar feeding on high backgrounds. The findings of the present study do not agree with this opinion, as the effectiveness of foliar feeding was highest in the variants where no fertiliser was applied. V.F. Sayko and V.S. Vishnevsky (2015) note the high efficiency of foliar feeding during the budding phase, explaining this by decreasing the reduction of the inherent generative components. These data are generally consistent with the findings of the present study, since according to the average three-year data, the second foliar application with a mixture of the fertilisers under study provided a considerable increase in the biological yield of seeds, but various patterns were observed in terms of years. Specifically, in the less favourable weather conditions of 2020 and 2021, the second fertilisation (at the beginning of budding) did not significantly increase seed yields. Therefore, the effectiveness of foliar feeding depends on the weather conditions of the year.

Generally, all researchers note the significance of such components of the nutrition system as pre-sowing fertilisation and foliar feeding. Therewith, there is a wide range of proposals for pre-sowing doses, phases, and tank mixtures for foliar application, which is due to the different growth and development conditions of mustard plants. Therefore, these issues require further investigation, especially as the market for pre-sowing and foliar fertilisers is constantly being updated with new, more effective products.

CONCLUSIONS

The findings of the conducted study strongly suggest the following conclusions: indicators of the elements of productivity of brown mustard plants of the Felicia variety were the highest in the variants of pre-sowing application of $N_{45}P_{30}K_{30}$ and $N_{45}P_{45}K_{45}$ in combination

with two instances of foliar feeding during microphases 21-23 and 50-53 according to the international scale with a mixture of carbamide and complex fertiliser Quantum Technical in a single dose of 10 kg/ha and 2.0 l/ha, respectively. The addition of mono-element water-soluble fertiliser Quantum Boron Active to the working solution during foliar application did not increase either plant productivity elements or biological yield of mustard seeds.

The highest biological yield of mustard seeds in the experiment – 2.79 t/ha, was formed in the variant of the combination of pre-sowing application of $N_{45}P_{45}K_{45}$ and carrying out two instances of foliar dressing with a mixture of carbamide (10 kg/ha) and complex water-soluble fertiliser Quantum Technical (2.0 l/ha). Therewith, when reducing the dose of fertilisers in pre-sowing application to $N_{45}P_{30}K_{30}$ in combination with the same variant of foliar feeding, the biological yield of seeds did

not decrease significantly, which strongly suggests the impracticality of increasing the dose of pre-sowing application of phosphorus and potassium from 30 kg/ha to 45 kg/ha in the active substance. Considering the wide range of fertilisers and their constant replenishment, different requirements and specifics of varieties, as well as significant fluctuations in fertiliser prices, it is advisable to continue research into optimising the nutritional regime of brown mustard, as a more in-depth study of this issue will help to increase its yield and competitiveness, and thus expand the area under cultivation.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Продуктивність рослин гірчиці сизої залежно від передпосівного внесення мінеральних добрив і позакоренових підживлень

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Анотація. Гірчиця сиза має високий генетичний потенціал насіннєвої продуктивності, реалізували який можна шляхом оптимізації елементів технології вирощування, зокрема системи живлення, що обумовлює актуальність досліджень. Мета досліджень полягала у визначенні комплексного впливу поєднання різних варіантів передпосівного внесення мінеральних добрив і позакоренових підживлень водорозчинними добривами на формування елементів продуктивності та біологічну врожайність насіння гірчиці сизої. Під час досліджень використовували польовий, лабораторний (для обліку структурних елементів урожаю) та

статистичний (для математичної обробки матеріалів) методи досліджень. Дослідження проводили в 2020, 2021 і 2023 рр. на базі фермерського господарства «КИРИЧЕНКО М» розташованого в центральній частині Борівського району Харківської області. У досліді порівнювали п'ять варіантів передпосівного внесення мінеральних добрив і п'ять варіантів позакоренових підживлень різними сполученнями водорозчинних добрив. Найбільша кількість насінин на рослині і найбільша їх маса була у варіантах поєднання передпосівного внесення $N_{45}P_{45}K_{45}$ і проведення двох позакоренових підживлень під час мікрофаз 21-23 і 50-53 сумішшю карбаміду і комплексного добрива Квантум технічні – 627,3 шт. і 1,97 г відповідно. Проте, ці показники істотно не відрізнялися від варіанту з меншою дозою передпосівного внесення добрив – $N_{45}P_{30}K_{30}$. У цілому по досліді найвища біологічна врожайність насіння гірчиці сизої в середньому за три роки була у варіантах поєднання передпосівного внесення $N_{45}P_{45}K_{45}$ і $N_{45}P_{30}K_{30}$ з двома позакореновими підживленнями різними сумішами добрив – 2,72-2,79 т/га. Виходячи з цього, оптимальним у досліді є варіант передпосівного внесення $N_{45}P_{30}K_{30}$ у поєднанні з проведенням двох позакоренових підживлень під час мікрофаз 21-23 і 50-53 сумішшю сечовини і досліджуваного комплексного водорозчинного добрива в разових дозах 10 кг/га і 2,0 л/га відповідно. Встановлено, що найвища біологічна врожайність насіння гірчиці в усі роки була на варіантах передпосівного внесення – $N_{45}P_{45}K_{45}$ і $N_{45}P_{30}K_{30}$. При цьому істотної різниці між цими варіантами по роках не було. Результати досліджень можуть бути використані аграрними підприємствами для корегування системи живлення (передпосівного внесення комплексних добрив і позакоренових підживлень), що забезпечить більш повну реалізацію генетичного потенціалу продуктивності гірчиці сизої

Ключові слова: технологія вирощування; сорт гірчиці сизої; система живлення; мінеральні добрива; структура врожаю; біологічна врожайність насіння; продуктивність; погодні умови



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Multiplex primers employment for detection of rinderpest virus RNA by PCR

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Abstract. Given the high contagiousness and rapid spread of the rinderpest virus, timely and accurate diagnosis plays a key role in preventing epidemics and taking measures to control the disease. The study aims to evaluate the efficiency of using multiplex primers in the polymerase chain reaction method for the detection of rinderpest virus ribonucleic acid. The study included the analysis of samples such as blood serum and conjunctival swabs from 50 animals with clinical manifestations

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of the disease. The experiment involved the collection of clinical samples such as blood serum and conjunctival washings. The results demonstrate the high specificity of the developed primers. These primers stand out because they use two pairs of the same gene region with different variable sequences that are specific for all strains of the rinderpest virus. In the polymerase chain reaction, both pairs of primers are used simultaneously at equal concentrations and under the same conditions. An additional polymerase chain reaction performed using these primers at the optimal annealing temperature confirmed the successful amplification and specificity of the primers. The absence of dimers and nonspecific products in the negative control confirmed the purity and reliability of the results. Thus, these results demonstrate that the use of these multiplex polymerase chain reaction primers allows for the efficient detection of the ribonucleic acid of the rinderpest virus of different strains. The developed multiplex primers represent an innovative method for the diagnosis of rinderpest virus with the potential for use in veterinary practice and animal disease control

Keywords: oligonucleotides; specificity; annealing; diagnostic efficiency; biological samples; amplification

INTRODUCTION

In today's world, which includes globalisation, climate change, large-scale breeding and increasing numbers of pets, animal diseases are becoming an increasing problem. These conditions favour the spread of infections, affecting not only the welfare of pets. Scientists face the challenge of effectively controlling and preventing epidemics, as the rapid spread of infections can have a serious impact on various sectors of society. Therefore, the development and implementation of accurate diagnostic methods are critical for the timely detection and control of diseases and the minimisation of their adverse effects, hence the research.

A. Safarov *et al.* (2021), canine distemper is a serious viral disease occurring in puppies and adult dogs. Common manifestations include increased body temperature, digestive disturbances and diarrhoea, sudden vomiting, lethargy, depression, loss of appetite, and symptoms involving the respiratory, eye, skin, or nervous system. In some cases, complications may occur in the form of pneumonia, which can be fatal, or paralysis. K. Altay *et al.* (2023) and K. Beus *et al.* (2023) state that accurate detection of the disease plays an important role in controlling and preventing the spread of the disease. The most effective method for diagnosing rinderpest today is polymerase chain reaction (PCR). This method detects the pathogen at early stages, even when there are no obvious signs of the disease. PCR is characterised by the possibility of multiple amplification of genetic material and visualisation using fluorescent dyes, which makes it an effective tool for early detection and accurate diagnosis. In the context of this method, a range of disagreements and opinions arise among researchers focusing on its efficiency, cost-effectiveness and comparative sensitivity compared to alternative approaches. The innovativeness of multiplex primers is manifested in their ability to amplify multiple RNA sites simultaneously. This increases diagnostic efficiency, speeding up the detection of rinderpest virus and providing a wider range of information about the presence of infection.

R. Uakhit *et al.* (2022) also note that the use of multiplex primers also involves saving resources such as reagents and time. A single reaction can simultaneously cover multiple genes. However, for all their efficiency, optimising multiplex PCRs can be challenging. The diversity of plague virus genes requires careful selection of primers to avoid competition between them, which may affect the reliability of the results. Y. Yamamoto *et al.* (2021) raised concerns about the sensitivity of multiplex PCR. Possible interactions between primers may reduce the sensitivity of the method, which requires careful validation of the results and comparison with alternative techniques. To ensure the validity of the results, additional tests and data verification are critical. This is particularly important in the case of multiplex assays, where it is potentially difficult to interpret the results. Given the advances in molecular biology, the use of PCR and multiplex primers represents a promising avenue in the development of diagnostic methods that allow more accurate and rapid detection of the causative agent.

Thus, the use of multiplex primers in PCR represents an innovative approach that can significantly improve diagnostic accuracy and efficiency. Such technological developments are of great importance in modern medical practice. The study aims to review and evaluate the efficacy of using multiplex primers in a polymerase chain reaction method for the detection of RNA of rinderpest virus. The main focus is to identify the advantages of this method compared to conventional approaches for the diagnosis of this highly contagious virus. Key objectives of the study include: selection of primers and analysis of genetic sequences; specificity assessment and development of diagnostic primers; evaluation of the sensitivity of the PCR method.

MATERIALS AND METHODS

The research was carried out between 2022 and 2023 at the Kyrgyz Research Institute of Veterinary Medicine named after Arstanbek Duisheyev и Kyrgyz National Agrarian University named after K.I. Skryabin laboratory.

The National Centre for Biotechnology Information (NCBI) bioinformation database was used to obtain genetic data of the plague virus. Oligonucleotide sequences were analysed in GenBank. Carnivore plague genes were analysed using the BLAST programme. The selection of primer parameters was performed using the MEGA 10 software package, which includes the Clustal program, as well as manual selection. As part of the experiment, samples such as blood serum and eye conjunctiva washes were collected from 50 animals with clinical manifestations of the disease. The dogs ranged in age from 2 months to 7 years.

In the preparation of biological materials for research, blood serum was used, which was obtained from the blood of animals, and collected in special vacuum tubes. The tubes with blood were left in the refrigerator at +4°C for 1-2 hours for complete clot formation. The serum was then transferred to a smaller tube and stored at -20°C until the time of testing. Special sterile cotton swabs were used to obtain flushes from animals. The sticks were used to wipe the mucous membrane and then placed in a tube with a physiological solution. After vortexing and centrifugation, the material was stored at -20°C. For the study, the methodology included the following steps:

1. RNA isolation of viruses using a commercial RIBO-sorb kit (AmpliSens) according to the instructions for use. These steps provided high-quality RNA for subsequent analysis steps.

2. PCR using selected primers: after obtaining RNA from the samples, a polymerase chain reaction was performed using selected primers. PCR parameters were optimised for maximum specificity and amplification efficiency.

3. Validation of PCR results: the PCR products obtained were subjected to agarose gel analysis to verify their size and purity.

4. Vaccine strain experiment: RNA from the Onderstepoort vaccine strain was used to optimise PCR conditions and validate the methodology. This step

provided additional data for the correct interpretation of the results and determination of the sensitivity of the method.

5. Reverse transcription: reverse transcription processes were performed using a commercial Qiagen kit (Qiagen, Germany) according to the instructions for use.

This integrated approach to methodology allows for the reliable acquisition and analysis of genetic data from the carnivore plague virus and maintains the accuracy and validity of the study results. The results were processed for reliability using multivariate analysis of variance MANOVA using Microsoft Excel software and the Statistica 10 software package. Differences in the obtained results are possible at a significance level of $p \leq 0.05$ using the Student's criterion. No animals were harmed during sampling.

RESULTS

Rinderpest virus undergoes an evolutionary development that can be traced at the phylogenetic level down to its older roots. Over time, under the influence of a variety of environmental factors, the rinderpest virus is periodically infected, resulting in the acquisition of new genetic properties. These changes are accompanied by nucleotide mutations, and as a result, the carnivore plague virus is currently represented by several genetic lineages (Trogu *et al.*, 2021). It is important to consider the variability of the virus gene when making a diagnosis. In this study, primers were selected to account for the different variations of the carnivore plague pathogen. After processing all data using Clustal software, an aligned gene was obtained. The study allowed the selection of a relatively conserved region suitable for primer design. However, the gene alignment showed differences in nucleotide sequence between strains of rinderpest virus. This makes the detection of identical gene regions a challenge. The most similar gene regions of this virus were adenine (A) and thymine (T) rich, which precluded their use. As a result, specific gene regions were selected, and appropriate primers were designed (Table 1).

Table 1. Nucleotide sequences of direct primers for the carnivore plague virus gene

Primer	Oligonucleotide sequence	GenBank position	Length of the resulting product in nucleotide pairs
D-1	5'-TCGTTCTCTATCCCTCCT-3'	3,519-3,539	681
D-2	5'-GGCTCATACCCAAGTCAGA-3'	4,180-4,200	

Source: compiled by the authors

For diagnostic use, the primers selected should have maximum specificity to specific regions of the sample. The low specificity of primers used in diagnostics may cause false negatives. For this reason, diagnostic primers are subject to specificity requirements to avoid errors in

the results. To develop diagnostic primers that would apply to all strains of rinderpest virus, their specificity was tested using computer programs. Genetic information on different strains of rinderpest virus was extracted from GenBank (Fig. 1 and 2). From the studies presented in

Figures 1 and 2, it can be seen that the selected primers were not found to be specific for all strains of the tested virus. The forward primer was identical for strains 1-3, 4, 7, 10 and 13, but did not show specificity to some strains at three sites: C (cytosine)→A, A→G (guanine) and

C→T. Similar to the forward primer, the reverse primer was found to be specific for certain strains of carnivore plague virus. It perfectly matches strains of lines 1-5, 9-11, and 15-19, but has differences in the sequence of nitrogenous bases at two and three sites.

0	G	G	C	T	C	A	T	A	C	C	C	C	A	A	G	T	C	A	G	A
1	-	-	-	-	A	G	-	-	-	-	T	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	A	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	A	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	A	G	-	-	-	-	T	-	-	-	-	-	-	-	-	-
9	-	-	-	-	A	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	A	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	A	G	-	-	-	-	T	-	-	-	-	-	-	-	-	-
15	-	-	-	-	A	G	-	-	-	-	T	-	-	-	-	-	-	-	-	-
16	-	-	-	-	A	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	A	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	A	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	A	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	A	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	A	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Figure 1. Evaluation of the forward primer specificity

Note: 0 – primer sequence; 1-21 – different strains of rinderpest virus strains

Source: compiled by the authors

0	T	C	G	T	T	C	C	T	C	C	T	A	T	C	C	C	T	C	C	T
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	G	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-
3	-	G	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-
4	-	G	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	G	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-
10	-	G	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-
11	-	G	-	-	-	-	-	C	-	-	-	-	-	T	-	-	-	-	-	-
12	-	G	-	-	-	-	-	C	-	-	-	-	-	T	-	-	-	-	-	-
13	-	G	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	G	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-
16	-	G	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Figure 2. Results of reverse primer specificity testing

Note: 0 – primer sequence; 1-21 – different strains of rinderpest virus strains

Source: compiled by the authors

Other variants of primer selection for gene sections without variations were also tested in the course of the work. However, it proved difficult to select primers with

absolute (100%) specificity when examining other gene sections. Some sections of the plague virus gene remained unchanged but contained excess adenine and

thymine or were too short. When selecting primers, their length and the ratio of nitrogenous bases play an important role, which significantly affects the accuracy of the polymerase chain reaction. For example, if adenine and thymine predominate, the annealing temperature may be low, which increases the risk of decreasing the specificity of primers and thus the quality of PCR analysis. However, too high guanine and cytosine content also affect the quality of PCR analysis, as excess of these bases can lead to the formation of secondary product structure and slow down the activity of DNA polymerase (Alfano *et al.*, 2020).

Under the influence of these factors, the sensitivity of the PCR method may be reduced, which may lead to false negatives. With this in mind, primers rich in G and C nitrogenous bases are favoured when selecting primers. By analysing all possible variants, another pair

of primers was designed considering the sequence differences between 3,519-3,539 and 4,180-4,200 using the multiplex PCR principle, in this case, two pairs of primers are involved in the analysis. When multiplex PCR is used to diagnose several types of diseases, it is necessary to provide different lengths of PCR products for each species. When designing primers for multiplex polymerase chain reactions, it is important to avoid mutual complementarity. In this case, the size of the polymerase chain reaction products will retain homogeneity since the goal is not to differentiate between strains of rinderpest virus but to identify the pathogen itself. Considering previous results, the design of primers was modified to make them universal for multiplex PCR. Thus, two primer pairs were obtained instead of one (Fig. 3).

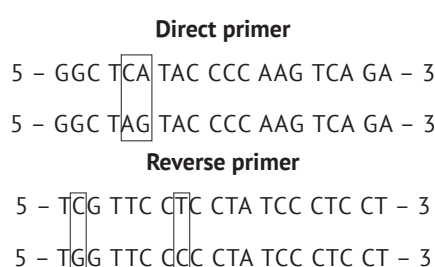


Figure 3. Multiplex primer pairs with modified sequences

Source: compiled by the authors

To adapt to the variability of the study site, the nitrogenous bases of the primers were modified. The forward primer was modified by replacing two sites (C→A, A→G), and the reverse primer was modified by replacing two sites (C→G and T→C). In subsequent studies, all four primers were applied simultaneously using multiplex PCR, which increases the probability of interaction between them. However, increasing the concentration of primers may favour the formation of dimers, which affects the specificity of the reaction. Dimerization results in the formation of a variety of PCR products, and false products may compete with the formation of the product under investigation. These problems are exacerbated when low-temperature annealing and excessive annealing times are used. Dimers have a significant impact on real-time polymerase chain reaction (PCR-RV) results, especially when using an asymmetric cyanine dye (SYBR Green I). When this dye is used, any product obtained during the reaction is considered a positive result. Therefore, dimers contribute to false positives when SYBR Green I is used in PCR-RV.

The primers proposed in the study, although almost identical, have slight differences in sequence, which provides a better diagnosis of the different genetic lineages of the rinderpest virus. The main principle determining specificity lies in the 3'-end of the primers. This end, according to the principle of complementarity, should be at least 4 nucleotide pairs

closer to the tested material. The complementarity of the 3'-end of the primer with the test material significantly affects the sensitivity of PCR. Therefore, this feature should be considered when selecting primers. Another characteristic feature is the fact that when annealing 3'-ends to each other, primers practically lose their efficiency, unable to interact correctly with the material under study. In such situations, the polymerase chain reaction may demonstrate a negative result. Taking all these features into account, the first two primers were analysed using the developed validation method. The results of this test allowed us to evaluate the specificity of the primers against different strains of rinderpest virus.

To develop the design of primers used in multiplex PCR, a grouping of rinderpest virus strains based on the similarity of nitrogenous bases was carried out. This allowed the design of four primers, two for each of the forward and reverse directions corresponding to each strain group. Genetic data from GenBank were used to form a group of 20 strains of rinderpest virus representing different genetic lineages of the virus. Before forming this group, many variants of the carnivore plague gene were analysed using BLAST software. From the entire dataset, 20 plague virus sequences representing different genetic lineages of the virus were selected and divided into four groups for subsequent specificity analysis using each primer.

The results of the reverse primer specificity study of variant No. 1 revealed that for one line of carnivore plague virus strains, complete specificity was not observed when nucleotide C was replaced by T (Fig. 4). However, in the remaining cases, a complete match was achieved. Similar results were obtained when analysing

the second variant of the reverse primer (Fig. 5). The specificity of the forward primer was also analysed, and the results showed that the first variant of the forward primer had mismatches at one site where C is replaced by T (Fig. 6). The second variant of the forward primer achieved complete similarity in all cases (Fig. 7).

0	T	G	G	T	T	C	C	C	C	T	A	T	C	C	C	T	C	C	T
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Figure 4. Specificity of reverse primer, variant No. 1

Note: 0 – primer sequence; 1-13 – different strains of rinderpest virus strains

Source: compiled by the authors

0	T	C	G	T	T	C	C	T	C	C	T	A	T	C	C	C	T	C	C	T
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Figure 5. Specificity of reverse primer, variant No. 2

Note: 0 – primer sequence; 1-7 – different strains of rinderpest virus strains

Source: compiled by the authors

0	G	G	C	T	A	G	T	A	C	C	C	C	A	A	G	T	C	A	G	A
1	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Figure 6. Specificity of the forward primer, variant No. 1

Note: 0 – primer sequence; 1-7 – different strains of rinderpest virus strains

Source: compiled by the authors

0	G	G	C	T	C	A	T	A	C	C	C	C	A	A	G	T	C	A	G	A
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Figure 7. Specificity of forward primer variant No. 2

Note: 0 – primer sequence; 1-7 – different strains of rinderpest virus strains

Source: compiled by the authors

In two cases, the primers obtained had some differences in nucleotide sequences, but these changes should not significantly affect PCR sensitivity. It is important to note that such small substitutions in the middle of the primer usually do not adversely affect PCR sensitivity. However, mismatches at the 3' end should be avoided during primer selection. Currently, a wide range of computer programs is available that greatly simplify the primer selection process. These programmes facilitate the experimental stage of primer design, allowing researchers to quickly select the necessary primers, determine annealing conditions for PCR reactions, and detect variability in gene regions based on sequenced gene variants. This information significantly reduces the time spent on experiments in laboratories.

Despite the abundance of available computer programs capable of identifying potential primers, their efficiency and performance can only be assessed through laboratory experiments. In the subsequent stage of the work, the selected primers were repeatedly processed using computer programs to establish the annealing temperature and melting temperature. For the initial determination of the annealing temperature, a gradient temperature regime is often used. Given the participation of two primer pairs in the reaction, the possibility of dimer and hairpin formation increases significantly. The appearance of dimers significantly affects the sensitivity of the polymerase

chain reaction. In addition, another important factor affecting the formation of dimers is the presence of the enzyme revertase. Revertase used in reverse transcription can promote dimer formation. To exclude this factor, revertase was inactivated after the reverse transcription reaction by incubating for 3 minutes at 95°C. Inactivated samples were used to perform gradient PCR. The selected temperature regime included the following values from 53°C to 57°C.

For polymerase chain reaction using the selected primers, a 20 µl reagent mixture was prepared with a total volume of 20 µl, including the following components: 4 µl of 5x buffer, 5.2 µl of deionised water, 3 µl of CDV-1 primer mixture (0.6 µM concentration), 3 µl of CDV-2 primer mixture (0.6 µM concentration), 0.4 µl of 10x dNTP mix, 1.2 µl of 25 mM MgCl₂, 3 µl of cDNA with a concentration of 25 ng/µl, 0.2 µl of Taq DNA polymerase. This configuration of the reaction mixture was used for subsequent PCR to amplify the gene under study. Reverse primers were mixed with reverse primers and forward primers were mixed with forward primers, the mixing ratio being 50:50. During PCR, annealing was performed at temperatures of 53°C, 54°C, 55°C, 56°C and 57°C for 40 seconds, elongation at 72°C continued for 45 seconds and denaturation at 95°C for 30 seconds. These conditions were used to perform PCR using positive samples known to contain the causative agent of carnivore plague (Fig. 8).

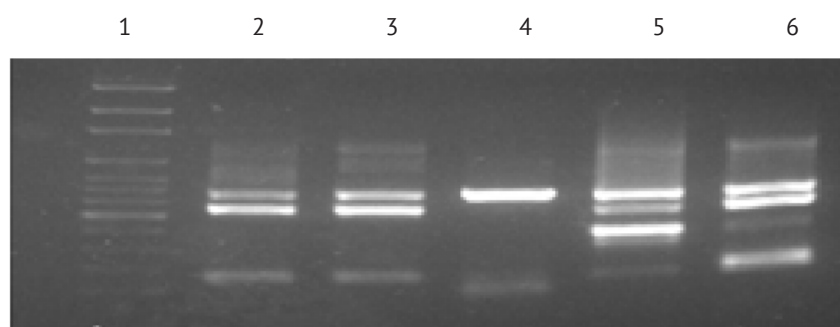


Figure 8. Spectra of amplification products at different temperature regimes

Note: 1 – 100 bp molecular weight marker; 2 – 53°C; 3 – 54°C; 4 – 55°C; 5 – 56°C; 6 – 57°C

Source: compiled by the authors

Different primer annealing temperatures were found to produce PCR products, but the optimum temperature to obtain a clear and specific result was 55°C. Different annealing temperatures (53°C, 54°C, 56°C, and 57°C) lead to the appearance of non-specific products, as shown by the luminescent bands at the level of 500-600 bp relative to the marker. The

annealing temperature of 55°C is optimal because at this temperature there are no non-specific products and the product obtained is at 681 bp, indicating the high specificity of the primer used. To confirm these results, an additional PCR was performed using newly selected primers and 55°C annealing temperature (Fig. 9).



Figure 9. Results of PCR product amplification of 681 nucleotide pairs in length

Note: Line 1 – molecular weight marker, lines 2 to 4 – known samples in advance, line 5 – negative control, line 6 – positive control

Source: compiled by the authors

Thus, the selected primers were successfully optimised for multiplex PCR. This is confirmed by the fact that the PCR products obtained are at the expected nucleotide length of 681 bp. No dimers or other non-specific products were detected during multiplex PCR. This is important because their presence can negatively affect the final results and accuracy of the assay. The negative control (sample No. 5) confirms the purity of the results. The absence of dimers and dimers in the negative control indicates that the selected primers do not possess self-sparking nucleotide sites. It was experimentally confirmed that the selected primer is successfully used in the polymerase chain reaction for the detection of the causative agent of plague in carnivores. Thus, the approach to selecting and optimising primers for multiplex PCR is successful and the results demonstrate the specificity and analysis purity.

DISCUSSION

Carnivore plague is a disease that has historically had a significant impact on domestic dogs. First described in ancient times, this infection still poses a serious threat to animal health, especially in regions with inadequate sanitary infrastructure. Rapid and accurate detection of the presence of virus in infected samples is essential to effectively control the spread of rinderpest (Uakhit *et al.*, 2022; Takeda *et al.*, 2020; Tan *et al.*, 2011). Carnivore plague is characterised by rapid spread in the carnivore population and remains a major challenge for veterinary medicine, requiring continuous improvement of diagnostic and prophylactic methods to con-

trol and prevent the consequences of epidemics. The contagiousness of the virus emphasises the need for rapid control measures in the event of outbreaks. Effective prevention, including vaccination and quarantine strategies, also plays an important role in managing the risks and limiting the negative consequences of rinderpest epidemics.

Studies conducted by authors such as K. Ergunay *et al.* (2022) and Y. Yamamoto *et al.* (2021) emphasise the difficulties in the treatment of carnivore plague. The peculiarities of this disease are the involvement of the central nervous system, which makes it difficult to treat. To date, no effective therapy exists, making carnivore plague a serious threat to animals. One of the factors hampering the development of effective therapies is the peculiarities of the reproductive cycle of the virus that causes the disease. These peculiarities create difficulties in the search for approaches capable of targeting the virus and interrupting its life cycle. In addition, differential diagnosis of rinderpest is important, given that many other diseases show similar clinical signs, such as parvovirus enteritis and other diseases. Distinguishing between these diseases is key to accurate diagnosis and the subsequent provision of appropriate treatment, as this study also confirms.

M. Karki *et al.* (2022) noted that the key to controlling rinderpest is early and accurate detection of the presence of the virus before it crosses the blood-brain barrier. This is of particular importance to pet owners and veterinary professionals who care for sick animals. Treatment depends on the form of the disease

(respiratory, intestinal, or cutaneous), so early and accurate diagnosis becomes crucial. Traditional methods for diagnosing rinderpest virus can have disadvantages such as low specificity or limited ability to detect different strains. Multiplex primer testing seeks to overcome these limitations and represents a promising and effective approach, providing more accurate and rapid detection of the virus while minimising the disadvantages of traditional methods.

The multiplex primer-based methodology for the diagnosis of rinderpest virus represents an advanced approach in veterinary medicine. Multiplex primers, short nucleotide sequences, are designed to detect multiple RNA variants of the virus, providing efficient detection and differentiation of different strains. This specificity for different strains is a key advantage of the method, allowing more accurate characterisation of the virus and targeted control measures. K. Beus *et al.* (2023) confirm the focus on optimising PCR reaction conditions. Thus, during PCR using multiplex primers, virus RNA is amplified, generating sufficient material for subsequent analysis. Experimental steps involve optimising reaction conditions, such as annealing temperature and PCR time, to ensure maximum specificity and efficiency. The resulting products are subjected to confirmation assays such as electrophoresis, using control samples to verify the results.

K. Altay *et al.* (2023) and R.M. Nayak *et al.* (2020), focused on innovative methods, and support efforts in the use of multiplex primers. The authors believe that polymerase chain reaction, as a high-tech method, allows not only the diagnosis of infection but also the differential analysis of the genetic material of the virus. The intervention of multiplex primers in the PCR process represents an innovative approach that allows simultaneous consideration of multiple sites of the virus RNA, which significantly increases the efficiency of testing. Z. Lanszki *et al.* (2021) and Y. Gedik *et al.* (2021), emphasising multiplex methods and their advantages, are also consistent with the discussion. Multiplex primers are designed to specifically amplify multiple genetic sites, making the method more sensitive and specific. This approach is particularly useful when the virus may manifest in different variants or when multiple strains need to be detected simultaneously. The use of multiplex primers also shortens the analysis time and reduces the likelihood of false positives, which is essential for rapid diagnostic measures, as also noted in the study conducted.

Polymerase chain reaction in the laboratory diagnosis of infections is characterised by high efficiency due to its rapidity, unrivalled sensitivity, and high specificity. It allows the detection of microorganisms even at very low concentrations, e.g. 1-10 pathogens in a sample. RNA of infectious agents is successfully extracted from various biological fluids, tissues, and from the environment such as soil, water, and food. PCR is

effective in detecting bacteria, fungi, parasites, and viruses (Saltik & Kale, 2020; Worsley-Tonks *et al.*, 2021). R. Uakhit *et al.* (2022) state that the PCR method is characterised by a sensitivity sufficient to detect 10-100 pg of viral RNA in a sample. This method allows rapid and quantitative detection of RNA even in unconcentrated samples. It also enables the examination of various surfaces (animal bodies, care items, soil, feed) for the presence of viruses. However, the author notes that the hypersensitivity of the method can sometimes lead to false positives.

F. Alfano *et al.* (2022), D.K. Yang *et al.* (2020), and A. Beineke *et al.* (2009) applied polymerase chain reaction to detect viral RNA in biological samples. According to the authors, the application of PCR is in the works to become a powerful tool for determining the presence and type of virus in a variety of biological samples, including tissues, fluids, and the environment. The results of their research propel PCR into the role of a key technique in the diagnosis of viral diseases, emphasising its importance in modern medical science and veterinary medicine. They open new horizons in the understanding of viral pathologies and provide the basis for the development of more effective treatment and prevention methods. I. Ricci *et al.* (2021) also believe that the use of multiplex primers in PCR represents a modern diagnostic method with improved specificity and sensitivity. The use of multiplex primers in the polymerase chain reaction method for the detection of RNA of rinderpest virus is an important method in veterinary diagnostics, providing effective detection and identification of different strains of the virus, which also confirms the conducted study.

All studies reviewed, including the present study, emphasise the need for effective control of rinderpest, and detection of the virus before central nervous system involvement, and encourage the use of innovative diagnostic methods. However, in comparison to the authors' results, the study conducted adds to the existing body of knowledge by bringing innovation in diagnostic methods for carnivore plague. Understanding the commonalities and differences helps to create a more complete picture of the issues, which is an important contribution to the field of veterinary medicine. Thus, the polymerase chain reaction method is a powerful tool for detecting and analysing the genetic material of microorganisms, including rinderpest virus. However, the efficiency of PCR directly depends on the use of specific and highly efficient primers. Therefore, multiplex primers represent a promising approach for the simultaneous detection of several virus genes, which increases the sensitivity and accuracy of diagnosis.

CONCLUSIONS

The carnivore plague virus exhibits phylogenetic branching due to the effects of various environmental factors. This variability is manifested through

nucleotide mutations, resulting in the formation of different genetic lineages of the virus. The study aimed to select multiplex primer pairs for the detection of RNA of carnivore plague virus by PCR represents a significant step in the field of veterinary diagnostics and animal disease control.

As a result of laboratory experiments, multiplex pairs of primers were selected for the detection of plague virus RNA using PCR in biological materials such as blood serum and eye conjunctiva washes containing the virus. A distinctive feature of the use of these primers is the use of two pairs of the same gene region with different variable sequences. These sequences are specific for all strains of carnivore plague virus. Both pairs of primers are applied simultaneously at equal concentrations and under the same conditions in the polymerase chain reaction, and the results obtained demonstrate the high specificity of the designed primers. Additional PCR performed using the selected primers and optimal annealing temperature confirmed the successful amplification

of specific sites and the high specificity of the applied primers. The absence of dimers and non-specific products in the negative control was observed, reinforcing the purity and reliability of the results obtained. Consequently, the results convince us that these primers used in multiplex PCR can effectively detect RNA of rinderpest virus of different strains circulating worldwide.

The developed multiplex primers represent an innovative and practically important method for the diagnosis of rinderpest virus, with great potential for application in veterinary practice and animal disease control. Further research on this issue should focus on the interaction of the rinderpest virus with other infections that may affect diagnosis and epidemiology.

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None.

CONFLICT OF INTEREST

None.

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Використання мультиплексних праймерів для виявлення РНК вірусу чуми м'ясоїдних методом ПЛР

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Анотація. З урахуванням високої контагіозності та швидкого поширення вірусу чуми м'ясоїдних, своєчасна та точна діагностика відіграє ключову роль у запобіганні епідеміям та вжиття заходів щодо контролю за захворюванням. Мета дослідження – провести оцінку ефективності використання мультиплексних праймерів у методі полімеразної ланцюгової реакції для виявлення рибонуклеїнової кислоти вірусу чуми м'ясоїдних. Дослідження включало аналіз зразків, таких як сироватка крові і змив з кон'юнктиви очей у 50 тварин з клінічними проявами захворювання. В рамках експерименту проводився збір клінічних зразків, таких як сироватка крові та змив з кон'юнктиви очей. Отримані результати демонструють високу специфічність розроблених праймерів. Ці праймери виділяються тим, що вони використовують дві пари однієї й тієї ж ділянки гена з різними варіабельними послідовностями, які є специфічними для всіх штамів вірусу чуми м'ясоїдних. У процесі полімеразної ланцюгової реакції обидві пари праймерів застосовуються одночасно за рівних концентрацій та однакових умов. Додаткова полімеразна ланцюгова реакція, проведена з використанням цих праймерів при оптимальній температурі відпалу, підтвердила успішну ампліфікацію та специфічність праймерів. Відсутність димерів та неспецифічних продуктів у негативному контролі підтверджує чистоту та надійність отриманих результатів. Таким чином, дані результати переконують у тому, що використання цих мультиплексних праймерів полімеразної ланцюгової реакції дозволяє ефективно виявляти рибонуклеїнову кислоту вірусу чуми м'ясоїдних різних штамів. Розроблені мультиплексні праймери представляють інноваційний метод для діагностики вірусу чуми м'ясоїдних з потенціалом для застосування у ветеринарній практиці та контролю захворювань тварин

Ключові слова: олігонуклеотиди; специфічність; відпал; ефективність діагностики; біологічні зразки; ампліфікація



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Utilisation of livestock by-products for resource-saving biogas production in industrial pork production

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Abstract. Modern energy systems are complex multi-component systems that use mechanical, thermal, and electrical energy. As fossil fuel reserves decline, interest in renewable energy sources is growing everywhere, which is driving research into biogas production technologies. The purpose of this study was to evaluate technological approaches to reduce resource costs to produce renewable energy sources from organic waste (manure) from pig farms. This study employed the bibliographic method

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of research, laboratory method, biochemical methods (determination of the chemical composition of manure and wastewater), statistical, mathematical (calculation of economic efficiency), multicriteria analysis, and analytical method. Based on the conducted research, a resource-saving technology for the preparation of wastewater for use in the production of renewable energy in industrial pork production was developed, which ensures a reduction in capital and operating costs for biogas generation. It was found that the settling of the initial wastewater with a moisture content of $96.94 \pm 0.18\%$ produces a sediment with a moisture content of $91.23 \pm 0.25\%$ and a liquid fraction with a moisture content of $98.86 \pm 0.42\%$. At the same time, the organic matter in the sediment extracted from the original wastewater is about 87%, and in the liquid fraction – about 98% of the dry matter. The technological process includes centrifugal and gravity separation of the initial wastewater into fractions (sediment and liquid fraction), gravitational thickening of the sediment and its dosing for methane digestion to produce biogas. It was shown that the application of the proposed approaches can reduce capital investments by about 30%. Based on the multicriteria analysis, it was found that the proposed biogas production technology has a significant advantage in terms of the complex efficiency indicator of each of the considered options $N(C_k)$ compared to the idealised one. For this technology, the objective function is the smallest according to the criteria considered and amounts to 0.1672, while the objective function of the baseline technology is 1.9 times worse. The findings of the study on the use of livestock by-products for resource-saving biogas production are planned to be used at pork production complexes and farms.

Keywords: renewable energy sources; bioenergy; wastewater; technology; sediment; resource conservation; capital investments

INTRODUCTION

Ukraine's energy sector is facing a challenging task – to reduce the consumption of natural gas and coal in electricity generation and replace them with renewable energy sources (RES) to the maximum extent possible. On the one hand, this would reduce the cost of production and improve its competitiveness in the global market. On the other hand, the combustion of fossil carbohydrates – coal, gas, oil, and their derivatives – is considered the main source of CO₂ emissions, which contributes to global warming. Therefore, the campaign to replace fossil fuels with other environmentally friendly fuels, such as biofuels (biomethane, biohydrogen, bioethanol, etc.) is growing worldwide (Dabirian *et al.*, 2023). This provides an impetus for the widespread use of RES (specifically, farm animal manure) processing plants for electricity or heat in the national economy and is a promising area (Skrylnyk *et al.*, 2020).

The need to feed humanity has contributed to the development of animal husbandry, the development of technologies for intensive farming of livestock and poultry, and the construction of complexes or large farms. As a result, a massive amount of livestock and poultry waste has been generated, which is the main source of agricultural pollution, especially in regions with a prominent concentration of livestock and poultry (Zhang *et al.*, 2019; Li *et al.*, 2022). According to M.J. Sukhesh and P.V. Rao (2019), in the United States, the amount of waste from livestock production is 130 times higher than human waste, which affects the local environment. Therefore, countries with developed livestock production need to develop and implement an effective strategy for the utilisation of livestock manure.

V. Burg *et al.* (2018) believe that manure, without quality processing, is a source of air and water pollution, but when integrated into the management chain, it can become a strong energy resource. The potential for biogas production from cattle manure in Europe is quite high, for instance, in Sweden it is estimated at about 3-6 TWh per year (Achinas & Everink, 2020). According to N. Yurchuk (2018), is an equipment that allows processing various types of organic raw materials into energy in the form of biogas and into highly effective organic fertilisers. They can also be used as wastewater treatment plants on farms, poultry farms, and processing plants, thereby improving their sanitary and hygienic conditions.

The European Union limits the amount of manure that can be applied to arable land. Therefore, in regions with a prominent concentration of livestock and a limited amount of arable land, such as large pig and poultry farms, it is necessary to remove manure or set up its processing. One of the elements of the manure processing system can be the biogas units. This will not only reduce the amount of dry matter to be exported but will also bring additional financial profit from the sale of biofuels. Studies conducted by scientists from different countries have made it possible to determine the approximate volumes of biogas output from agricultural waste processing (Lutkovska & Zelenchuk, 2023).

In Ukraine, livestock and poultry waste, specifically manure, can be another major and environmentally sound source for biofuel production. Thus, according to A. Doronin (2019), the potential biogas production in Ukraine from cattle waste in 2017 could be up to 1,920 mln m³/year, pigs – up to 971 mln m³/year,

poultry – up to 1,863 mln m³/year. According to G.M. Kaletnik and I.V. Honcharuk (2020), in 2020, the livestock population in Ukraine was within 3.1 mln heads of cattle, 5.7 mln heads of pigs, and 220.5 mln heads of poultry, from which about 92.8 mln t of animal by-products were produced. According to the calculations, the total potential for biomethane production from livestock waste will reach approximately 2.2 mln toe. And according to N.V. Pryshliak (2021), in 2019, about 40.65 mln t of waste was produced from animals in the private sector of Ukraine. If they are used in the biogas units, the theoretical biogas output could reach 1,760.3 mln m³.

According to the authors, the main obstacle to the introduction of bioenergy plants in industrial pork production is the considerable cost of creating and operating such systems, as well as the suboptimal characteristics of the material for the fermentation process. Thus, the purpose of this study was to develop and evaluate approaches to reducing resource costs in the production of renewable energy sources (biogas) in industrial pork production. However, high moisture content has a negative impact on biogas production, and therefore some work is needed to increase the dry matter content of the biomass fed to the biogas unit. This will help improve the quality of the output product, increase biogas yields, and increase the economic efficiency of the plants. One of the ways to optimise the substrate for methane digestion is to separate the pig manure into sediment and liquid fraction. This became the subject of this study. The purpose of this study was to develop and evaluate approaches to reducing resource consumption during the production of renewable energy sources (biogas) in industrial pork production.

MATERIALS AND METHODS

The wastewater studies were carried out at the Hlobinskyi Pig Farm LLC, Poltava Oblast, in 2016-2018. The technology of industrial pork production at Hlobinskyi Pig Farm LLC prescribes feeding pigs with complete feed according to sex and age groups in a liquid-feeding fattening shop. The microclimate was maintained by an automated ventilation system. The receiving tank was supplied with effluent from the buildings containing machines with partially raised slotted floors placed on bathtubs with valves. When the valves were raised, the effluent flowed to the central collectors and from there to the receiving tank. The receiving tank was equipped with a hydraulic bubbling system, which operated using centrifugal pump pressure. The outgoing wastewater was separated in a filter press.

The outgoing wastewater was sampled at the complex from the receiving tank. Therewith, liquid and solid fractions were selected after separation of the initial effluent on the filter press. In laboratory conditions, the effect of the settling process on the moisture content of the sediment obtained in the laboratory plant was investigated (Fig. 1).

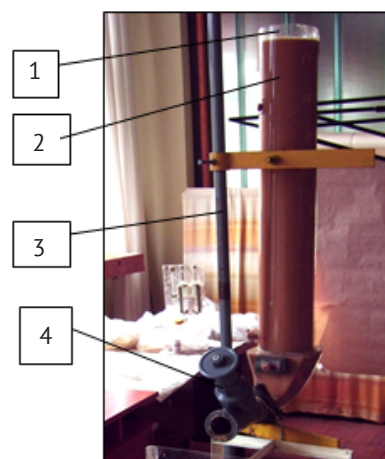


Figure 1. Laboratory installation for studying wastewater settling

Notes: 1 – organic glass unit body; 2 – outlet drains; 3 – tripod; 4 – latch

Source: compiled by the authors

During the experiment, the volume of the unit's body was thoriated to investigate the process of wastewater settling using a measuring cylinder. Before feeding the source wastewater to the laboratory unit, it was homogenised and sampled for moisture content. The moisture content of the sediment formed during sedimentation was monitored after 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5; 5.0 hours. After a respective period of time, during which the initial wastewater was settled, the sediment and liquid fraction were removed through valve 4 (Fig. 1). Sediment and liquid fraction samples were taken to determine moisture content and other parameters. The moisture content of the sediment was determined following DSTU ISO 6496:2005 (2006), using the following equation (Turovsky, 1982):

$$\frac{V_1}{V_2} = \frac{100 - W_2}{100 - W_1}, \quad (1)$$

where V_1 is the volume of output effluents, V_2 is the volume of sediment from output effluents, W_1 is the humidity of output effluents, W_2 is the humidity of sediment from output effluents. The volume of sediment was determined as the difference between the volume of the initial effluent and the volume of the liquid fraction. The volume of the liquid fraction was determined as the product of the cross-sectional area of the laboratory vessel and the height of the liquid fraction column.

The wastewater was assessed for moisture and dry matter (DSTU ISO 6496:2005, 2006), ash (DSTU ISO 5984:2004, 2006), and crude fibre (DSTU ISO 6865:2004, 2006). During the study, a method was developed to ensure a reduction in capital investment in the production of renewable energy sources in industrial pork production, based on ensuring the optimal value of the substrate parameters for methane fermentation. The substrate for methane digestion was

obtained from the original wastewater upon gravity settling and compaction. The effectiveness of the proposed approach to ensuring a reduction in capital investment in the development and implementation of technologies for the preparation of wastewater for use with the production of renewable energy sources (biogas) in industrial pork production was assessed, considering capital investment and operating costs. The annual economic effect of the proposed technology was determined according to the following formula (DSTU 46-012-2000, 2001):

$$E_{an} = (O_B + E_I K_{IB}) - (O_N + E_I K_{IN}), \quad (2)$$

where E_{an} is the annual economic effect of implementing the development; O is the operational costs in the basic ($_B$) and new ($_N$) variant; E_I is the coefficient of efficiency of innovative investments; K_I is the investments in the basic ($_B$) and new ($_N$) variant.

The method of multicriteria analysis was applied using the assessment of the integral criterion of the distance to the target. For this, an approach was used that included the aggregation of all criteria into one comprehensive performance indicator for each of the alternative variations (Piskun *et al.*, 2020).

RESULTS AND DISCUSSION

During the experiment, the effect of the settling process on the moisture content of the sediment obtained from the original wastewater in the laboratory plant was investigated (Fig. 2). With the concentrate type of feeding and the litterless technology of pig housing, outgoing effluents with a moisture content of $96.94 \pm 0.18\%$ were obtained. During the separation of the original wastewater on the filter press, a liquid fraction with a moisture content of $98.73 \pm 0.12\%$ was obtained, and a solid fraction with $73.16 \pm 0.18\%$ moisture content.

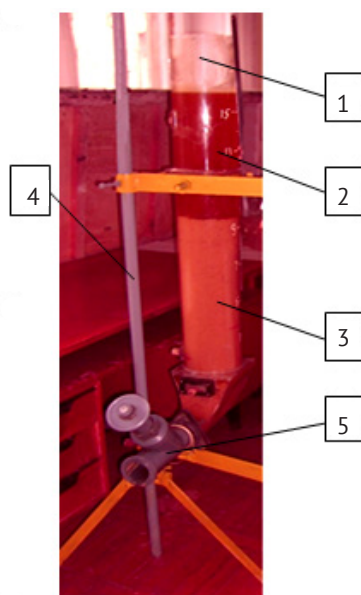


Figure 2. Study of the effect of the settling process on the moisture content of the sediment produced in a laboratory plant

Notes: 1 – Organic glass unit body; 2 – liquid fraction; 3 – sediment; 4 – tripod; 5 – latch

Source: compiled by the authors

Studies have shown that the settling of the liquid fraction obtained during the separation produces a precipitate with a moisture content of $92.57 \pm 0.47\%$ and a liquid fraction with a moisture content of $98.49 \pm 0.29\%$. That is, when the liquid fraction was stored in the storage pond, which was obtained from the original wastewater after separation in the filter press, a sediment with a moisture content of about 92% was formed. The organic matter in the effluent was $71.83 \pm 3.98\%$ of the dry matter content. At the same time, the organic matter in the sediment that was separated from the liquid fraction after separation of the original wastewater was about 98%, and in the liquid fraction – about 99% of the dry matter. When the initial wastewater was settled

with a moisture content of $96.94 \pm 0.18\%$, a sediment with a moisture content of $91.23 \pm 0.25\%$ and a liquid fraction with a moisture content of $98.86 \pm 0.42\%$ were formed. That is, during the storage of the original wastewater in the storage pond, a sediment with a moisture content of about 92% was formed. At the same time, the organic matter in the sediment extracted from the original wastewater was about 87%, and in the liquid fraction – about 98% of the dry matter.

Based on these and other studies, the provisions of the utility model patent “Method of wastewater treatment” (Piskun & Piskun, 2010), a variant of the process scheme for methane digestion of the initial wastewater in industrial pork production was developed, ensuring

a reduction in construction and operation costs while generating renewable energy sources (biogas). The technological process includes centrifugal and gravity separation of the initial wastewater into fractions (sediment and liquid fraction), gravity thickening of the

sediment obtained during centrifugal and gravity separation and periodic discharge of the thickened sediment for dosing when it is fed to methane digestion to produce biogas. When biogas is used in a generator, electricity and heat are produced (Fig. 3).

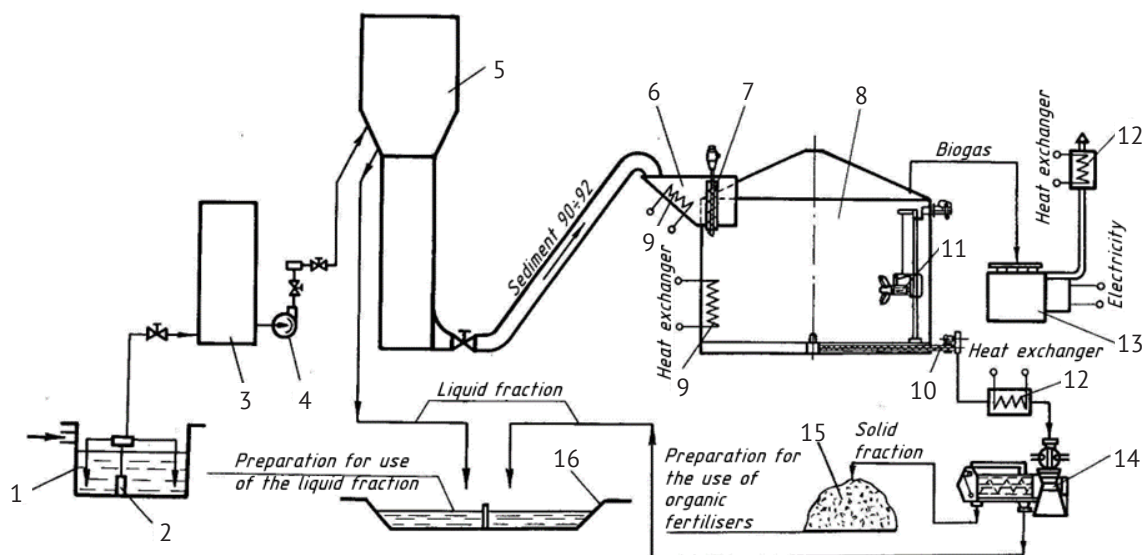


Figure 3. Diagram of a resource-saving biogas production line for industrial pork production

Notes: 1 – receiving tank; 2, 4 – sanitary pumps; 3 – intermediate tank; 5 – device for centrifugal-gravity separation of the initial wastewater; 6 – dosing tank; 7 – device for dosed sediment supply; 8 – digester; 9 – heat exchanger; 10 – sediment discharge device; 11 – stirrer; 12 – heat recuperator; 13 – power generation unit; 14 – filter presses; 15 – solid fraction pile; 16 – storage pond

Source: N.V. Piskun and V.I. Piskun (2010)

The technological process of obtaining renewable energy sources (biogas), according to the scheme developed in this study, is as follows. The wastewater from rooms with slotted floors and bathtubs is conveyed by collectors to the receiving tank 1, from where it is transferred to the intermediate tank 3 by means of a pump 2. Subsequently, pumps 4 convey the wastewater to the device for centrifugal and gravity separation of the original wastewater 5, where the wastewater is separated into a liquid fraction and sediment, which is compacted to a moisture content of 90–92% and subsequently fed to the dosing tank 6. The liquid fraction is fed to the storage pond 16. The sediment from the dosing tank 6 is periodically fed to the methane digester 8 by the sediment dosing device 7 according to the technological regime of methane digestion. The resulting biogas is fed to the power generation unit 13. The fermented sediment from the digester 8 is fed to the filter press 14, where it is separated into solid and liquid fractions using the sediment discharge device 10. The solid fraction is delivered to the site, where a solid fraction pile 15 is formed, undergoes biothermal treatment, and is subsequently used as organic fertiliser. The liquid fraction goes to the storage pond 16 and, after quarantine, is used to fertilise agricultural land. The resulting biogas

is converted into electricity and heat in the power generation unit 13. They can be used in the electrical and heating systems of an enterprise. Surplus electricity can be supplied to the external power grid through a transformer substation and a distribution point.

The design parameters of the digester, solid fraction pads, and liquid fraction storage tanks are determined using the following analytical relationships. According to the known factual data characterising the quality of separation into individual elements of the technological line, such as the mass M and moisture W of the effluents entering the treatment and received at the exit, the mass M_p and moisture W_p of the liquid fraction and the mass M_o and moisture W_o of sediment, V_o is the volume of sediment considering the approaches developed by N.G. Kovalev *et al.* (1983). Based on the balance equations for the mass flow rate of the current through the separation element, the following data are obtained:

η_p, η_o – efficiency of separation into liquid fraction and sediment; C_p, C_o – the concentration of the liquid fraction of effluents and sediment. If M, W, W_p, W_o are given, the main characteristics of the separation quality are determined according to Equations 3-7:

$$M_T = \frac{M^*(W_p - W)}{W_p - W_o}, \quad (3)$$

$$M_P = \frac{M^*(W-W_O)}{W_P-W_O} \text{ or } M_P = M - M_O, \quad (4)$$

$$C_P = 1 - 0.01W_P; C_T = 1 - 0.01W_O; C = 1 - 0.01W, \quad (5)$$

$$\eta_P = 1 - \frac{(M_P * C_P)}{M * C} = 1 - \frac{M_P * (100 - W_P)}{M * (100 - W)}, \quad (6)$$

$$\eta_T = 1 - \frac{(M_O * C_O)}{M * C} = 1 - \frac{M_O * (100 - W_O)}{M * (100 - W)} \text{ or } \eta_T = 1 - \eta_P. \quad (7)$$

The presented equations make it possible to determine the volume of sediment and subsequently the volume of digesters based on factual data on the outgoing effluent, considering the digestion modes. Estimation of the cost of processing the initial wastewater in industrial pork production with bedding-free housing and renewable energy sources (biogas) for the baseline and new variants is presented in Table 1.

Table 1. Costs of wastewater treatment to produce biogas

Indicators	Variants			
	New		Basic	
	UAH	%	UAH	%
Volume of wastewater treated	146,000 tonnes			
Capital investments:	17,549,496	100.0	25,801,300	100.0
Production costs:				
remuneration of labour	102,000	6.19	84,000	3.58
deductions for social events	23,500	1.43	19,320	0.82
electricity costs	142,884	8.67	214,620	9.15
Heat energy costs	119,837	7.27	199,728	8.51
amortisation total	877,475	53.24	1,290,065	54.98
Maintenance – total	350,510	21.27	516,026	21.99
Low-value assets	11,500	0.69	6,000	0.25
Total production costs	20,400	1.24	16,800	0.72
Total production costs (prime cost)	1,648,106	100.0	2,346,559	100.0
Cost reduction, %	29.8		–	

Source: compiled by the authors

An assessment of wastewater treatment technologies for use at a pork production complex for biogas production showed that to process 146,000 tonnes per year, capital investments under the basic technology amounted to UAH 2,346,559, and under the proposed technology – UAH 1,648,106. In other words, the proposed technology for preparing liquid manure for use in renewable energy production will reduce capital investment by 29.8%. Based on the data obtained, a multicriteria analysis of wastewater treatment

technologies for use in industrial pork production was conducted. The results are presented in Table 2. The analysis results suggest that the proposed biogas production technology has a considerable advantage in terms of the complex efficiency indicator of each of the considered options $N(C_k)$ compared to the idealised one. For this technology, the objective function is the smallest according to the criteria considered and amounts to 0.1672, while the objective function of the baseline technology is 1.9 times worse.

Table 2. Results of multicriteria analysis of wastewater treatment technologies for use in industrial pork production

Indicators	Variants	
	New	Basic
Capital investments:	1	1.4702
Production costs:		
remuneration of labour	1.2143	1
deductions for social events	1.2163	1
electricity costs	1	1.5021
heat energy costs	1	1.6667
amortisation total	1	1.4702
Maintenance – total	1	1.4722
Low-value assets	1.9167	1
Total	9.3373	10.5617
$N(C_k)$	0.1672	0.3223

Source: compiled by the authors

The current research is consistent with the findings of other authors, but is more in-depth in determining the effectiveness of using pig by-products for biogas production. The vast majority of authors publish fragmentary research results. For instance, N. Pantsyreva (2019) reports that one breeding pig with a litter of 20-24 piglets (weighing up to 30 kg) produces about 14.5 kg of manure per day. A fattening pig with a live weight of 30-110 kg produces an average of 4.5 kg. Up to 65 m³ of biogas can be produced from a tonne of dry matter of pig manure. According to M. Linnuk and B. Ruban (2011), on farms and complexes with hydraulic manure removal, the moisture content of biomass is 95-97%, and in fattening shops it can reach 98-99%.

Biogas is a combustible gas mixture consisting of 50-70% methane (CH₄), which is formed from organic

compounds in an anaerobic microbiological process. Biogas also contains 30-40% carbon dioxide (CO₂) and small amounts of hydrogen sulphide (H₂S), ammonia (NH₃), hydrogen (H₂) and carbon monoxide (CO) (Teng *et al.*, 2022). Biogas is a versatile renewable energy source, as it is suitable for electricity and heat production. To produce biogas in industrial pork production, it is advisable to use by-products with a moisture content of 90-92%. However, the moisture content of wastewater at pig farms can reach 97-98%. Even when pigs are kept on a slotted floor with baths, the wastewater has a moisture content of 95-97%. Therefore, the use of conventional biogas production technologies in industrial pork production leads to high capital investment and operating costs (Table 3) due to, for instance, suboptimal parameters of the fermentation effluent.

Table 3. Main indicators of biogas production technologies for industrial pork production ZORG and BIOTHANE

Indicators	ZORG (Germany)	BIOTHANE (Netherlands)
Process duration, days	30	1
Bioreactor volume, m ³	3,600	3,000
Number of bioreactors, pcs./volume	9/400	3/1000
Volume of wastewater for treatment, m ³	945	945
Process temperature, °C	35/55	35

Source: authors' development based on technical data from biogas unit manufacturers

Upon analysing the practices of implementing the biogas units (based on the technology and equipment of BTG, the Netherlands) by the "Agro-Oven" company on a farm with a population of 14,000 pigs, Yu. Matveev and G. Geletukha (2004) found that the construction costs amounted to USD 413.3 thous. Two methane tanks with a volume of 1,000 m³ each were built to process 80 t of manure per day. The average fermentation time is 25 days. Practical experience has shown that wastewater with a moisture content of 97-98% can be subjected to the fermentation process. At the same time, the amount of dry matter in the fermentation substrate was increased to only 6-7%, which was achieved through the use of recycled materials. According to M. Lyubin *et al.* (2011), the best results of fermentation of fermentable raw materials are achieved at 30-32°C and humidity of 90-95%. As reported by T.L.I. Vergote *et al.* (2020), the dry matter content of pig manure in commercially managed pigs is usually less than 10%.

One of the producers of biogas units, the Public Foundation "Fluid" (n.d.), also believes that the composition of feedstocks affects biogas production. The moisture content of the raw materials loaded into the biogas unit must be at least 85% in winter and 92% in summer. If pig manure alone is used, the cycle will take 10-12 days, and if the liquid manure is optimised by adding plant material, the time required for fermentation will increase to 40 days or more. This approach

will increase the required volume of digesters, which will lead to a considerable increase in the cost of equipment. It is estimated that the price increase will be about 4.4-6.7 times. Obtaining the optimum moisture content of the methane digestion substrate by thickening allows reducing capital investments in the construction of biogas units. This coincides with the data provided by the authors of the study in Table 1. According to H.V. Zhuk (2022), the introduction of innovative technologies developed, among other things, at the Gas Institute of the National Academy of Sciences of Ukraine will allow for the stable production of 500 mln m³ of methane annually. L. Sakun *et al.* (2020) report that most biogas units pay off in 4-7 years, and therefore government support in the form of guaranteed purchases of energy from bioenergy facilities is economically beneficial for investors.

Thus, the use of pig by-products for biogas generation is a promising way to increase the efficiency of the industry, reduce production costs, reduce the amount of land for manure storage, and improve the environment. The proposed approach also reduces the cost of wastewater treatment.

CONCLUSIONS

It was found that in the production of pork at the Hlobinskyi Pig Farm LLC, Poltava region, using a concentrate feeding type and litterless pig housing technology, the outgoing effluent has a moisture content of

96.94±0.18%. Settling of the initial wastewater with a moisture content of 96.94±0.18% produces a sediment with a moisture content of 91.23±0.25% and a liquid fraction with a moisture content of 98.86±0.42%. The organic matter in the sediment extracted from the effluent was about 87%. It was shown that the separation of the initial wastewater on a filter press produces a liquid fraction with a moisture content of 98.73±0.12%. When the liquid fraction obtained during the separation is settled, a precipitate with a moisture content of 92.57±0.47% and a liquid fraction with a moisture content of 98.49±0.29% are formed.

A variant of the scheme of the process of methane fermentation of the initial wastewater in industrial pork production has been developed, ensuring a reduction in construction and operation costs for the generation of renewable energy sources (biogas). The technological process includes centrifugal and gravity separation of the initial wastewater into fractions (sediment and liquid fraction), gravity thickening of the sediment obtained during centrifugal and gravity separation and periodic discharge of the thickened sediment for dosing when it is fed to methane digestion to produce biogas. When biogas is used in a generator, electricity and heat are produced. Equations are presented that allow determining the volume of sediment and subsequently the volume of digesters based on factual data on the outgoing effluent, considering the digestion modes.

Evaluation of the proposed technology of wastewater treatment for use at a complex for industrial production of pork with the production of renewable energy sources suggests that the use of the proposed technology will reduce capital investments by about 30% and obtain an annual economic effect from the implementation of the proposed technology in the amount of UAH 1 mln 255 thous. Based on a multicriteria analysis, it was found that the proposed biogas production technology has a substantial advantage in terms of the complex indicator of efficiency of each of the alternative options $N(C_k)$ compared to the ideal one. For this technology, the objective function is the smallest according to the criteria considered and amounts to 0.1672, while the objective function of the baseline technology is 1.9 times worse. Further work on the use of livestock by-products for resource-saving biogas production in industrial pork production is aimed at implementing and investigating the proposed approaches in production conditions.

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CONFLICT OF INTERESTS

The authors of this study declare no conflict of interest.

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Використання побічних продуктів тваринництва для ресурсозберігаючого отримання біогазу за промислового виробництва свинини

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Анотація. Сучасні енергетичні системи є складними багатокомпонентними системами, що використовують механічну, теплову та електричну енергію. У міру скорочення запасу викопного палива інтерес до відновлюваних джерел енергії повсюдно збільшується, що актуалізує дослідження технологій отримання біогазу. Метою дослідження було оцінити технологічні підходи для зниження ресурсовитрат за отримання відновлюваних джерел енергії з органічних відходів (гною) свинарських комплексів. В роботі було використано бібліографічний метод дослідження, лабораторний метод, біохімічні методи (визначення вмісту хімічного складу гною та стоків), статистичний, математичний (розрахунок економічної ефективності), метод багатокритеріального аналізу, аналітичний метод. На підставі проведених досліджень розроблено ресурсозберігаючу технологію підготовки стоків до використання з отримання відновлюваної енергії за промислового виробництва свинини, з забезпеченням зменшення капітальних та експлуатаційних витрат при генерації біогазу. Встановлено, що при відстоюванні вихідних стоків вологістю $96,94 \pm 0,18$ % утворюється осад вологістю $91,23 \pm 0,25$ % та рідка фракція вологістю $98,86 \pm 0,42$ %. Одночасно, органічна речовина в осаді, який виділено з вихідних стоків, становить біля 87 %, а в рідкій фракції – біля 98 % від сухої речовини. Технологічний процес включає: відцентрово-гравітаційне розділення вихідних стоків на фракції (осад та рідку фракцію), гравітаційне згущення осаду його дозування при подачі для метанового зброджуванням із одержанням біогазу. Показано, що застосування запропонованих підходів дозволяє знизити капітальні вкладення біля 30 %. На основі багатокритеріального аналізу встановлено, що за комплексним показником ефективності кожного із розглянутих варіантів $N(C_k)$ у порівнянні з ідеалізованим, запропонована технологія отримання біогазу має значну перевагу. Для неї цільова функція за розглянутими критеріями є найменшою і становить 0,1672 при тому, що цільова функція технології базового варіанту гірша в 1,9 раза. Результати проведених наукових досліджень по використанню побічних продуктів тваринництва для ресурсозберігаючого отримання біогазу плануються використовувати на комплексах і фермах з виробництва свинини.

Ключові слова: відновлювані джерела енергії; біоенергетика; стоки; технологія; осад; ресурсозбереження, капітальні вкладення



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Automated remote sensing system for crops monitoring and irrigation management, based on leaf color change and piecewise linear regression models for soil moisture content predicting

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Abstract. Plants can serve as biological sensors if their "readings" and the feedback they provide us through changes in the colour of their leaves can be correctly interpreted. The study aims to predict soil moisture and, as such, the need for irrigation, using nonlinear mathematical models, describing the relationship between RGB and HSL colour model components and soil moisture and temperature. Nonlinear mathematical models used in the study are based on piecewise linear regression with breakpoint and soil moisture prediction using colour components and soil temperature with a deviation of $\pm 6\%$. A system for automated irrigation was created and its control program was made, the basic control law of which is based on non-linear piecewise linear models. The automated irrigation management system includes a remote crop monitoring subsystem and an irrigation management subsystem. The program processes the photo received from the camera and activates the actuators when watering is needed. Compared to manual data collection in the first part of the study, the program calculates the average RGB model values from images in the studied row of tomato plantations with an accuracy of over 99% for the R and G components and over 92% for the B component. The program also predicts soil moisture with 98% accuracy. The practical significance of the water-saving efforts of this study lies in the development of a program-controlled automated irrigation system that utilizes plants as biological sensors, employing nonlinear mathematical models based on leaf colour changes to accurately predict soil moisture

Keywords: biosensors; precision irrigation; RGB colourimetry; image processing; digitalization; bioinformatics

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INTRODUCTION

People knew when their crops required water or treatment by their appearance (e.g., wilting, wrinkling) or their colour (lightening or darkening of their green colour) for centuries. Precision irrigation offers numerous benefits in modern agriculture. This targeted approach also improves crop yields and quality while minimizing environmental impact. Additionally, precision irrigation systems can be remotely controlled and monitored, allowing farmers to make real-time adjustments based on weather conditions and soil moisture levels, ultimately enhancing overall farm productivity and sustainability.

Soil moisture refers to the amount of water present in the soil, typically measured as a percentage of the soil's weight. Monitoring soil moisture is crucial from a precision irrigation perspective as it allows farmers to optimize their water usage. By determining the exact moisture levels in the soil, farmers can apply irrigation precisely, providing just the right amount of water needed. This not only conserves water resources but also ensures that crops receive adequate hydration for optimal growth and yield. Monitoring soil moisture levels helps prevent over-irrigation, which wastes water and causes environmental issues, as well as under-irrigation, which can harm crop health and productivity.

However, irrigation efficiency and water resource preservation are often lower than expected (Zahoor *et al.*, 2019; Laureti *et al.*, 2021). It is estimated that agriculture uses over 70% of the world's freshwater supplies, with about half of this being lost and wasted (United Nations Development Programme, 2021). Furthermore, during the irrigation process, most of the water is absorbed by the soil whereas plants get only a small portion. Tomatoes lose over 90% of absorbed water through transpiration from their leaves, making them more vulnerable to water stress than other parts of the plant, such as fruit (Zhao *et al.*, 2020), therefore, they are more sensitive and a suitable indicator of water stress.

There are two widely recognized traditional approaches for monitoring irrigation: groundwater monitoring (Wood & Cherry, 2021) and plant water monitoring (Simbeye *et al.*, 2023). Various methods for assessing soil moisture are highlighted by several authors, including the gravimetric approach (Reich *et al.*, 2021), TDR dielectric methods (Gnatowski *et al.*, 2018), FDR dielectric methods (Kang *et al.*, 2019), and neutron probe. Measurement techniques for assessing plant water status encompass ZIM probes, dendrometers, infrared thermometry measurements, pressure chambers, infrared gas analysers, pulse methods, and porometers. E. Serrano-Finetti *et al.* (2023) suggest employing non-invasive plant leaf water content monitoring using electrical impedance spectroscopy.

The study describes a different, indirect, intelligent, quick approach based on physical observation of plants with the naked eye, used by farmers for a long time, which also has scientific potential. A method that

provides information that the plant is well watered and that eliminates the drawbacks of the available sensors. This method must be sufficiently accurate to ensure optimal irrigation and water saving.

Modern studies (Atanasov, 2021) state that plants require irrigation when their leaves turn dark green and lighten as the soil moisture increases. However, the present study does not include cases of infected or diseased plants with discoloured leaves, but only healthy ones. Atanasov (2021) also proves that the colour change is an early indicator of water stress, which can be detected within half an hour to an hour after irrigation depending on the height of the plant, or it can be monitored continuously with an automated monitoring system proposed late in the present study. The same aforementioned study also demonstrated that young leaves (at the top of the plant) are more informative regarding the need for watering (they provide smaller errors when predicting soil moisture). Therefore, twice as many RGB and HSL colour samples were taken from them in the studies.

The study demonstrates the potential of the natural world. The study author suggests that plants can become reliable living biosensors – essentially high-tech biogadgets. They employ leaf colour as an indicator, and a smart and cost-effective solution for directly measuring soil moisture by analysing colour changes in tomato and other plants' leaves grown in a greenhouse environment or outdoors can be created on this basis. As such, the study aims to build an interactive model to predict soil moisture levels and, as a result, provide optimal irrigation timing. This study also seeks to employ the natural traits of plants for further use. All of this can be achieved by investigating the type of functional dependence describing the relationship between the average value of the parameters of the RGB and HSL colour components (R_{avg} , G_{avg} , B_{avg} , H_{avg} , S_{avg} , L_{avg}) and the factors of soil moisture (θ) and soil temperature (T).

MATERIALS AND METHODS

Methodology of the experiment. The experimental approach involves conducting research in heated glass greenhouses situated in Plovdiv, Bulgaria, at coordinates 42.18265, and 24.73989. These greenhouses meticulously control all environmental variables and ensure the well-being of plants by maintaining optimal conditions. To provide winter heating, hot water is sourced from a nearby thermal power plant using steam heating. The greenhouse dimensions are approximately 50 m by 35 m, with 15 columns of 3 m in length and each row spanning about 2 m in width. Tomatoes are cultivated year-round, yielding two harvests – one from January to July and another from August to December.

The investigated indeterminate tomatoes are one variety – Panekra. They were planted at the end of July. The experiments were conducted in September – 24 hours after irrigation and 24 hours before irrigation.

The study object is the leaf mass of tomato plants (*Solanum lycopersicum*) and the influence of microclimate parameters in the greenhouse (humidity and soil temperature). Leaf mass was examined for four quality factors – young leaves (C_1) before (A_1) and after irrigation

(A_2), old leaves (C_2) before and after irrigation. Young leaves (Fig. 1 – left and Fig. 2 – left) are the new, underdeveloped leaves at the top of the plant. Old leaves (Fig. 1 – right and Fig. 2 – right) are large, fully developed leaves at the bottom of the plant.



Figure 1. Photos of young (left) and old leaves (right) after irrigation



Figure 2. Photos of young (left) and old leaves (right) before irrigation

Source: photos by the author

Throughout the measurements, wireless sensors were employed to monitor soil moisture and temperature, a handheld portable colourimeter (PCE-RGB2) was utilized for colour measurement, and a device (PCE-EM 883) was employed to measure air humidity, temperature, and luminance. The calibration of the soil moisture sensor for the specific greenhouse soil type, completed as outlined by J. Starr and I. Paltineanu (2002) for calibrating capacitive sensors, was conducted, as the greenhouse soil is of an alluvial-meadow nature.

As both factors θ and T are measurable, the study delved into the relationship between them and the average values of each component in the RGB and HSL colour models through multivariate regression analysis.

The influence of qualitative factors, such as “before irrigation”, “after irrigation”, “young leaves”, and “old leaves”, were investigated using multivariate analysis of variance. The examination of normal distribution utilized the entire set of obtained experimental data (random variables), while in regression and dispersion analysis, the averaged values of the RGB and HSL colour components for each plant (R_{avg} , G_{avg} , B_{avg} , H_{avg} , S_{avg} , L_{avg}) were employed.

Sample size. In the present study, 168 RGB and HSL tomato leaf colour samples were taken manually using a colourimeter before and 168 after irrigation. Regression and variance analysis were performed and models of the dependence between colour components and θ and T were obtained. The RGB readings for each plant

are rounded to the second digit, although the RGB components have no fractional part. Soil moisture (θ) and soil temperature (T) were measured repeatedly and averaged, close to the plant and the root, in a layer of soil 20–30 cm, which is also the root habitat.

Conducting the experiments. The experiment took place under diverse weather conditions. In conditions of clear and sunny weather, the greenhouse registered a humidity level of 73% and a temperature of 25.7°C. Concurrently, outside the greenhouse, the humidity was 65.8%, and the temperature remained at 25.5°C, with an illumination range of 39–47 kLx within the greenhouse. Additionally, during cloudy and rainy weather, the greenhouse experienced increased humidity at 84%, a lower temperature of 19.8°C, and illumination within the range of 5.2–6.2 kLx. Outside the greenhouse, the humidity increased to 85.8%, and the temperature dropped to 16.8°C.

Based on the experience gained during the experiments, the following experiment plan or the following data collection methodology was created and named “12-9-6-3”: twelve randomly selected plants (several

from each of the three sections of the row) were studied – nine for mathematical model creation and three to test the model (before irrigation). Measurements used six-fold repeatability for young leaves and three-fold repeatability for old leaves – six colour values were taken randomly from different leaves from the crown (top) of each plant examined and three colour values were randomly taken from the lowest their leaves.

To facilitate the process, the plants are marked with an easily visible marking, e.g. red thread. Measured data is entered into a purpose-built field log. The experiment was conducted at the end of a specially dedicated row. The experiment section is 15.53 m long with controlled irrigation using two valves. Near the first valve, inside the greenhouse, as well as for the entire greenhouse, the irrigation rate is 30 m³/acre. The irrigation rate between the first and second valve is 20 m³/acre and 10 m³/acre near the second valve to the concrete path. Fertilization is carried out by dissolving 20 kg of fertilizer and using the solution for drip irrigation with water – 20 m³/acre (Fig. 3):

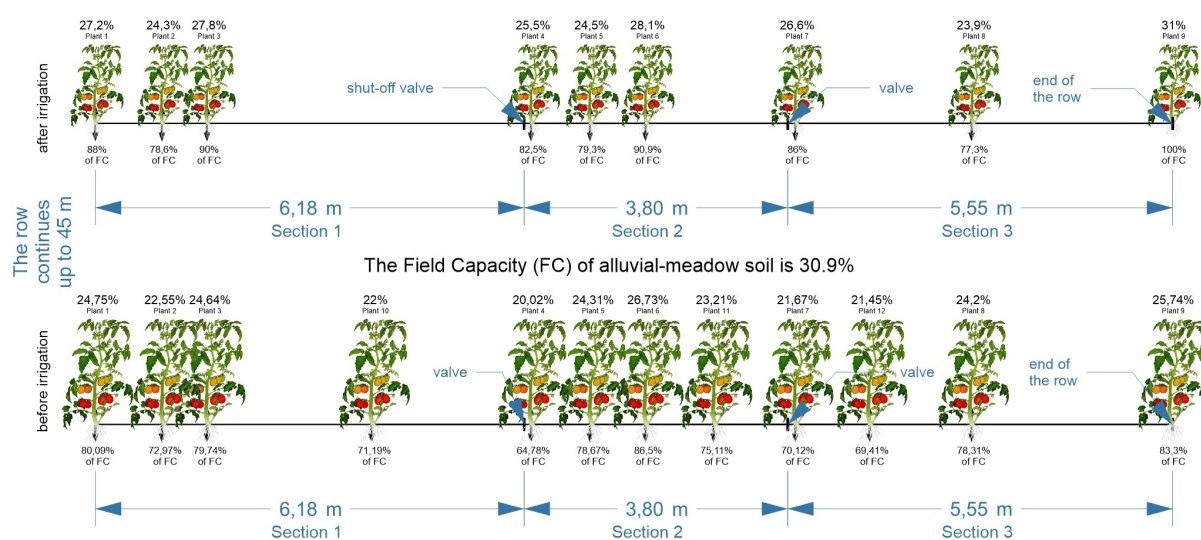


Figure 3. Visualization of the studied plants and methodology “12-9-6-3” at the row in the greenhouse intended for this purpose

Source: compiled by the author

Multivariate analysis of variance. The variance analysis demonstrated that both “young/old leaves” and “before/after irrigation” factors have a significant influence on the colour component R_{avg} . The “young/old leaves” factor has a stronger influence. Only the “young/old leaves” factor has a proven influence on G_{avg} and B_{avg} colour components. The components R_{avg} , G_{avg} and B_{avg} have the largest values (lighter green colour when combined) in the older leaves after irrigation, which demonstrates that leaves darken when dry.

This study determines a model with functional dependence between the studied parameters, where the coefficient of determination R^2 is $\geq 80\%$, as suitable, which means that it is capable of predicting soil

moisture. That is why the non-linear estimation approach is used to find the relationship between the set of independent variables (θ and T) as predictors and the dependent variables (colour components) which vary over the independent variables.

Modeling with Piecewise linear regression with breakpoint. This method works as follows: the model is divided into two parts and the formula is solved using the Quasi-Newton nonlinear method, which is an iterative, iterative, nonlinear optimization method that greatly minimizes inconsistencies and errors in colour prediction, and which is used to minimizing the sum of squared errors (Araújo-Paredes *et al.*, 2022). Breakpoints are fundamental when using piecewise regression, as

they are points in the range of X where the behaviour of Y changes, thus the name “breakpoint”. In some cases, the breakpoint can be known, but normally it is not. In such cases, there is a need for a method to determine it.

In the Quasi-Newton method, the process of constructing a model to predict leaf colour involves calculating the first-order derivative of the function at a given point to ascertain its slope. Subsequently, the second derivative indicates the rate at which the slope changes at that point and its direction. The Quasi-Newton method iteratively evaluates the function at various points during each step, computing both first and second-order derivatives. These derivatives are crucial for determining the minimum of the loss function. The choice of methods to minimize the loss function depends on the objective, with the least squares method being the most prevalent. This method entails squaring the difference between predicted and observed values. The end goal is to minimize the disparity between the observed and predicted values (Araújo-Paredes *et al.*, 2022).

RESULTS AND DISCUSSION

Investigating the distribution of colour components (random variables). During the normal distribution check, almost all RGB component values studied were found to have a Gaussian distribution, except for B in young leaves after irrigation. A normal distribution in HSL for the following colour components was present – S and L in young leaves after irrigation, S and L in young leaves before irrigation and H , S and L in old leaves before irrigation. For those colour components that have a Gaussian distribution, prerequisites are

available for moving on to the next steps: regression and variance analyses.

Multivariate analysis of variance. In the HSL colour model, both “young/old leaves” and “before/after irrigation” factors have a significant influence on the H_{avg} component. The component H_{avg} has the greatest value in young leaves before irrigation. None of the two factors “young/old leaves” and “before/after irrigation” had a proven influence on the S_{avg} component. Only the factor “young/old leaves” has a proven influence on the L_{avg} component. The components H_{avg} and S_{avg} have the greatest values in young leaves before irrigation. The component L_{avg} has the largest values in old leaves after irrigation.

Multivariate (bivariate) regression analysis. Regression analysis concluded that multiple linear regression and quadratic polynomial regression modelling were inappropriate (unsatisfactory coefficient of determination and non-significant models). The studied environmental factors also have an inherent non-linear behaviour concerning leaf colour. In other words, the variation of the θ and T data does not follow any recognizable linear combination concerning leaf colour. Therefore, it is difficult to model a required dynamic relationship using conventional linear methods such as multiple linear regression. A universal non-linear model is believed to best describe the colour components at different soil moistures and temperatures.

Modelling with Piecewise linear regression with breakpoint. The following coefficients of the empirical equation were obtained using this method. The general form of the model, calculated using the least squares method, is as follows:

$$y = (b_{01} + b_{11}x_1 + \dots + b_{m1}x_m)(y \leq b_n)(b_{02} + b_{12}x_1 + \dots + b_{m2}x_m)(y > b_n), \quad (1)$$

Alternatively, using the parameters R_{avg} , G_{avg} , B_{avg} , H_{avg} , S_{avg} , L_{avg} , θ and T , the model is as follows:

$$\{R_{avg}, G_{avg}, B_{avg}, H_{avg}, S_{avg}, L_{avg}\} = (b_{01} + b_{11}\theta + b_{21}T) \text{ (for } \{R_{avg}, G_{avg}, B_{avg}, H_{avg}, S_{avg}, L_{avg}\} \leq \text{breakpoint } b_0) \\ \text{Or } (b_{02} + b_{12}\theta + b_{22}T) \text{ (for } \{R_{avg}, G_{avg}, B_{avg}, H_{avg}, S_{avg}, L_{avg}\} > \text{breakpoint } b_0), \quad (2)$$

In this way, two separate linear regression equations are calculated – one for the y values that are less than or equal to the breakpoint (b_0) and one for y values greater than the breakpoint. For the R_{avg} colour component in young leaves after irrigation, according to model (2), the coefficient of determination was 98.67%. It indicates that 98.67% of the variation in R_{avg} is attributed to changes in the θ and T percentages. The remaining 1.33% is attributable to factors not included in the model. The conclusions

are similar for the other investigated colour components possessing a normal distribution – G_{avg} , S_{avg} and L_{avg} .

A very important conclusion was also established – the physical definition of the breaking point coincides with the arithmetic mean value of the given colour component (in this case breakpoint = 119.13). The coefficients of the models concerning the remaining colour components in young leaves after irrigation according to model (2) are summarized in Table 1:

Table 1. Coefficients and breakpoint for R_{avg} , G_{avg} , S_{avg} and L_{avg} models in young leaves after irrigation

Model variable	Coefficients	R_{avg}	G_{avg}	S_{avg}	L_{avg}
Constant	b_{01}	868.17	2.35	-0.106	0.781
θ	b_{11}	-3.80	-12.44	-0.007	-0.003
T	b_{21}	-31.49	20.90	0.024	-0.029
Constant	b_{02}	10.35	28.84	0.455	0.052

Table 1. Continued

Model variable	Coefficients	R_{avg}	G_{avg}	S_{avg}	L_{avg}
θ	b_{12}	-1.06	-1.24	-0.002	-0.0005
T	b_{22}	6.66	6.99	-0.007	0.0036
	breakpoint	119.13	140.09	0.235	0.111
R		0.99	0.99	0.88	0.99
Variations covered (%)		98.67%	98.03%	77.54%	99.47%
R^2		0.99	0.98	0.78	0.99

Source: compiled by the author

This iterative approach is well-suited for scenarios involving multiple independent variables and a dependent variable (colour component) with values both above and below the breakpoint. The residual values are maintained at acceptably small levels through a nonlinear optimization method, ranging between -1.443 and 1.094. The predicted values closely align with the observed values. All data points from the experiments fall within or very near the narrow confidence interval (at a confidence level of $\gamma=0.95$), and the regression line closely tracks the experimental points.

The residual values, distributed uniformly and within acceptable limits, contribute to the model's reliability. The high R^2 value of 0.99 for R_{avg} in young leaves after irrigation amplifies a significant dependence of the colour component on the variables incorporated into the model. The points cluster closely around the straight line, suggesting a normal distribution of residuals. Similar reasoning can be extended to all other considered colour components in old leaves after irrigation based on model (2). The coefficients of the models related to colour components in young leaves before irrigation are summarized in Table 2.

Table 2. Coefficients and breakpoint for R_{avg} , G_{avg} , B_{avg} , S_{avg} and L_{avg} models, young leaves before irrigation

Model variable	Coefficients	R_{avg}	G_{avg}	B_{avg}	S_{avg}	L_{avg}
Constant	b_{01}	104.72	137.12	57.18	0.357	0.112
θ	b_{11}	-2.12	-2.59	-0.59	-0.0023	-0.0016
T	b_{21}	2.48	2.57	1.63	-0.0032	0.0014
Constant	b_{02}	-746.30	-1727.72	131.70	0.383	0.105
θ	b_{12}	9.26	19.02	0.25	0.006	-0.132
T	b_{22}	33.59	74.11	-2.98	-0.0127	0.189
	breakpoint	108.74	133.89	77.35	0.264	0.113
R		0.98	0.99	0.98	0.90	0.77
Variations explained (%)		96.98%	97.51%	95.14%	81.44%	59.22%
R^2		0.97	0.98	0.95	0.81	0.59

Source: compiled by the author

Figure 4 also shows acceptably small residual values, with predicted values very close to the observed values:

Model is: (Young leaves before irrigation.sta) Dep. Var. : R			
	Observed	Predicted	Residuals
1	100.83	99.91	0.92
2	115.50	115.50	-0.00
3	95.50	98.48	-2.98
4	108.50	109.56	-1.06
5	104.33	100.72	3.62
6	129.33	129.33	-0.00
7	107.33	106.53	0.80
8	117.00	117.00	0.00
9	100.33	101.64	-1.30

Figure 4. Measured, and predicted values and residuals for R_{avg} in young leaves before irrigation

Source: compiled by the author

Figure 5 also demonstrates that all the experimental data points fall within or very close to the narrow confidence interval (at a confidence level of $\gamma=0.95$)

and that the regression line passes close to the experimental points:

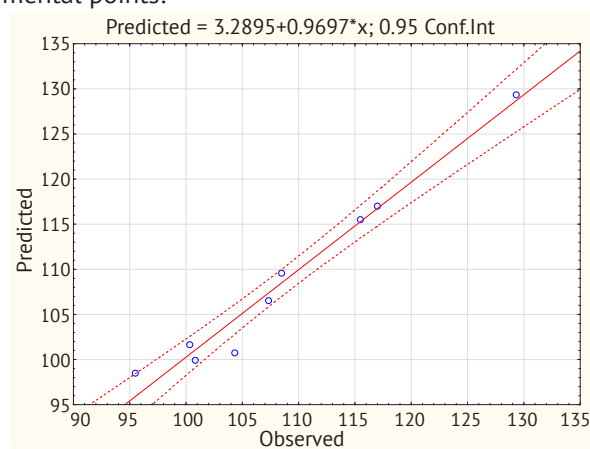


Figure 5. Scatter plot – predicted versus measured values of colour component R_{avg} at young leaves before irrigation, model (2)

Source: compiled by the author

Figure 6 demonstrates that the residual values are within acceptable limits and with a uniform distribution. The high value of $R^2=0.99$ for R_{avg} in young leaves after irrigation indicates that the colour component is significantly dependent on the variables included in

the model. The points are located close to the straight line, i.e. it is possible to assume that the residuals have a normal distribution. The coefficients of the models regarding the colour components of old leaves before irrigation are summarized in Table 3:

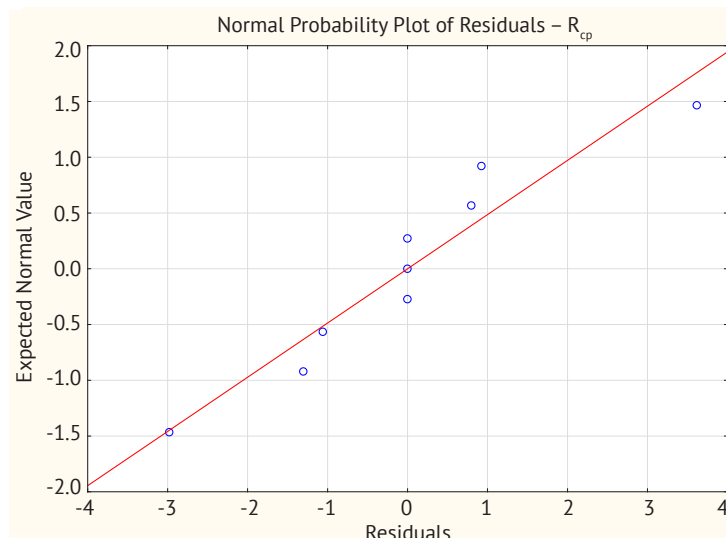


Figure 6. Normal probability plot of the residuals of model (2) for R_{avg} at young leaves before irrigation

Source: compiled by the author

Table 3. Coefficients and breakpoint for R_{avg} , G_{avg} , B_{avg} , H_{avg} , S_{avg} and L_{avg} models, old leaves before irrigation

Model variable	Coefficients	R_{avg}	G_{avg}	B_{avg}	H_{avg}	S_{avg}	L_{avg}
Constant	b_{01}	122.18	169.45	122.02	0.288	0.092	0.146
θ	b_{11}	0.53	1.16	0.42	0.0005	-0.005	0.0005
T	b_{21}	0.02	-2.16	-1.25	-0.0033	0.009	-0.0016
Constant	b_{02}	-785.96	-512.82	-222.52	0.374	0.283	-0.385
θ	b_{12}	2.89	2.96	4.80	-0.0005	-0.0034	0.0064
T	b_{22}	44.99	31.73	11.75	-0.0063	-0.0007	0.0192
	breakpoint	139.15	158.89	112.37	0.239	0.172	0.133
R		0.93	0.95	0.97	0.88	0.96	0.95
Variations explained:		87.42%	91.13%	93.54%	77.10%	92.82%	89.96%
R^2		0.87	0.91	0.94	0.77	0.93	0.90%

Source: compiled by the author

Verification of the operability of the developed model (2). Next, the performance of model (2) was tested, examining θ as the dependent variable and the colour component and soil temperature as the independent variable. The goal is for the farmer to determine the plant colour and know whether to irrigate (i.e. how wet it

is – an indirect smart method). To this end, RGB and HSL values were taken from additional control plants before irrigation (Plants 10-12) during the experiment to verify the need for irrigation. With their help, the adequacy of the model (2) was tested. Analytically, model (2) transforms concerning θ as follows, yielding model (3):

$$\theta = \left(\{R_{avg}, G_{avg}, B_{avg}, S_{avg}, L_{avg}\} - b_{01} - b_{21}T \right) / b_{11} \quad (\text{for } \{R_{avg}, G_{avg}, B_{avg}, S_{avg}, L_{avg}\} \leq \text{breakpoint } b_0) \quad (3)$$

$$\text{Or } \left(\{R_{avg}, G_{avg}, B_{avg}, S_{avg}, L_{avg}\} - b_{02} - b_{22}T \right) / b_{12} \quad (\text{for } \{R_{avg}, G_{avg}, B_{avg}, S_{avg}, L_{avg}\} > \text{breakpoint } b_0),$$

The results of model (3) testing are presented in Table 4:

Table 4. Approbating the model (3) in young leaves before irrigation

	R_{avg}	G_{avg}	B_{avg}	S_{avg}	L_{avg}	$\theta_{measured}$	T
Plant 10	108.83	131.67	81.50	0.236	0.104	22.00	19.03

Table 4. Continued

	R_{avg}	G_{avg}	B_{avg}	S_{avg}	L_{avg}	$\theta_{measured}$	T
$\theta_{calculated}$	23.32	20.93	25.30	26.13	21.65		
Predict. error	+5.99%	-4.85%	+14.98%	+18.88%	+13.77%		
Plant 11	118.67	146.33	81.67	0.284	0.111	23.21	18.96
$\theta_{calculated}$	24.63	24.66	25.13	23.63	17.22		
Predict. error	+6.14%	+6.23%	+8.28%	+1.82%	-9.20%		
Plant 12	110.50	133.50	80.67	0.246	0.105	21.45	18.89
$\theta_{calculated}$	24.01	20.09	20.37	21.98	20.90		
Predict. error	+11.92%	-6.36%	-5.05%	+2.42%	+10.66%		

Source: compiled by the author

During the approbating of the model (3) on older leaves before irrigation of large soil, moisture prediction errors (between -66% and 45%) were obtained, therefore they were not demonstrated in this study. Therefore, the older leaves are not suitable for determining the need for irrigation. Young leaves before irrigation are more informative because the error is acceptably small, confirming the result's accuracy.

Description of the components of the automated irrigation system. The model of the remote sensing system for monitoring the tomato crop in the greenhouse and automatic control of the drip irrigation system

(irrigation start and stop) consists of the following elements (Fig. 7):

1) Solar panel for autonomous power supply of the camera (40 W); 2) Autonomous 4G IP camera (dust and waterproof); 3) micro-Sim and microSD cards; 4) Protection of the camera lens against condensation (from the high humidity in the greenhouse); 5) Rodent proof cables; 6) Accumulator (20 Ah); 7) Drip irrigation system; 8) Crops greenhouse-grown indeterminate tomato plants; 9) A remote computer executing the control function embedded in a program written in Python; 10) A remote server where a snapshot is stored (e.g. every half hour).

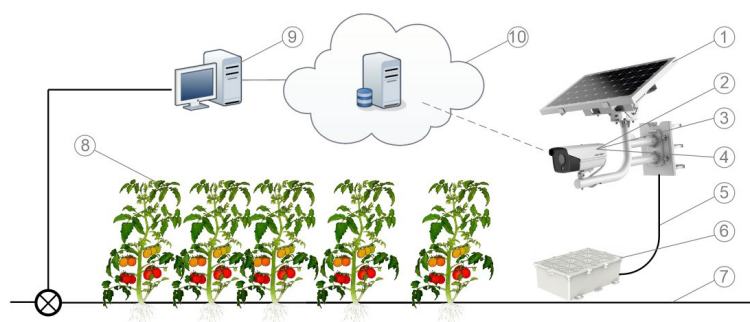


Figure 7. A model of a remote sensing system for crop monitoring and automated irrigation

Source: compiled by the author

The camera can be set to automatically take a picture of the crop at a certain time interval (for example half an hour during the daylight hours) through the web interface of its software. This means that to save energy, the camera does not need to be on at all times, but only during the day every half an hour. The cropped photo can be stored in two ways. The first is locally, on the camera's microSD card. The second: is by installing a micro-SIM card in the designated slot, the camera can be connected to the GSM cellular network and send the photos at a certain interval to a cloud storage service or a remote server. Connecting to the GSM cellular network is done by configuring the correct network settings: e.g. through the web interface of the camera, the name of the access point - APN (Access point name) must be set, among other appropriate network settings:

username, password, IP address, subnet mask, gateway, and DNS (if the cellular network requires them). Setting the camera to send the pictures to a cloud service or a remote server is also done through the web interface of the camera. IP address or domain name of the server where the photos will be uploaded, upload interval, file format and image resolution are set. Finally, the photo upload schedule is enabled in the settings.

The image sent to a remote server is automatically retrieved remotely via a script on another computer, processed by a Python-based program, and if the soil moisture calculated from the colour of the leaves is below a certain threshold level, an actuator in the greenhouse is remotely activated to start and subsequently to stop the irrigation system. In the following Figure 8 is presented an example photo of the crop in the greenhouse, taken

during the conduct of the experiments and the acquisition of the experimental data. Field experiments were conducted on row 2 of the image (the right one). If the camera has a wider field of view, more rows can be covered and processed simultaneously.



Figure 8. Photo obtained from the camera monitoring the crops in the greenhouse

Source: compiled by the author

Description of the image extraction and processing procedure and the program. Python is a powerful and versatile programming language that has become increasingly popular among scientists and researchers in recent years. Python can be used to create compact and simple code whereas other programming languages require more resources and coding time.

Irrespective of the scientific domain, Python proves valuable for researchers in data analysis, visualization, and model development to advance their studies. The developed program focuses on image processing and soil moisture computation for two rows of tomatoes, subsequently regulating irrigation in one of the rows (specifically, row 2 on the right, where the field experiments were conducted) based on the calculated values. The program operates through the following sequence: Initially, it imports essential modules or libraries, such as OpenCV (cv2) for image processing, urllib.request for fetching an image from a URL, numpy for array manipulation, and serial for establishing serial communication with Arduino.

The process begins by retrieving an image from a URL and converting it into a numpy array using

OpenCV's `cv2.imdecode()` function. Subsequently, the program generates two binary masks for distinct regions of interest in the image, specifically the areas corresponding to young leaf regions. The polygonal points defining these regions are manually set during pre-calibration. The `cv2.fillConvexPoly()` function is called to delineate irregular quadrilaterals around each region (refer to Fig. 9). Finally, `cv2.bitwise_and()` is used to apply the masks to the image, resulting in a masked image that exclusively displays the two regions of interest (Fig. 9).

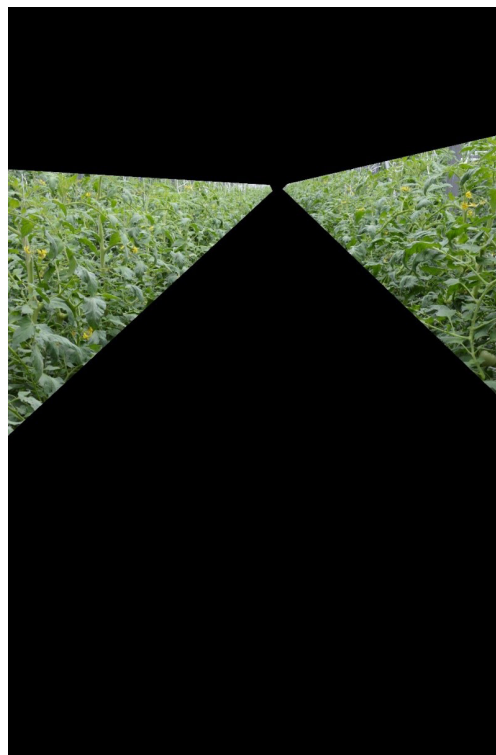


Figure 9. Software-masked image showing only the two young leaf regions

Source: compiled by the author using the author-developed program

Subsequently, a scaling factor is computed to adjust the size of the masked image, ensuring it is 1080 pixels tall – the standard resolution height of a modern monitor. The resizing operation is executed through the `cv2.resize()` function. The program then showcases the resized masked image using `cv2.imshow()`, awaits a key press with `cv2.waitKey()`, and saves the masked image locally via `cv2.imwrite()`. Next, the program counts non-zero pixels in each mask (provided for informational purposes) using `cv2.countNonZero()` and determines the mean RGB colour values for each region using `cv2.mean()`.

For the two rows, the program computes the soil moisture value based on their respective mean green colour values using the non-linear model (3), which forms the core of the control law. This model serves as a

regulator, influencing the actuator to initiate and cease irrigation. According to the assessments in Table 4, the G_{avg} colour component is chosen for soil moisture calculation and irrigation management. This colour component yields the smallest errors in soil moisture prediction (ranging from -6% to 6%, contingent on whether G_{avg} surpasses or falls below the breakpoint). Any prediction errors are rectified in the soil moisture calculation, along with an adjustment factor for the specific soil type (in this instance, the resulting soil moisture readings must be multiplied by a coefficient of 1.1). Ultimately, the program outputs the results of the soil moisture calculation and the irrigation decision for user inspection. Communication with the Arduino board is facilitated using the serial module, transmitting a message to initiate or halt irrigation in the two monitored rows of plantations based on the computed soil moisture value. The program's source code is available on GitHub in the "living-biosensors" repository at this link (n.d.).

Results after the program implementation (Program output)

Row 1:
Mean color values (RGB): [118.79, 142.88, 93.75]
Number of pixels: 2321756
Average Soil Moisture: 25.03
Soil moisture is sufficient in Row 1. Irrigation not started.

Row 2:
Mean color values (RGB): [109.1, 133.79, 83.38]
Number of pixels: 1926333
Average Soil Moisture: 23.45
Soil moisture is sufficient in Row 2. Irrigation not started.

The precision and accuracy of operation of the described manual measurements with a calibrated colourimeter meter were unambiguously confirmed by the calculations with an image processing program, which also confirms the correctness of the calculation methods used, work methods and the sufficiency of the sample used, as shown in the next comparative Table 5:

Table 5. Comparison of the obtained average RGB values and the soil moisture in the manual measurements and with the help of a program

Measurement method	Number of measurements	RGB averages	Soil moisture
With hand-held measuring devices	54	[109, 133, 77]	23.85
From photo and program	1 926 333 pixels	[109, 134, 83]	23.45

Source: compiled by the author

The average value of the colour components of young leaves before irrigation obtained from several dozen manual measurements with a calibrated colourimeter and obtained from nearly 2 million pixels in row 2 is almost the same. Only the B component diverges, but the accuracy is still above 92%, while R and G it is above 99%. Compared with the handheld measurements, the program predicts the soil moisture with 98% accuracy and activates the actuators if watering is needed. Python allows an automatic program execution, e.g. every half hour using task scheduling tools such as cron (on Unix-based systems) or Task Scheduler (on Windows) to schedule the script to run automatically at set intervals.

Remote access to the greenhouse irrigation system and its start and stop can be done using a GSM card for sending commands via SMS messages and a device that can receive and execute SMS commands (Arduino or other controllers with a GSM receiver). Arduino is a popular microcontroller platform that can be used to control various devices, including irrigation systems. There are various other controllers on the market such as Raspberry Pi, BeagleBone and other microcontroller boards that can be used to control greenhouse irrigation systems. An in-depth state-of-the-art review (Atanasov, 2023) confirmed what was found in previous research (Atanasov, 2021), that there is no information that leaf colour has been used for predictive models of soil moisture, including in the model, as well as soil

temperature usage as a dependent factor. Similar studies conducted demonstrate the following results:

Q. Li *et al.* (2022) investigated the interconnection between soil water content, different stages of wheat drought stress, canopy temperature, and spectral response characteristics. C. Ru *et al.* (2020) explored the effectiveness of the Crop Water Stress Index, which relies on leaf temperature, as a tool for assessing the water status of grapevines. N. Chandel *et al.* (2022) introduced a non-invasive approach utilizing computer vision and thermal-RGB imagery to detect water stress in winter wheat crops. Their method incorporated deep learning and function-approximation models to classify crops based on stress levels, utilizing thermal-RGB images and various input parameters. P. López-García *et al.* (2022) conducted experiments to evaluate the impact of different water qualities and initiation times for irrigation on crop growth. They employed Artificial Neural Network models trained on multispectral and RGB data to simulate and analyse water stress in crops. J. Fernández-Novales *et al.* (2021) focused on evaluating the water status of grapevines using measurements of leaf water potential and canopy temperature. They created spatial-temporal maps to examine changes in water status over time and across different locations.

R. Dhillon *et al.* (2019) developed a methodology for forecasting Plant Water Stress (PWS) in almond and walnut trees using a continuous leaf monitoring system. By

gathering data on leaf temperature and environmental factors with the Leaf Monitor system, they achieved a significant average reduction of 39% in water usage compared to the fixed 100% evapotranspiration replacement irrigation approach across all trees. Analysing the colour of strawberry leaves proves to be an effective method for assessing soil conditions and protecting strawberry crops from excessive environmental nutrients, which can lead to financial losses (Madhavi *et al.*, 2022). Their primary goal was to develop machine learning models, specifically multiple linear regression (MLR) and gradient boost regression (GBR), capable of simulating variations in the colour of strawberry leaves. To achieve this, they gathered precise measurements of soil physicochemical properties in the rooting zones from the largest and most diverse coloured leaves of the strawberry plants, using a multifunctional soil sensor. Additionally, they collected 400 strawberry leaflets at various stages of vegetative and reproductive growth, capturing individual leaves using a digital imaging system. Ultimately, their findings indicated that the GBR model outperformed the MLR model, achieving superior performance metrics. F. Hahn *et al.* (2021) focused on mango trees, utilizing dendrometers and capacitors equipped with Teflon clamps to measure Leaf Water Content and investigate the impact of water availability on mango production.

H. Skoneczny *et al.* (2020) explored the potential of non-invasive proximal hyperspectral remote sensing to differentiate between the leaves of apple trees in three states: healthy, infected, and dry. This distinction was achieved by utilizing spectral bands and indices. J. Rodriguez-Perez *et al.* (2018) compared two regression models, ordinary least squares regression and functional linear regression, to predict Leaf Water Content in grapevines using reflectance data and specific wavelengths. T. Zhao *et al.* (2020) employed hyperspectral imaging to assess the water status in tomato leaves, calculating vegetation indices to evaluate Leaf Water Content in various parts of the tomato plants. These studies underscore the promise of advanced techniques in monitoring Plant Water Stress and overall plant health, offering potential benefits for enhanced crop management.

CONCLUSIONS

A significant discovery emerged, highlighting the significance of the breakpoint's physical meaning – it aligns with the arithmetic mean value of the specified colour component. The regression analysis produced two noteworthy non-linear models, namely model (2) and the piecewise linear regression with breakpoint technique. These models demonstrate remarkable accuracy in predicting colour component values based on soil moisture and temperature, as evidenced by the high coefficient of determination (R^2) achieved. The confirmation of a non-linear relationship between the studied dependent variable (colour component) and the two independent variables (θ and T) has been convincingly established.

Model (3) and the piecewise linear regression with breakpoint method prove to be effective in accurately predicting soil moisture values, particularly in young leaves, based on leaf colour and soil temperature. The low error rates in forecasting ($\pm 6\%$) underscore the suitability of young leaves in determining the need for watering. This validates the methodology of sampling twice as many RGB colour samples from young leaves during the research compared to older leaves, which are found to be unsuitable for assessing watering needs due to less informative results.

Models (2) and (3) form the foundation for the development of an automated crop monitoring system designed to assess watering requirements and control the irrigation system automatically. The precision and accuracy of manual measurements using a calibrated colourimeter are unequivocally confirmed through calculations with an image-processing program. This further affirms the accuracy of the employed calculation methods, work procedures, and the sufficiency of the sample size.

The average colour component values of young leaves before irrigation, derived from both manual measurements with a calibrated colourimeter and analysis of nearly 2 million pixels in the row where field experiments were conducted, align closely. While there is a slight divergence in the B component, the accuracy remains above 92%, with R and G components exceeding 99%. The Python-based program, with a 98% accuracy rate, predicts soil moisture and triggers actuators if there is a need for watering, highlighting the effectiveness of the developed system.

The study results may not solve all subsequent engineering and other problems, as the abovementioned methods do not work in the following situations: when due to diseases, the colour of the leaves of the plants changes; when the plants grow, the camera should change its tilt and/or move up automatically; evening time, when there is no natural light, logically, measurements and calculations cannot be carried out. While the primary application of the study is in terrestrial agriculture, the principles and technologies developed and the ability to predict and manage soil moisture using plant-based sensors can be adopted in outer space exploration and farming. Automated irrigation systems, as described in the paper, could be adapted for use in controlled environments on spacecraft or extraterrestrial bases, ensuring optimal conditions for plant growth.

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CONFLICT OF INTEREST

None.

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Автоматизована система дистанційного зондування для моніторингу посівів та управління зрошенням, що базується на зміні кольору листя та кусково-лінійних регресійних моделях для прогнозування вмісту вологи в ґрунті

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Анотація. Рослини можуть слугувати біологічними сенсорами доти, доки ми можемо правильно інтерпретувати їхні «показання» та зворотній зв'язок, який вони надають нам через зміну кольору їхнього листя. Метою даного дослідження було прогнозування вологості ґрунту і, відповідно, потреби в зрошенні на основі створених нелінійних математичних моделей, що описують взаємозв'язок між компонентами кольорних моделей RGB і HSL та вологістю і температурою ґрунту. Ці нелінійні математичні моделі базуються на кусково-лінійній регресії з точкою розриву і прогнозують вологість ґрунту за допомогою кольорних компонентів і температури ґрунту з похибкою $\pm 6\%$. Створено систему автоматизованого поливу та написано програму керування нею, де основним законом керування є створені нелінійні кусково-лінійні моделі. Система автоматизованого управління зрошенням включає підсистему дистанційного моніторингу стану посівів та підсистему управління зрошенням. Програма обробляє фото, отримане з камери, і активує виконавчі механізми, якщо є потреба в поливі. У порівнянні з ручним збором даних у першій частині дослідження, програма розраховує на основі зображення середні значення RGB-моделі в досліджуваному ряду томатних плантацій з точністю понад 99 % для R- і G-компонентів і понад 92 % для B-компонента. Програма також прогнозує вологість ґрунту з точністю 98 %. Практичне значення роботи з точки зору водозбереження полягає в розробці програмно-керованої автоматизованої системи зрошення, яка використовує рослини як біологічні сенсори, застосовуючи нелінійні математичні моделі, засновані на зміні кольору листя, для точного прогнозування вологості ґрунту

Ключові слова: біосенсори; точне зрошення; RGB-колориметрія; обробка зображень; оцифрування; біоінформатика



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Developing dairy farming and improving product quality

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Abstract. The need to find ways to improve the quality characteristics of milk as a basis for increasing the competitiveness of dairy products in the global market determines the relevance of the study. Ukraine's dairy industry is one of the critical ones in the agricultural sector of the economy, as it provides the country's population with vital foodstuffs. The purpose of this study was to substantiate the areas of solving organisational and economic problems of development of the dairy industry to meet the needs of the domestic market with quality dairy products and increase export

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potential. The study was conducted to identify the main reasons for the transformational changes that have taken place in dairy farming and to outline the promising areas for the development of this industry in the context of improving product quality. This study focused on European and national requirements for the quality of raw milk. The following methods of economic research were used in the analytical material: economic and statistical, comparative analysis, graphical, logical generalisation, systematic approach, systemic and structural analysis, calculation, and design. The study analysed the data of agricultural enterprises and households engaged in milk production in Ukraine for 2010-2022 and recalculated the purchase prices for raw milk in relation to the European quality indicators. The findings of this study showed that ensuring the competitiveness of dairy products depends on the quality of milk. It was found that it is higher in European countries, and therefore high standards for the quality of raw milk become a barrier to entry into European markets for most domestic dairy processing enterprises. Accordingly, the state's strategy should focus on increasing the production of high-quality raw milk and growing export potential by forming specialised milk production cooperatives for dairy processing enterprises. The practical significance of the obtained results is that the author's recommendations can be used by agricultural enterprises or private households in their everyday activities

Keywords: milk; quality of dairy products; international standards; producers; release price

INTRODUCTION

The problem of producing high-quality milk is multifaceted. In scientific discussions, various methods are discussed to determine the quality characteristics of products and their impact on the efficiency of the industry. In production and meeting the growing needs of society, the requirements for the quality characteristics of dairy products are constantly increasing. Therefore, for Ukrainian dairy products to be competitive in international markets, they must meet the safety and quality standards that have already become the norm for the world's leading producers.

Ensuring the quality, safety, and competitiveness of products is a key factor in the development of the dairy industry. Thus, M. Nikolaenko and L. Bal-Prylypko (2020) note that the guarantee of product quality is compliance with international ISO standards, which are actively implemented by Ukrainian dairy processing enterprises. M. Rahman and D. Hryzo (2021) note that the dairy and dairy processing industries in Ukraine have many issues that hinder their effective development and specify that domestic milk producers are trying to use modern European technologies, because in many developed countries, dairy producers adhere to stricter milk standards than in Ukraine. P. Borawski *et al.* (2020a) emphasise the significance of the dairy sector for the economy of the EU, which is the largest producer of milk in the world. Furthermore, European producers are focused on producing safe dairy products in compliance with certain limits on the number of somatic cells and bacteria in raw milk.

I. Fedulova (2018) considers the entry of dairy processing enterprises into foreign markets as a major factor in the current stage of dairy farming development and notes that the small-scale sector of the industry cannot fully meet all the needs of the processing industry with high-quality raw milk, which is mostly of the second grade. The author notes that the only strategic vector for the development of dairy

farming can be cooperation of private farms and strict adherence to production technologies to obtain milk of proper quality. A. Popescu and E. Angel (2019) also believe that improving the quality of milk, primarily in terms of the number of bacteria and somatic cells, is an opportunity to increase profits in dairy farming. Scientists say that improving milk quality will increase customer confidence and demand for dairy products. O. Shpychak *et al.* (2022) emphasise the need for fundamental changes in the organisation and management of the dairy farming system and the creation of a suitable market infrastructure. Scientists believe that the development of the milk market environment by a considerable number of small farms leads to a decline in the quality and, consequently, price indicators of products. K. McGarr-O'Brien *et al.* (2023) have an extraordinary perspective on this issue and characterise the sustainability certification standards used in dairy production. They emphasise the diversity of existing standards, allowing farmers to choose the one that suits their beliefs or stage of development.

O. Kozak (2020) examines the factors that have a major stimulating impact on the development and further structural changes in the dairy industry. The author also notes that even in the context of the war waged by the Russian aggressor, Ukraine stays part of the global market for milk and dairy products. Despite the shock at the beginning of the war, the domestic industry managed to restart in the new reality and, just as before the full-scale invasion, is experiencing global trends in the development of the milk and dairy products market. In this context, P. Borawski *et al.* (2020b) also consider the impact of the global crisis on the dairy market.

However, the relevance of the issues under study does not diminish due to a considerable number of issues that impede the development of the industry, including martial law, the COVID-19 pandemic, declining consumer demand in the country, rising unemployment,

increased production costs caused by the crisis in the energy system, and the depreciation of the hryvnia, which necessitates further comprehensive research and development of recommendations for their solution. Thus, the purpose of this study was to analyse the current state of the Ukrainian milk market, determine the optimal price level depending on the quality of raw milk in agricultural enterprises and substantiate the ways to improve the efficiency of the dairy industry in the context of increasing its export potential.

MATERIALS AND METHODS

The study was conducted for 2010-2022 based on data and indicators from the State Statistics Service of Ukraine (n.d.), the Association of Milk Producers – a non-profit non-governmental professional association of farms specialising in dairy farming (n.d.), the methodology of the National Research Centre “Institute of Agrarian Economics” – “Expected indicators of production costs, performance, and profitability in agricultural enterprises of Ukraine in 2022: methodology and calculations (December 2022)” (Lupenko *et al.*, 2023), the findings of previous own studies and research of other Ukrainian scientists, namely, I. Fedulova (2018), O. Petrychenko and V. Rossokha (2018). The study also used available statistical data from the following official websites: CLAL, an Italian consulting company on dairy economics that analyses the dairy market and its development trends, generalising through information and training activities (CLAL.it., n.d.), and the Ministry of Agrarian Policy and Food of Ukraine (Ministry of Agrarian Policy and Food of Ukraine, n.d.) on the financial and economic activities of agricultural enterprises in Ukraine.

The economic research was based on the following principles: the choice of its area – research on the development of dairy farming and improvement of product quality; formulation of goals – identification of causes and consequences of changes in the state of the dairy industry, outlining the prospects for its development in the context of improving product quality; accumulation of facts and bringing them into a certain order – collection and processing of statistical information, systematisation of such information in the form of tables and graphs; sustainable theoretical generalisations – analysis of research and publications over the past 3-5 years; development of measures to determine the level of milk price depending on the quality of raw milk.

The dialectical method of cognition formed the theoretical and methodological framework of the study. A systematic and comprehensive approach was also used to investigate Ukrainian and foreign scientific provisions on the fundamental principles of analysing the state of dairy farming and factors influencing the quality of raw milk to improve its competitiveness. The information on the state of the domestic dairy industry was processed, described and summarised by selecting

information from the Internet and printed media. To fulfil the purpose of this study, the following methods were used: monographic method – to investigate and systematise Ukrainian and foreign scientific achievements and practices in determining the main provisions of functioning and development of the dairy industry of Ukraine; method of system-structural analysis – to analyse the economic activity of agricultural enterprises engaged in milk production; method of cause-and-effect analysis – to identify urgent problems in the activities of enterprises in this industry; calculation and constructive method – to calculate the main indicators of development and economic efficiency of milk production in agricultural enterprises of Ukraine; method of logical generalisation – to formulate conclusions and proposals. The dynamics of milk production in Ukraine by major commodity producers and indicators of its economic efficiency were investigated using economic-statistical and economic-mathematical methods. Microsoft Excel software was used for statistical processing of the study results.

The requirements for raw materials necessary to produce dairy products were compared following the national standard DSTU 3662:2018 (2019). To adjust the selling price of milk, Methodological regulations on the organization of state statistical monitoring of the supply of milk to processing enterprises (n.d.) were considered.

RESULTS AND DISCUSSION

Since 1991, the situation in Ukraine's dairy industry has been radically different from what is happening globally. While in 1991, the world produced 440 million tonnes of milk, in 2021 it was 726 million tonnes. In 1991, Ukraine's gross milk yield was 24.5 million tonnes, and in 2022 it was 7.7 million tonnes. Thus, global milk production increased by 1.5 times, while in Ukraine it decreased by three times (Zhupinas, 2023). One of the factors of negative impact on milk supply is a constant decrease in livestock, which leads to a decrease in dairy production, conflicting interests of milk producers and processors, and a decrease in per capita consumption of milk and dairy products (Cherednichenko & Pashchenko, 2018). The dairy industry suffered significant losses in the first months of the war in 2022.

The occupation of the territories and hostilities meant that a considerable number of livestock was simply destroyed by the Russian invaders, and another part of the farm was forced to be sold for nothing or transported to safer regions. Thus, the industry lost 1 million tonnes of milk due to the war (Association of milk producers in Ukraine, n.d.). An analysis of the dynamics of milk production in Ukraine over 2010-2022 suggests that in all categories of farms, the average annual decline rate is 2.3%, including 2.0% and 3.7% in agricultural enterprises and households, respectively (Table 1).

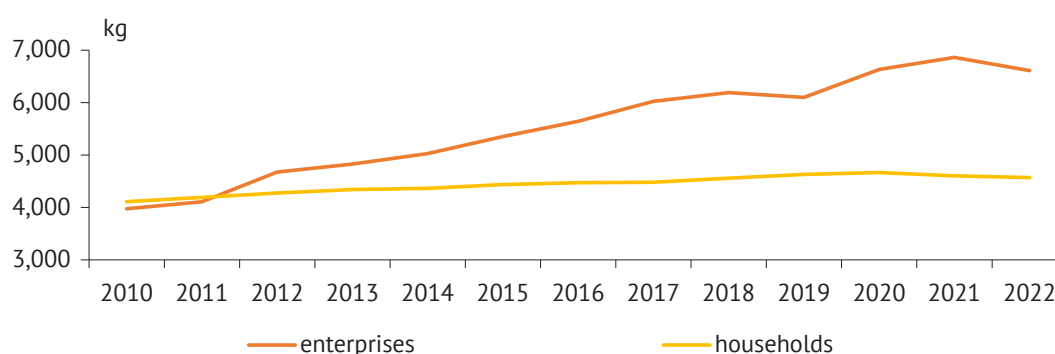
Table 1. Dynamics of milk production in Ukraine, thous. t

Years	Farms of all categories	incl.			
		enterprises		households	
		thous. tonnes	% of total	thous. tonnes	% of total
2010	11,249	2,217	19.7	9,032	80.3
2011	11,086	2,246	20.3	8,840	79.7
2012	11,378	2,535	22.3	8,842	77.7
2013	11,488	2,583	22.5	8,906	77.5
2014	11,133	2,648	23.8	8,485	76.2
2015	10,615	2,669	25.1	7,946	74.9
2016	10,387	2,711	26.1	7,676	73.9
2017	10,281	2,766	26.9	7,515	73.1
2018	10,064	2,756	27.4	7,309	72.6
2019	9,663	2,729	28.2	6,935	71.8
2020	9,264	2,761	29.8	6,502	70.2
2021	8,714	2,768	31.8	5,946	68.2
average annual growth (decline) rate, %	-2.3	2.0	–	-3.7	–
2022	7,768	2,644	34.0	5,124	66.0
2022 to 2021, %	89.1	95.5	–	86.2	–

Source: calculated by the authors of this study based on data from the State Statistics Service of Ukraine (n.d.)

In 2022, all categories of farms produced 7,768 thous. t of milk, agricultural enterprises – 2,644 thous. t, households – 5,124 thous. t, which is 10.9%, 4.5%, and 13.8% less than in 2021, respectively. Thus, households have reduced milk production the most substantially. This is explained by the fact that they were forced to sell or abandon their livestock when fleeing the war from the occupied territories and the active combat zone.

An analysis of livestock productivity over 2010–2022, both in agricultural enterprises and households, suggests its growth. Thus, in 2022, the milk yield per cow in agricultural enterprises was 5,119 kg, while in households – 6,611 kg, which is 66.3% and 11.2% more than in 2010, respectively. Thus, according to the research, agricultural enterprises provided a much higher level of productivity than households and had an average growth rate of 5.1% compared to 1% for households (Fig. 1).

**Figure 1.** Average annual milk yield per cow in Ukraine, kg

Source: compiled by the authors of this study based on data from State Statistics Service of Ukraine (n.d.)

In 2022, interruptions in the supply of feed and veterinary medicines and power outages led to a decline in cow productivity, which ultimately led to a decrease in gross milk production. In 1990, there were 643 dairy plants in Ukraine, and as of 1 January 2022, only 140 of them had received permission to produce

dairy products, of which 111 were operating. Since the outbreak of full-scale hostilities, the dairy processing industry has lost 38 enterprises in the occupied and temporarily occupied territories, and therefore as of 1 December 2022, only 73 were operating, which is almost 9 times less than in 1990. The total capacity of

the remaining enterprises is 4.9 mln t of raw materials. However, they are still under-utilised with raw materials, and therefore their unit costs are much higher than if they were fully utilising their capacity. The logistics aspect also plays a major role. Provided that the farm produces 15-20 t of milk, the processor is ready to take the raw materials even 300 km away. However, there are many small milk producers in Ukraine whose transportation is not very profitable for the processor (AgroTimes, n.d.).

An analysis of milk supply to Ukrainian processing enterprises in 2010-2022 shows that its quantity has decreased. Thus, in 2022, 2,740 thousand tonnes of milk were supplied, which is 42.8% less than in 2010. Notably, the structure of milk supply has changed considerably over the relevant period: while in 2010, 41.7% of milk came from agricultural enterprises and 56.1% from households, in 2022 the share of agricultural enterprises increased to 86.7%, and the share of households decreased to 13.3% (Table 2).

Table 2. Milk supply to Ukrainian processing enterprises, thous. t

Indicators	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Supply of milk and dairy products for processing, total	4,793	4,615	4,716	4,570	4,647	4,251	4,183	4,348	4,179	3,800	3,512	3,198	2,740
of which were purchased	4,537	4,547	4,692	4,545	4,617	4,090	3,710	3,928	3,809	3,462	3,289	3,032	2,657
incl. purchased from: enterprises	1,890	2,030	2,278	2,325	2,428	2,413	2,512	2,689	2,720	2,610	2,556	2,478	2,305
share of enterprises, %	41.7	44.6	48.6	51.2	52.6	59.0	67.7	68.5	71.4	75.4	77.7	81.7	86.7
households	2,544	2,155	2,007	1,824	1,737	1,346	1,198	1,239	1,089	851	733	554	353
share of households, %	56.1	47.4	42.8	40.1	37.6	32.9	32.3	31.5	28.6	24.6	22.3	18.3	13.3
other business entities	302.6	362	407	396	452	331							
Received on a tolling basis	42.1	59	15	6	7	138	441	396	347	318	203	148	74.8
Own-produced milk that was processed	13.9	9	10	19	23	24	32	25	25	20	20	18	7.8

Source: calculated by the authors of this study based on data from the State Statistics Service of Ukraine (n.d.)

One of the key factors in incentivising dairy producers is the price, the increase of which affects the efficiency of the enterprise (Bal-Prylypko *et al.*, 2022). Today, due to the involvement of intermediary structures, the purchase price for dairy products increases by 15-20%. Finished products are also sold from the processing plant to retailers through intermediary structures, which are usually recommended by the retailers where the products are sold. Depending on the range of products and the corresponding surcharges for the sale of dairy products in the retail network, the wholesale price of the company's

dairy products increases by 25-60%. Often, the same product passes through two or three of these structures, which leads to an increase in price. Retailers sell at their own margin, and producers have no other alternative.

As a result of price distortions, raw milk is sold for virtually nothing, and prices for industrial products and services are so high that milk production for most farms becomes unprofitable or even detrimental. Thus, in 2022, agricultural enterprises sold milk at UAH 10,678 per tonne, while the retail price of milk in the retail network was UAH 35-42 per 1 litre (Fig. 2).

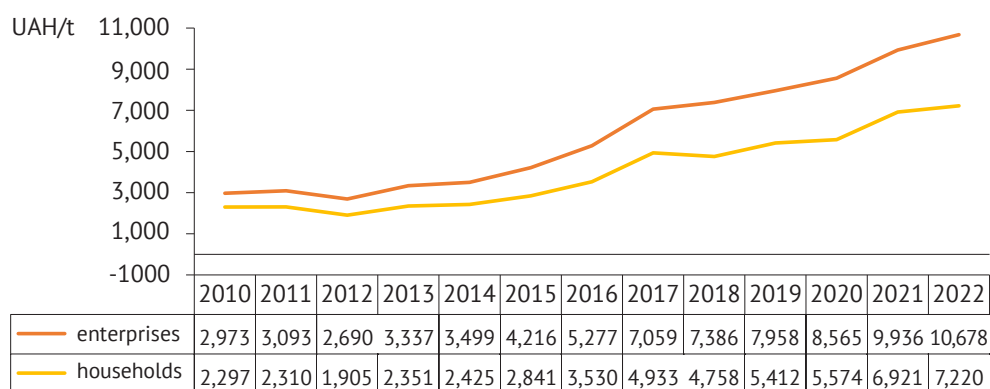


Figure 2. Average milk purchase price, UAH/t

Source: calculated by the authors of this study based on data from the State Statistics Service of Ukraine (n.d.)

In March 2022, the situation in dairy farming was more critical than before due to the disruption of the

supply chain for raw milk, finished products from dairy farms and dairy processing plants, which resulted in a

4.6 EUR/100 kg decrease in purchase prices. Due to the increase in exports in May-July 2022, the price of raw milk stabilised at 37.6-37.8 EUR/100 kg. However, as a result of the dollar's appreciation against the hryvnia in July, the price of milk dropped to 32 EUR/100 kg. Subsequently, the strengthening of the hryvnia and increased export activity led to an increase in the price of milk in hryvnia terms, and in November the price of milk reached 36 EUR/100 kg (Association of milk producers in Ukraine, n.d.).

At the end of 2022, purchase prices for raw milk were 40% lower than in the EU and 11% lower than in 2021. The Ukrainian dairy market is affected by the following factors that constrain milk price increases: reduced demand for dairy products, rising production costs due to the energy crisis, and lower real incomes, which leads to a decrease in dairy consumption in the short term. However, there is underutilised capacity in

the dairy industry that could be used to further develop the market, depending on the strength of external and internal demand. It is believed that due to the diversification of foreign markets, support of the Ukrainian consumer as a result of the reduction of the VAT rate on milk and dairy products and the implementation of the School Milk programme, favourable conditions will be created for the recovery of the Ukrainian dairy industry (Association of milk producers in Ukraine, n.d.).

Over 2010-2022, milk production at agricultural enterprises has been consistently profitable. Milk retained its status as the most profitable livestock product. Thus, in 2022, the profitability of production of this product in agricultural enterprises was 17.4% (Fig. 3). However, the authors believe that achieving 20% profitability is insufficient for expanding business and attracting considerable investment resources.

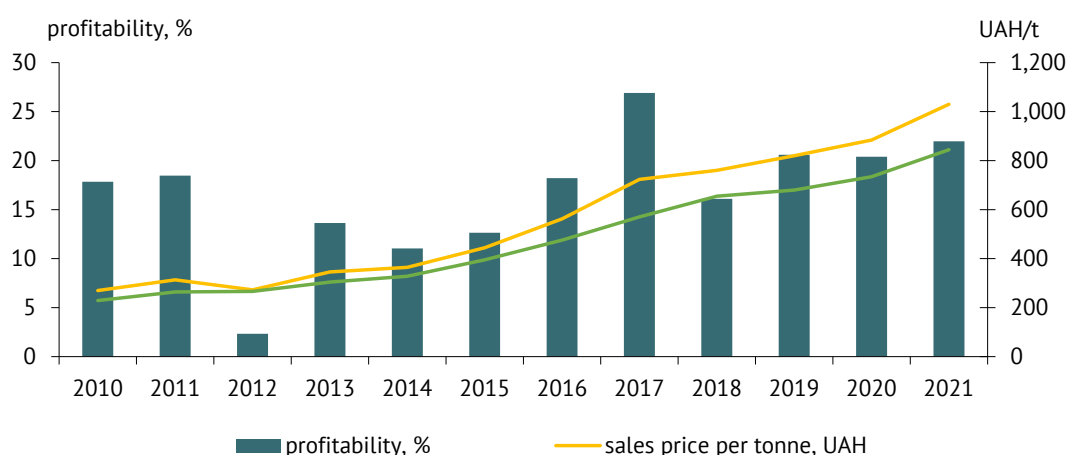


Figure 3. Indicators of economic efficiency of milk production

Source: calculated by the authors of this study based on data from the State Statistics Service of Ukraine (n.d.)

Dairy farming faces a significant risk of becoming unprofitable, as the industry has been stagnating for a long time and requires not only government support but also a systematic approach to effective strategic decisions at the level of each business entity, regions, and the entire country. Approximation to European standards requires compliance with new requirements for raw materials needed for dairy production. Ukrainian producers were given time, the so-called transition period, to bring the quality of cow's milk up to the required standards. In 2018, a new national standard DSTU 3662:2018 (2019) was introduced, which reinforced the requirements for the raw milk production process and its quality. In the grading system, milk is divided into grades (extra, premium, first) (Stepasyuk, 2020).

However, the implementation of these requirements for the safety and quality of milk and dairy products was postponed for two years until 1 January 2023 (Order of the Ministry of Agrarian Policy and Food, 2019). The extension of the transitional period for certain

measures on state control of raw milk production and marketing was intended to provide time for milk producers and processors to harmonise their relations and create an effective organisational mechanism for the Ukrainian dairy industry. The state should be actively involved in the implementation of the Strategy for the Development of Dairy Farming and the Dairy Processing Industry, the development of relevant policies and the distribution of functions of competent authorities, including the State Service of Ukraine for Food Safety and Consumer Protection (Avercheva, 2021).

An analysis of milk supply to processing enterprises shows that milk produced by agricultural enterprises is of much higher quality. Thus, the share of milk that meets the requirements of the extra grade is 47.2%, while the highest and first grades are 32.6% and 19.3%, respectively. The quality of raw milk supplied for processing by households is quite different, with no extra milk at all, only 3.9% of milk of the highest quality, and the vast majority of milk being of the first and second quality, namely 77.1% and 19% (Fig. 4).

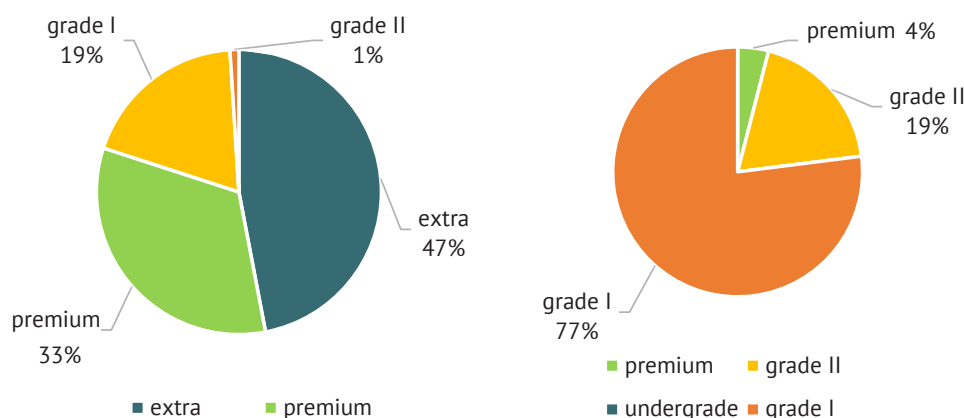


Figure 4. Structure of milk sold by agricultural enterprises and households, 2022

Source: calculated by the authors of this study based on data from State Statistics Service of Ukraine (n.d.), Clal.it (n.d.).

It was found that the quality of milk sold by agricultural enterprises tends to improve (Table 3). And this is natural, as producers seek to earn more income, and the price difference between grade I and grade II ranges within 10-20%, while between grade I and non-grade

milk this deviation is over 30%. In addition, agricultural enterprises already have the proper conditions (minimal contact with air and prompt cooling) to produce such milk, and most of them breed highly productive cows with proper housing, feeding, and veterinary care.

Table 3. Quality of milk supplied for industrial processing, by grade

Indicators	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Agricultural enterprises												
Volume of milk supplied, thous. t	1,919	2,160	2,193	2,298	2,287	2,375	2,533	2,560	2,428	2,348	2,253	2,107
in terms of milk with basic fat content	2,030	2,278	2,325	2,428	2,413	2,512	2,688	2,720	2,610	2,556	2,478	2,305
including by grade:												
– extra	113	120	227	223	248	366	441	586	710	885	973	1089
specific weight, %	5.6	5.3	9.8	9.2	10.3	14.6	16.4	21.6	27.2	34.6	39.3	47.2
– prime grade	669	711	798.7	821	850	923	987	1036	938	874	875	750
specific weight, %	33.0	31.2	34.4	33.8	35.2	36.7	36.7	38.1	35.9	34.2	35.3	32.6
– grade I	1,157	1,311	1,182	1,251	1,197	1,056	1,018	895	844	776	614	444
specific weight, %	57.0	57.6	50.8	51.5	49.6	42.0	37.9	32.9	32.3	30.4	24.8	19.3
– grade II	83	128	113.7	129	113	160	235	194	97	16	16	21
specific weight, %	4.1	5.6	4.9	5.3	4.7	6.4	8.7	7.1	3.7	0.6	0.6	0.9
Undergrade	8	7	3.7	5	5	6	7	9	21	5	0.5	0.2
specific weight, %	0.4	0.3	0.2	0.2	0.2	0.3	0.3	0.3	0.8	0.2	0.0	0.0
Share in purchased milk, %												
– fat	3.6	3.59	3.6	3.59	3.59	3.6	3.6	3.6	3.66	3.7	3.74	3.72
– protein	x	x	x	3.07	3.05	3.06	3.06	3.1	3.13	3.16	3.19	3.20
Households												
Volume of milk supplied, thous. t	2,094	1,954	1,771	1,699	1,312	1,161	1,200	1,054	822	708	531	337
in terms of milk with basic fat content	2,155	2,007	1,824	1,737	1,346	1,198	1,239	1,089	851	733	554	353
including by grade:												
– prime grade	7.6	4.4	1.223	1.7	1.0	0.6	1.6	2.3	0.8	1.6	0.7	13.7
specific weight, %	0.4	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.1	3.9
– grade I	521	341	238.9	205	119	108	110	136	102	586	436	272
specific weight, %	24.2	17.0	13.1	11.8	8.9	9.0	8.9	12.4	12.0	79.9	78.7	77.1
– grade II	1,573	1,508	1,507	1,452	1,163	1,028	1,081	904	708	135	108	67
specific weight, %	73.0	75.1	82.6	83.6	86.4	85.8	87.2	83.0	83.2	18.4	19.6	19.0

Table 3. Continued

Indicators	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Undergrade	55	154	76.9	79	63	61	46	47	40	10.8	9.1	0.1
specific weight, %	2.5	7.7	4.2	4.5	4.7	5.1	3.7	4.3	4.8	1.5	1.6	0.0
Share in purchased milk, %												
– fat	3.5	3.49	3.5	3.48	3.49	3.51	3.51	3.51	3.52	3.52	3.54	3.56
– protein	x	x	x	2.93	2.92	2.93	2.95	2.96	2.94	2.96	2.94	2.96

Source: calculated by the authors of this study based on data from State Statistics Service of Ukraine (n.d.), Yu. Lupenko et al. (2023)

It is much more difficult to bring milk from private households to a high quality, as the conditions of cows in such farms and the milking process do not always ensure that raw milk is of a high quality. In some cases, contamination of milk with microorganisms can occur even during milking due to non-compliance with sanitary standards. All this leads to contamination of milk with undesirable microflora, and due to imperfect procurement and logistics, milk from the private sector does

not meet the requirements of regulatory documents. An analysis of milk supply to processing companies shows that in 10 months of 2022, extra grade milk was processed 10% more than in 2021 (Fig. 5). In other words, in 2022, 46% of milk supplied by agricultural enterprises was extra grade milk. Thus, in 2022, the share of extra grade milk produced by agricultural enterprises in Ukraine increased by a greater percentage than in the EU but did not reach their level.

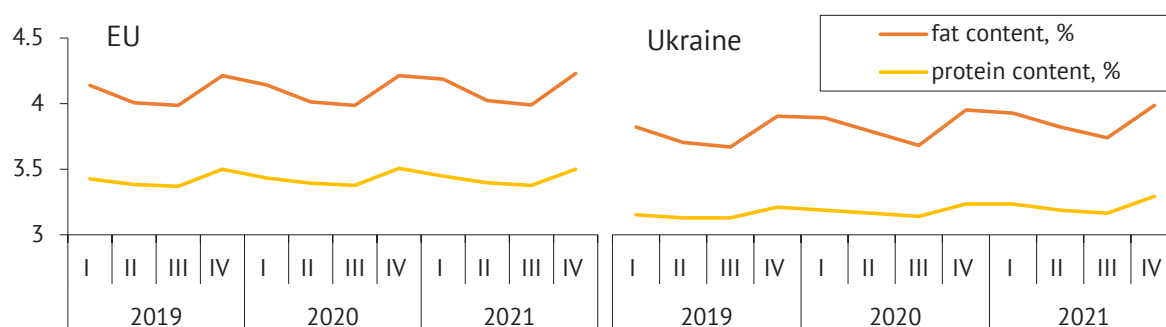


Figure 5. Milk quality (fat and protein content) in the EU and Ukraine in 2019-2021 (by quarters)

Source: calculated by the authors of this study based on data from State Statistics Service of Ukraine (n.d.), Clal.it (n.d.)

As noted earlier, Ukrainian standards are considerably inferior to European ones. When it comes to the quality of Ukrainian and European milk, these

parameters differ significantly. Thus, fat content, protein, somatic cell count, and bacterial contamination in Ukraine are considerably lower than in Europe (Table 4).

Table 4. Main parameters of milk quality in Ukraine and Europe

Country	Fat content, %	Protein content, %	SCC, cm ³	Bacterial contamination, cm ³
Ukraine	3.4	3.0	<400000	<100000
Europe	4.2	3.4	<249999	<24999

Source: DSTU 3662:2018 (2019)

It was found that the higher level of raw milk prices in the world is conditioned by higher requirements for product quality – fat, protein, antibiotics, somatic cell count, and bacterial contamination. Considering the European fat content of 4.2% and protein content of 3.4%, the authors recalculated the purchase prices for raw milk in Ukraine relative to the European ones, using the methodology described below (CLAL.it). The pricing of milk and dairy products should factor in the quality of the product (fat and protein content as 40:60), which is incentivised by premiums and surcharges.

$$P = \left(\frac{P_{EU}}{P_{UA}} \cdot 0.6 + \frac{F_{EU}}{F_{UA}} \cdot 0.4 \right) \cdot Pr_f, \quad (1)$$

where P_{EU} is the average protein content for EU countries, %; P_{UA} is the protein content in Ukraine, %; F_{EU} is the average fat content for EU countries, %; F_{UA} is the fat content in Ukraine, %; Pr_f – the factual price of milk in Ukraine, EUR/t.

Comparing the raw milk purchase prices recalculated to the basic indicators, it was found that their factual level in Ukraine (43.47 EUR/t in October 2023 in European countries, against 39 EUR/t in Ukraine for the corresponding period) is lower than in most European countries (Fig. 6).

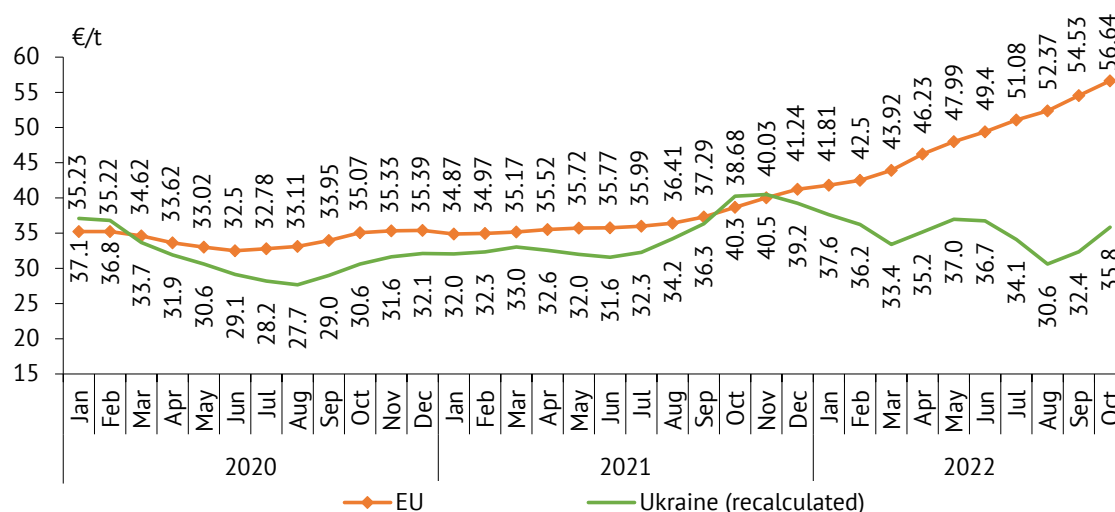


Figure 6. Average milk price in the EU and Ukraine (converted to EU milk quality), EUR/t

Source: calculated by the authors of this study based on data from State Statistics Service of Ukraine (n.d.), Clal.it (n.d.)

Thus, at the end of 2022, purchase prices for milk in Ukraine were 40% lower than in the EU and 11% lower than in 2021. Ukraine's dairy market is under pressure from a range of factors that are holding back milk price growth: declining domestic consumer demand; rising unemployment, which indicates a decline in dairy consumption in the near future; rising production costs due to the destruction of the energy system by Russia's shelling; depreciation of the hryvnia, etc. (Association of milk producers in Ukraine, n.d.).

There are numerous discussions among scientists about the readiness of Ukrainian milk producers to comply with European standards for fat (4%) and protein (3.4%) in milk. During the Soviet era, the only basic indicator of milk quality was fat content. The protein content indicator appeared a little later due to the lack of animal protein in the diet. Ukraine has the lowest basic indicators of fat and protein among post-Soviet countries. These requirements are not only consumer but also economic in nature. Thus, Israel does not accept Ukrainian milk for processing, even extra grade milk. The authors of the present study believe that achieving these standards will enable Ukrainian producers to join the so-called "elite" of milk producers.

Analysts have different opinions on compliance with standards. Y. Sivov *et al.* (2020) note the need to comply with such standards, but note that as a result of their application, the profitability of an average dairy farm may decrease by 12-14%. M. Bugai (2020) believes that Ukrainian dairy producers have all the capacities to produce products according to European standards without fantastic investments. An equally essential issue in the dairy industry is the dictates of retailers towards dairy producers. J. Britt *et al.* (2018) predict an increase in production and safety in the dairy industry through consolidation, modernisation, and specialisation. It is noted that large dairy farms

will increase the level of automation to reduce costs, which will affect their profitability. The authors envisage a transition from the usual export of surplus to an increase in the production of value-added products that will be adapted to particular tastes and traditions. O. Shpychak *et al.* (2022) believe that to ensure the production and sale of high-quality milk, it is necessary to reorganise its production by concentrating the number of cows in farms. Government support mechanisms can play a vital role in this regard.

According to L. Tulush, Head of the Analytical Department of the Union of Dairy Enterprises of Ukraine (2021), state support measures, entrepreneurs' efforts and consideration of international practices will help ensure food security in Ukraine, reduce dairy imports, and introduce advanced resource-saving technologies to produce high-quality dairy products. If the quality of their products is maintained, domestic producers will be able to expand their sales geography by developing new international markets.

The state should facilitate the implementation of financial incentives for the development of the dairy industry to stabilise milk production. There is a shortage of raw milk in Ukraine, which prompts processing companies to consider various options for maximising their capacity utilisation. Otherwise, Ukraine will become more dependent on dairy imports, which will increase threats to food security. Thus, negative processes in the industry will become irreversible if the state does not intervene in this situation in the coming years (Tulush, 2021). N. Shyian and V. Kolosha (2020) also note that with the joint efforts of the state, milk producers, and processors, the Ukrainian dairy market has a real potential to run a profitable business and become a powerful exporter of dairy products to the European market and other countries. The findings of the present study and the conclusions of the above-mentioned

authors suggest that, given the strategic course towards European integration, the dairy industry of Ukraine should be guided by European product quality standards. A vital factor in fulfilling the purpose of the strategy is to improve the quality of the raw material base through concentration of livestock and joint efforts of the state, milk producers, and processors.

CONCLUSIONS

The conducted study suggests that, since Ukraine has been granted EU candidate status, the issue of the quality of domestic products is of particular importance. Given that European standards for raw milk procurement are several orders of magnitude higher than Ukrainian standards, it is necessary to harmonise state standards for these products with international requirements and introduce certain measures to improve the culture of milk production, delivery, and marketing to dairy processing enterprises.

An equally urgent issue is private households, which should be transformed into family dairy farms (cooperatives) to preserve the livestock, because second-grade raw milk, which is mostly produced by these farms, cannot be used to produce export-oriented products, which is 75% of all milk produced in Ukraine. Statistics show that more than half of the world's milk is processed by cooperatives, as this business model best serves the interests of raw milk producers and the processing industry.

It was found that the competitiveness of dairy products depends on the quality of the raw material base. Strict quality requirements for raw milk limit the ability of most Ukrainian dairy processing companies to enter European markets. Therefore, government mechanisms of influence should be aimed at supporting Ukrainian producers and promoting the formation of specialised milk cooperatives. The authors of this study propose to consider the quality parameters of products when forming the price of milk of Ukrainian

producers using surcharges and extra charges to balance it against the basic indicators of raw milk quality in European countries. Comparing the raw milk purchase prices recalculated to the basic indicators, it was found that their factual level in Ukraine in October 2023 was 39.0 EUR/100 kg, and in European countries – 43.47 EUR/100 kg. Thus, the price of milk in Europe is 11.5% higher than the factual price offered by Ukrainian producers, which is an incentive for them to improve product quality and, consequently, generate higher profits.

The analysis of the current state of dairy farming suggests that to boost the dairy business, it is necessary to increase state support for the industry. The fact that the purchase price of milk from both agricultural enterprises and households, which is supplied to dairy processing plants, is almost three times lower than the final retail price is unacceptable. Therefore, the development of the dairy industry in the long term is possible only if the interests of all market players – producers, processors, and the state – are considered and they work closely together.

Further research will be aimed at a detailed analysis of the set of factors that directly affect the selling price of milk and substantiation of a fair distribution of the price between producers, processors, and trade. In the future, special attention should be paid to marketing strategies and innovative capabilities of the dairy business. Thus, the introduction of an effective mechanism for implementing the proposed measures with government support will be the driving force behind the accelerated development of the dairy industry.

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None.

CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Анотація. Необхідність пошуку шляхів підвищення якісних характеристик молока як основи підвищення конкурентоспроможності молочної продукції на світовому ринку зумовлює актуальність дослідження. Молочна галузь України є однією з важливих у аграрному секторі економіки, так як забезпечує населення країни життєво необхідними продуктами харчування. Мета дослідження – обґрунтувати напрями вирішення організаційно-економічних проблем розвитку молочної галузі для задоволення потреб внутрішнього ринку якісною молочною продукцією та збільшення експортного потенціалу. Дослідження проводилися з метою виявлення основних причин трансформаційних змін, які відбулися в молочному скотарстві та окреслення перспективних напрямків розвитку даної галузі в контексті підвищення якості продукції. У фокусі даного дослідження – європейські та національні вимоги до якості молоко-сировини. При роботі з аналітичним матеріалом використовувалися такі методи економічних досліджень: економіко-статистичний, порівняльного аналізу, графічний, логічного узагальнення, системного підходу, системно-структурного аналізу, розрахунково-конструктивний. У статті проаналізовано дані сільськогосподарських підприємств і господарств населення України, які займаються виробництвом молока за 2010-2022 роки та здійснено перерахунок закупівельних цін на молоко-сировину відносно європейських показників якості продукції. Результати проведеного дослідження свідчать, що забезпечення конкурентоспроможності продукції молочної галузі залежить від якості молока. Встановлено, що вона є вищою в європейських країнах, тому високі стандарти щодо якості молочної сировини стають бар'єром входження на європейські ринки для більшості вітчизняних молокопереробних підприємств. Відповідно, стратегія держави повинна орієнтуватися на збільшення виробництва молочної сировини високої якості та зростання експортного потенціалу шляхом формування спеціалізованих кооперативів із виробництва молока для молокопереробних підприємств. Практична значимість отриманих результатів полягає в тому, що авторські рекомендації можуть бути використані сільськогосподарськими підприємствами чи особистими господарствами населення в їхній пересічній діяльності

Ключові слова: молоко; якість молочної продукції; міжнародні стандарти; виробники продукції; ціна релізації



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Agricultural and resource economy of Ukraine and problems for economic growth

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Abstract. Ukraine's economy is highly dependent on the agricultural sector; it is the main component of the country's international specialisation in the international arena and is one of the main representatives of budget revenues. Thus, its research remains relevant, as does the investigation of historical prerequisites and prospects for this. The purpose of the study was to assess the latest trends in the development of the industry and, based on them, form recommendations for the development of the country, in particular, in wartime conditions. The main methods used in the study were analysis, historical analysis, forecasting, and abstraction. Within the framework of the study, some statistical data describing the latest trends in the development of the agricultural sector of Ukraine were analysed, including international trade in industry products, the amount of land planted with agricultural crops, the amount of livestock products produced. It was found that there were positive trends in the development of agriculture in the country, especially in the field of crop production. However, the events of 2014 and 2022 have led to political and economic instability in the country, resulting in stagnation (in the case of animal husbandry) and a decrease in growth rates (for crop production). Considering the difficulties that exist for the development of crop production in war conditions, separate recommendations were proposed for the development of national policy. These include minimising the negative impact of

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corruption (developing institutions), promoting innovation, and attracting investment. Although some such actions are already being implemented by the state in the form of various support measures, they are still not enough to solve all the problems of development in the sector. The paper brings new knowledge to the field of research in the agricultural sector, in particular in Ukraine. This knowledge will allow for more effective policy-making for both public sector representatives and entrepreneurs

Keywords: innovation; international trade; entrepreneurship; finance; agriculture

INTRODUCTION

The agricultural sector as a whole plays an important role in the global and national economy, ensuring food security, creating jobs, and positively influencing the social, economic and environmental components of society. The agricultural sector is the main source of food products for the population, which becomes very important in conditions of various crises that can lead to an increase in prices for these products on the market. Developed agriculture in general strengthens the strategic power of the state, provides raw materials to other industries, and also has a positive impact on the socio-cultural landscape of individual regions. In modern conditions, this industry requires innovation and the latest technologies to increase productivity and sustainable development. Agriculture remains one of the main industries in Ukraine. This is related both to some geographical factors (for example, the high fertility of local soils) and historical ones (the country was often a component of empires or other associations, remaining their raw material appendage). Nevertheless, even in modern conditions, this area has many difficulties that complicate its development.

A significant number of researchers have studied the current features of the development of the agricultural sector in Ukraine. J.A.T. Silva *et al.* (2023) considered agricultural production in Ukraine in the context of a full-scale Russian invasion. Researchers described the significant damage that the agricultural sector received due to military operations and the economic consequences of the war, but did not indicate recommendations that the state authorities can take to reduce the existing negative impact. O.V. Zakharchuk *et al.* (2021) assessed the current state of agricultural development in Ukraine and highlighted the main macroeconomic factors that influenced decision-making in this area. In addition, they offered solutions in the context of improving the efficiency of activities for businesses in agriculture in Ukraine. O. Tomilin *et al.* (2023) investigated the current state and dynamics of changes in land resources in agriculture in Ukraine. They wrote about the importance of maintaining the sectoral structure of agro-industrial production and achieving macroeconomic equilibrium by maintaining the most effective intersectoral proportions in the national economy and coordinating national interests. The features of modern conditions for the development of national policy, in turn, were evaluated by I.P. Dynnyk (2022). The researcher noted the main areas

of modern state agrarian policy, in particular, stabilisation of agro-industrial production, ensuring food security, supporting agricultural markets, improving food supply, maintaining balance with other sectors of the economy, protecting domestic producers. However, he gave quite a few recommendations to improve the effectiveness of policy implementation. L. Moldavan *et al.* (2023) investigated the sustainable development of Ukrainian agriculture in the context of climate change. Researchers drew attention to the problems of specialised monocultural farming, which is managed by large agro-industrial companies to make a profit. In view of this, it was proposed to switch from monocultural production to reduce the negative environmental effect of the activities of large agricultural enterprises.

Thus, the purpose of this study was to assess the latest trends in the development of agriculture in Ukraine. This is important to be able to better understand the current state of the sector and more effectively form public policy in it, conduct business.

MATERIALS AND METHODS

The study was conducted on the time interval from 2001 to 2023, since this period allows the best assessment of recent trends in the development of agriculture in Ukraine (choosing a longer period may lead to distorted results, given that the factors of the country's secession from the Union of Soviet Socialist Republics played a significant role in the 1990s). The main source for such information was the State Statistics Service of Ukraine (Areas, gross harvests..., 2023). Information from this service was used to assess the natural characteristics of agricultural development in the country, and to assess the net income of enterprises. The data that characterised international trade in agricultural products was taken from another service, Trade Map, through easier and more complete access to data.

It was decided to aggregate and summarise the data provided by the State Statistics Service as much as possible to simplify the understanding and presentation of data on the development of agriculture in Ukraine. However, sometimes there were difficulties with this. For example, in the context of crop production, information was provided on the volume of gross production and crop yields separately by group. Indeed, this gives a better understanding of the general situation separately for each of them, but it would be

inappropriate to provide such data in a study. Thus, it was decided to aggregate them. Thus, the indicator of

the average change in the yield of agricultural products was calculated as follows:

$$C_n = \left(\frac{(I_{n1} * I_{n2} * \dots * I_{nm})^{\frac{1}{m}}}{(I_{(n-1)1} * I_{(n-1)2} * \dots * I_{(n-1)m})^{\frac{1}{m}}} \right) * 100 - 100) * C_{n-1}, \quad (1)$$

where C_n – value of the indicator in the period n ; I_n – value of the indicator that appears when finding the percentage of yield growth of a certain type of agricultural product; m – number of indicators; n – period; C_0 – 100.

This approach allowed estimating the average change in agricultural yield in the country. Although it does not evaluate the weight component of each type of product separately, it provides significantly more correct indicators (in terms of agricultural characteristics) than other approaches based on available data and their aggregation needs. A similar approach has become typical for further analysis of livestock products.

The paper also examined the volume of international trade in agricultural products of Ukraine, and the income of local enterprises. To estimate export and import data, the study used data from the Trade Map source adjusted for the inflation rate, the value of which was taken from Macrotrends (U.S. Inflation Rate..., 2023). Data on the income of enterprises were taken from the State Statistics Service of Ukraine, and also adjusted for the level of inflation published by the same service. Data in the context of financial results of agricultural companies were available only for the period since 2010, which explains this time limit when analysing this indicator.

The main approach that was applied during the study was systematic. It allowed the study to analyse the

selected elements within a single system, where they interact with each other. This enabled a more accurate assessment of current trends in the study of the Ukrainian agricultural sector. A significant number of different research methods were also used. The historical method was used to investigate data on the state of the agricultural sector of the Ukrainian economy in recent years and form conclusions on this issue for the current state of the country's agricultural sector. Using the forecasting method, assumptions were formed about how this sector might develop in the future, and the country's economy as a whole. Abstraction also allowed limiting the number of factors that were considered during the study to assess the development of agriculture. This, in turn, helped to increase the effectiveness of the study by improving its accuracy and clarity.

RESULTS

The trends described above can be seen using statistical data. Although their duration is limited to the period since independence, and often even shorter, they can reveal some trends that are characteristic of Ukraine's current situation. First, it is worth considering individual indicators that characterise both animal husbandry and crop production in natural terms. They are shown in Figure 1.

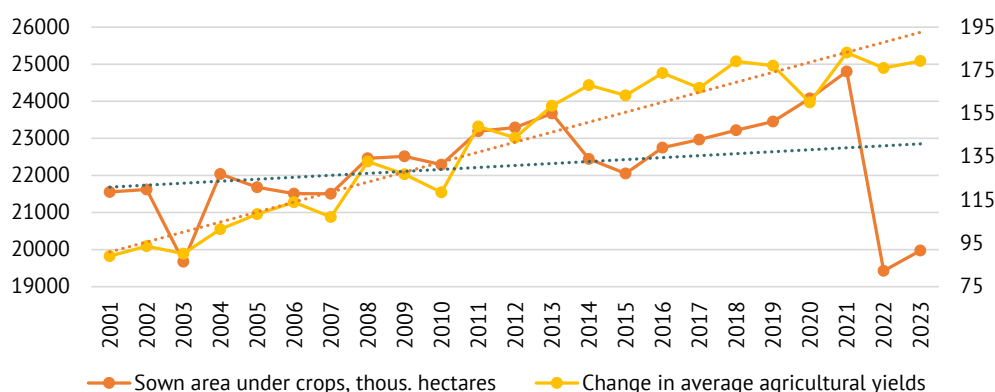


Figure 1. Data on the area of land planted with agricultural crops and information on the average yield of agricultural products in Ukraine in the period from 2001 to 2023

Source: compiled by the authors based on data from the State Statistics Service (Areas, gross harvests..., 2023)

As can be seen from Figure 1, the area under agricultural land gradually increased over time, including the average yield, which, in general, indicates the gradual development of this area. This was due to the export orientation of the agricultural sector of Ukraine and the simultaneous increase in demand for grain products, rising food prices. A significant decrease in acreage is

observed in 2014 due to the difficult political situation, the beginning of the events on the Maidan, the annexation of Crimea, and the beginning of the war with Russia. All of this has led to instability, reduced investment, and increased risks to the agricultural sector, while lower food prices have reduced farmers' motivation to expand their acreage. A similar situation is observed in

2022, but its volumes are much larger: the inflow of investment to the country significantly decreased, there were restrictions on trade in agricultural products, and

some territories were temporarily occupied. All this caused the loss of acreage. Information describing the field of animal husbandry can be seen in Figure 2.

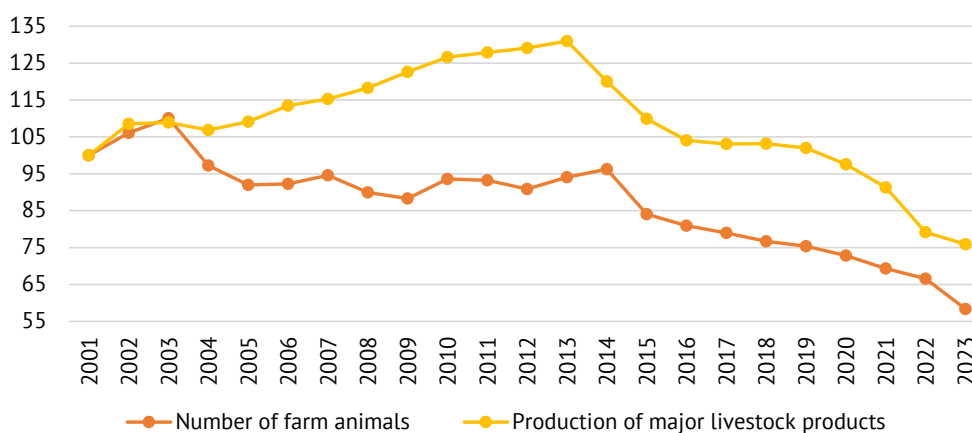


Figure 2. Data on changes in the number of farm animals and production of the main types of livestock products in Ukraine in the period from 2001 to 2023

Source: compiled by the authors based on data from the State Statistics Service (Areas, gross harvests..., 2023)

As can be seen from Figure 2, there is a trend in the country for a gradual decrease in the number of farm animals and the production of livestock products, which is generally a sign of stagnation of the industry. This is conditioned by the fact that environmental conditions in the country are more favourable for growing crop production, rather than animal husbandry. Thus, most farmers are gradually moving to growing grain. As for the production of livestock products, it grew until 2014 simultaneously with a decrease in the number of farm animals, which suggests that the reason for the growth is precisely the improvement of livestock productivity in the country, which is associated with the gradual recovery of the country after the collapse of the Union of Soviet Socialist Republics (USSR), attracting foreign investment, updating

the technological base in the industry. However, since 2014, there has been a gradual decline, which is also associated with the beginning of economic and political instability caused by the annexation of Crimea by Russia and the war in Donbas. The situation in 2022 only worsened due to the beginning of a full-scale invasion and even greater problems for farmers, both economic, such as restrictions on international trade and inflation, and military, namely the destruction of infrastructure and the loss of territories for growing livestock products. After analysing the data, it can also be seen from Figure 1 that the country has become more specialised in crop production, rather than animal husbandry, which causes changes in the structure of agricultural production. The income of agricultural enterprises in Ukraine is shown in Figure 3.

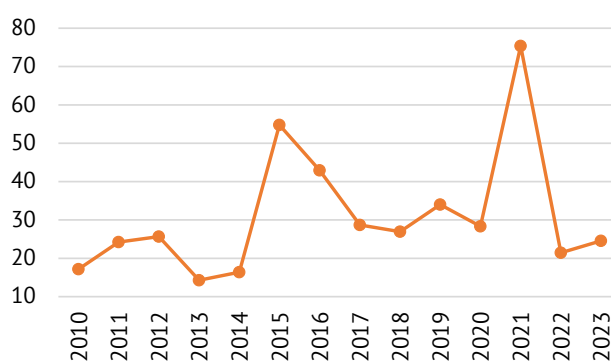


Figure 3. Net income of agricultural enterprises in Ukraine (adjusted for inflation) in the period from 2010 to 2022

Source: compiled by the authors based on data from the State Statistics Service (Areas, gross harvests..., 2023)

As can be seen from Figure 3, the net income of companies in the agricultural sector in Ukraine gradually increased between 2010 and 2021, even

considering inflation. In some years, both declines and increases can be seen, but they are all related to climatic factors (which affected yields) and agricultural

prices on international markets. In general, this is a factor in the gradual development of the sphere. However, there was a significant drop in 2022, which led to a real stagnation of the sector to the state it was in 10 years ago, which, as repeatedly noted above, was conditioned

by limited opportunities for development on the part of farmers due to the beginning of a full-scale Russian invasion. It is also worth considering information on the export and import of products by Ukraine in the field of agriculture. These data are shown in Figure 4.

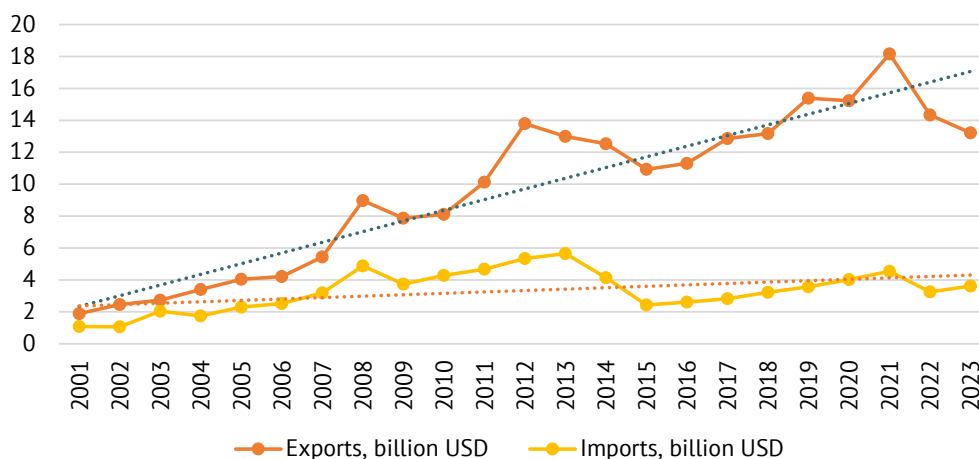


Figure 4. Exports and imports of Ukraine in the field of agriculture for the period from 2001 to 2023, adjusted for inflation, USD billion

Source: compiled by the authors based on data from the State Statistics Service (Areas, gross harvests..., 2023)

As can be seen from Figure 4, the country's foreign trade in agricultural goods also increased or remained at the same level, even considering inflation. Since the increase in foreign trade is a factor in the development of a particular sphere, the same can be said about agriculture. The trend generally remained upward, except for some years, namely 2008, 2014, and 2022, which was caused by various difficulties (namely, the global financial crisis, the outbreak of war with Russia and a full-scale invasion). The decline in 2022 and 2023 is characteristic: although it is significant, given the situation in the country and restrictions on the export of products abroad, it could be significantly greater if mechanisms were not created for the export and import of products by the country.

Thus, it can be concluded that agriculture in Ukraine is generally developing rapidly, especially crop production. However, there are serious problems in the country that slow down this process, in particular, low production efficiency (outdated methods and technologies are often used in the country), gradual loss of land resources (including due to military conflicts), high levels of corruption and low development of institutions, price instability, lack of infrastructure (Usman *et al.*, 2020). All these difficulties are compounded by the fact of military operations, and therefore, it is difficult to propose effective components of state policy that would avoid a significant number of negative existing effects. This is due to the fact that any support from the state from a financial standpoint cannot be relevant at such a time, since the maximum budget volumes should be directed to front-line needs. Thus, the most relevant

recommendation is to minimise the negative impact of the corruption component on the sector and on the country's economy as a whole (Zulfikar *et al.*, 2019; Zhai *et al.*, 2020). This can be ensured both by the activities of the bodies responsible for combating corruption and through changes in legislation that would make this activity impossible.

A special role in the modern development of agriculture in Ukraine is played by innovation, or rather difficulties that do not allow it to develop. Thus, most projects require investment, but agriculture often has limited access to financial resources, which does not allow them to implement any technologies. In addition, the country has a weak legal framework to ensure all the necessary processes related to the introduction of innovative technologies and their further scaling. The problems of legislation also include difficulties in the context of land reform. In general, corruption, along with weak institutional development (as mentioned above), also has a negative impact: they create uncertainty for investors, which reduces their desire to invest. All the above factors have not allowed the country to form a strong innovation base in a high-quality way all the time of independence. However, all of them have become even more relevant in the context of a full-scale invasion, given that agricultural enterprises receive lower profits, new (military) risks have appeared, economic, social and political instability has increased. Thus, for the qualitative development of the country in this area, it is necessary to provide high-quality conditions for investors to finance (Yadav, 2020; Tang *et al.*, 2021). It is worth noting that ensuring the introduction of innovations in

agriculture is one of the most urgent opportunities for countering the negative effects of war: for example, losses in territories that were previously sown with agricultural products can be levelled by increasing the fertility of areas that are still under control.

As of now, the state is making significant efforts to support the agricultural sector in Ukraine. Thus, on November 9, 2023, a grant programme was adopted for creating businesses in the agricultural sector, in particular, for creating greenhouses, creating or developing horticulture, berry growing, and viticulture (Grants for business..., 2023). Grants were issued in amounts of up to UAH 7 million for the greenhouse business, and hundreds of millions of UAH for horticultural enterprises (depending on the area of planted gardens). In addition, there are some benefits in the field of insurance (Agricultural insurance, 2023), for land reclamation (Melioration, 2023), providing farmers with temporary grain storage facilities and equipment for loading/unloading hoses (Programs to provide..., 2023) and the like. Special attention should be paid to some other separate types of assistance. So it is allocated for 1 ha of land cultivated by a farmer, and for the maintenance of cattle: it is important to note that this programme is also supported by the European Union, which allocated an amount of EUR 50 million for it (Financial support for..., 2023). However, support is provided not only in this area, but also in others, in particular – small and medium-sized producers. In addition, back in 2021, programmes were formed to simplify interaction with banks and reduce the cost of loans (Lowering the cost..., 2023; Interaction with banks, 2023). Thus, the state is already taking actions aimed at supporting agriculture and its further development. However, after the end of the war, the number of such positions is expected to increase, as the budget will release funds that were previously used in the defence sector. Such a policy will accelerate the pace of recovery of the sector, which will have a positive impact on the development of the country's economy as a whole.

Active work is also underway to attract international support. Thus, the International Conference on the Restoration of Ukraine in Lugano, Switzerland, held on July 4-5, 2022, was devoted to solving the problems of the Ukrainian economy. Prime Minister Denys Shmyhal presented a project to restore Ukraine, according to which USD 37 billion will be allocated to the agricultural sector from the total budget of USD 750 billion for 10 years. The plan includes USD 4 billion for an irrigation system on 1 million hectares, USD 7.7 billion to increase the production of high-value agricultural products, USD 1.6 billion to restore war-damaged land, USD 5.5 billion to increase the production of livestock products, and USD 1 billion for the transition of the Ukrainian economy to the agricultural sector to "green" development. The document defines the need to attract USD 6.5 billion by 2032 for the reconstruction of 10.5

thousand post-war Ukrainian agricultural enterprises (Draft Ukraine Recovery..., 2022).

Considering the near future, the state budget of Ukraine for 2024 includes more than UAH 41 billion for programmes to support the national economy, some of which is allocated to agricultural enterprises (Business aid for..., 2023). Given that Ukraine needs immediate economic development instead of post-war plans, the budget aims to cover almost all defence spending from tax revenues. The Ministry of Economy seeks to improve the public procurement system to effectively use budget funds and attract more Ukrainian businesses, and the government plans to expand business participation in the provision of social services, encouraging small and medium-sized businesses in communities to offer services such as social support for military personnel.

DISCUSSION

Given the current trends in the development of the agricultural sector in Ukraine in the future, the situation in agriculture is expected to continue to deteriorate until the war is over. Ukraine will continue to specialise in crop production, namely wheat, and the industry itself will become a driver of economic development and post-war reconstruction of the country. In the field of animal husbandry, there may also be a recovery in the event of an influx of new investment after the end of the war. However, all this will be possible primarily through the implementation of high-quality public policies aimed at supporting these two sectors and ensuring the inflow of foreign investment using the tools already mentioned above. The forecast will also differ depending on when military operations will stop.

The impact of the war on Ukraine's agriculture and global food security was assessed by V.A. Verhunov (2020). Researchers stressed the significant negative impact on the agricultural sector of Ukraine by the Russian-Ukrainian war, which has been going on since 2014 and was aggravated by the invasion of 2022. The war disrupted the sowing season, destroyed agricultural land, and complicated distribution routes, including important seaports and land routes. These disruptions extend to transportation, affecting the movement of agricultural products, with additional problems caused by the fuel and energy crisis and rising fertiliser costs. Although the researchers did not give clear digital values of the damage that Ukraine and the world suffered due to the war, they write about the need for further assessments of it, and also note the long-term negative impact of the conflict on the global economy. Above, attention was paid primarily to the study of the development of agriculture in Ukraine: as it was shown, it was largely negative, which can be seen both in the analysis of the values of international trade and natural indicators that characterise the agricultural sector. However, a similar situation has had a negative impact on the global economy, causing the risk of a food crisis.

Although such an outcome was avoided, however, representatives of state authorities around the world should continue to pay attention to this problem in order to prevent the emergence of global or regional hunger.

The role of agriculture was also studied by G.W. Meijerink and P. Roza (2007) and M. Tudi *et al.* (2021). Researchers have considered significant changes in recent years regarding the role of agriculture in the economic development of developing countries. They emphasised the importance of agriculture and the links between the agricultural and non-agricultural sectors, agricultural and non-agricultural activities, as well as rural and non-rural regions. The researchers noted that “new” agriculture focuses on high-value crops, quality food, and modern distribution channels, including exports, health standards, organic food, and certification. They were concerned about how in the future small farms and poor farms from remote regions will be able to develop effectively in similar conditions. This is particularly important given that “local” agriculture as a whole plays an important role in how local communities can develop from both an economic, social, and environmental perspective. Researchers noted the need for state support for such farms to ensure their sustainable development and readiness to use the latest technologies. In today's highly competitive environment, it is important to prevent the decline of the agricultural sector in countries to maintain their own strategic food security. The paper draws attention to the interdependence between agriculture and other sectors, and the numerous cross-sectoral links through which agricultural growth supports overall economic growth and provides benefits beyond economic performance. The paper above also highlighted the role of agriculture for regional development, as it can provide jobs for a significant number of people in rural areas. However, in Ukraine, its role is much broader due to the fact that the industry is the main one in the context of international specialisation (Singh *et al.*, 2021). The state budget and foreign exchange revenues to the country largely depend on it. Thus, state supervision of it is particularly important.

L.R. Magdalena *et al.* (2022) and R. Abbasi *et al.* (2022) evaluated the possibilities of using agricultural and industrial waste as raw materials for reuse in industry. Researchers considered the concept of a circular economy, focusing on the food and agricultural industries in Poland and related waste. They highlighted a significant problem in the country related to food waste, and described the importance of implementing the concept of a circular economy in the country. Researchers mentioned various ways to dispose of agricultural waste, such as the production of biofuels, biochar, and biosorbents. All of this applies to the construction of bio-processing plants where food waste can be converted into a range of biofuels or bio-products, emphasising the importance of sustainable development and

resource efficiency. Thus, the researchers emphasised the importance of environmental waste management practices and the importance of converting agricultural and food waste into valuable resources in accordance with the principles of a cyclical economy and sustainable environmental policy. In turn, A. Sengupta (2022) studied the current state of agriculture in India and assessed future prospects for its development. The researcher emphasised the importance of sustainable agricultural production, which depends on the efficient use of resources, including soil, water, genetics, and climate. It was noted that the country had a significant number of problems in the sector, which, in particular, led to a significant number of negative consequences, such as impoverishment of the population and a high proportion of farmers' suicides. The paper describes that the solution of these difficulties can be achieved through the development of the latest technologies and ensuring a model of sustainable development in this area. The above paper did not describe the need to solve environmental problems in the field of agriculture, although it should be recognised that this is an important component of state policy in the long term. The reasons for not paying enough attention to this issue are that there is a war going on in the country, and therefore, the state should first of all focus on solving issues of the front. However, environmental problems can also be solved by introducing the latest technologies, the importance of which was mentioned in the paper. In addition, this component will become relevant again after the end of the war.

K. Jha *et al.* (2019), N. Yaqoob *et al.* (2022) examined the relationship between consumption of major food crops and their impact on overall agricultural productivity. Researchers wrote that climate variability and grain prices are now significant factors influencing food choices, which leads to a decrease in the consumption of carbohydrate-rich grain products per capita. Researchers see the solution to these difficulties (as one of the options) as an increase in production volumes due to the use of the scale effect. However, this currently seems unlikely, given the reduction in the area of land that can be used for agriculture due to the spread of urban influence. Another option is to develop and use the latest technologies, which is a more efficient and realistic option in modern conditions. In the framework of the study above, the need for the use of the latest technologies in the context of agricultural development in Ukraine was also considered, in particular, considering the military aspect. This can really increase the efficiency of the industry, which is very important in the context of the loss of a certain part of the territories and the actual impossibility of developing the sphere extensively.

CONCLUSIONS

The study analysed the development of the agricultural sector in Ukraine in the period from 2001 to 2023. The

analysis of certain statistical data, including the area of planted land, the number of farm animals, international trade volumes and some others, showed both positive trends and challenges faced by the agricultural sector of Ukraine. Thus, a general trend towards a gradual increase in the area of agricultural land and average yields was gradually noted, in particular, due to export-oriented practices that reflect the development of the sector over time. However, after 2014 and 2022, due to the intensification of the war with Russia, significant difficulties arose in the agricultural sector, which led to a deterioration in all the indicators analysed in the paper.

In light of these trends, Ukraine faces serious obstacles to agricultural development, including poor efficiency, loss of land resources, corruption, institutional deficiencies, price volatility, and infrastructure inconsistencies. The ongoing war exacerbates these problems, making it difficult to propose effective public policies in the face of limited financial resources aimed at the urgent needs of wartime. To solve them, representatives of the public sector must change their policies. For example, it is important to minimise the negative impact of corruption on the sector (and the economy as

a whole), promote innovation, and create conditions for further investment attraction, both during and after the war. The country's innovative development is hindered by limited access to financial resources, a weak legislative framework, and uncertainty for investors. Efforts to counter the negative consequences of war should prioritise high-quality funding conditions and encourage the introduction of innovative technologies. Now the state has initiated separate measures to support the agricultural sector, including grants for creating businesses and various benefits. It is aimed at supporting the sector and international support. However, it is still important to strengthen the country's ability to support not only agriculture, but also other sectors of the economy. Further consideration of the country's agriculture from other sides remains relevant, in particular, consideration of the sphere in the context of sustainable development, and assessment of its innovative capabilities.

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CONFLICT OF INTEREST

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Аграрно-сировинна економіка України і проблеми для економічного зростання

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Анотація. Економіка України сильно залежить від аграрної сфери; вона є основною складовою міжнародної спеціалізації країни на міжнародній арені та є одним із основних представників доходів бюджету. Таким чином, її дослідження залишається актуальним, так само як і вивчення історичних передумов та перспектив для цього. Ціллю роботи стало оцінити останні тенденції розвитку галузі та на їхній основі сформулювати рекомендації для розвитку країни, зокрема – в умовах воєнного часу. Основними методами, що були використані під час дослідження, став аналіз, історичний, прогнозування та абстрагування. В рамках роботи були проаналізовані окремі статистичні дані, що характеризують останні тенденції розвитку аграрного сектору України, серед яких міжнародна торгівля продукцією галузі, кількість засаджених земель сільськогосподарськими культурами, кількість виробленої продукції тваринництва. Було виявлено, що в країні спостерігалися позитивні тенденції в розвитку сільського господарства, особливо сфери рослинництва. Тим не менш, події 2014 та 2022 років призвели до політичної та економічної нестабільності в країні, що призвело до стагнації (у випадку із тваринництвом) та зниження темпів росту (для рослинництва). Зважаючи на складнощі, що існують для розвитку рослинництва в умовах війни, було запропоновано окремі рекомендації для формування державної політики. Вони включають мінімізацію негативного впливу корупції (розвиток інституцій), сприяння розвитку інноваційності та залучення інвестицій. І хоча окремі такі дії вже впроваджуються з боку держави у вигляді різного роду заходів підтримки, однак їх досі недостатньо для вирішення всіх проблем розвитку в секторі. Робота приносить нові знання в сферу досліджень аграрного сектору, зокрема в Україні. Вони дозволять більш ефективно формувати державну політику як для представників державного сектору, так і для підприємців

Ключові слова: інновації; міжнародна торгівля; підприємництво; фінанси; сільське господарство



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The formation of a mechanism to improve the competitiveness of the sugar industry in the Republic of Kazakhstan

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Abstract. Agriculture plays an important role in the development of any country, which makes it important to study and consider the peculiarities of its development. One of the components of the agricultural sector is the sugar industry, which makes its analysis relevant. Thus, the research aims to form methods for increasing the level of competitiveness of sugar industry products. The study was conducted within the framework of the Republic of Kazakhstan, considering the features that characterize the development of the industry in the country. The main method of the study can be considered modeling. In addition, it is worth noting the analysis, historical method, induction, and others. Thus, the research considered the main trends in the volume of beet cultivation in Kazakhstan. It was shown that the number of tons of production in a section of 1 hectare in the country increases over time, which is a good indicator of the development of the industry. The mechanism

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of increasing the competitiveness of sugar products was also shown and substantiated. The authors concluded that it is possible to reach better results for the enterprises of the sector if the state has a more active influence on the activity of the companies in the aspect of economic assistance, as well as if it provides technological infrastructure development, personnel training, an increase of production possibilities, usage of new varieties of beet for increasing the level of fertility, etc. Thus, this study creates new knowledge in the context of studying the peculiarities of the state-stimulating policy, as well as provides an opportunity to take a different look at the sugar industry market in Kazakhstan, and the features of its development

Keywords: entrepreneurship; management; macroeconomics; market research; agriculture

INTRODUCTION

Agriculture plays a key role in the development of the country. It is the main source of food, providing the population with the necessary food resources; it contributes to the reduction of unemployment, providing employment for many people, especially in rural areas; it is a resource base for other sectors of the economy. All this is the reason why so much attention is paid to the development of this component in modern economic literature. However, the agricultural sector as such accommodates the production of many types of products, one of which, in particular, is sugar production. In the framework of this study, its current state and possibilities of improvement were studied on the basis of the data of Kazakhstan.

Sugar is a very common and frequently used product in everyday life (White, 2018). Most of it is made from sugar cane, as this is the cheapest way to produce it. Therefore, it is not surprising that its biggest suppliers in the world are Brazil, India, Thailand, China, Pakistan, Vietnam, and similar countries. At the same time, highly developed countries, particularly Australia, Ireland, and Denmark, remain its biggest consumers (Nguyen *et al.*, 2022). Sugar production, although less efficient, is also possible with other crops, in particular – beets, which are grown in Kazakhstan. In this case, the global distribution of production of this product by culture is as follows: about 9 countries produce sugar simultaneously from cane and beet, 43 – only from beet, and 71 – only from cane.

Sugar production is inherently one of the components of the agricultural sector. Nevertheless, it has some peculiarities related to the industry and the peculiarities of the crop (Honma *et al.*, 2020). For example, sugarcane is very sensitive to the external environment, which produces frequent fluctuations in supply and price in the international market (Zhao *et al.*, 2020). In addition, there has been a recent overproduction of this product worldwide along with its denial of the new crop as a failure and the increased use of sugar substitutes (Voora *et al.*, 2020; Leal & Teodoro, 2020).

Although the sugar industry is the leading industry only in some countries, its product is produced in one quantity or another almost everywhere. Sugar production in Kazakhstan is not very high: in 2012 it was 6%, and in 2022 – 7%, which makes the country virtually

import-dependent (Akaeva, 2022). Thus, it is relevant to consider in more detail the current state of the sugar industry in the Republic of Kazakhstan (RK), as well as to describe the possibilities of increasing its competitiveness. The study of the current state of the sugar industry and the prospects of its future development has engaged many scientists. It is worth noting the work of N. Aguilar-Rivera (2022), which considered some features of the functioning of the sugar industry in the world, as well as evaluating the future of the industry. Q.U.A. Raza *et al.* (2021) also studied the general trends of the industry in the world.

As such, the research aims to form a certain set of rules and principles to increase the competitive advantages of sugar industry products in the Republic of Kazakhstan. The object of the article is the sugar industry and the activities of its subjects.

MATERIALS AND METHODS

The main method that was used in the study was analysis. The reason for this was the significant amount of processed information, as well as the conclusions formed on its basis. In addition, an important role is played by the historical method, due to the study of trends in the development of sugar beet sugar production in the Republic of Kazakhstan. It is also worth noting that the work widely used the method of induction to form general conclusions about the state of the sugar industry in the country based on certain known facts about it and its characteristic statistical data. Also widely used statistical method of research for processing data arrays, as well as the graphical method for their simplified and more convenient image and subsequent analysis.

The method of modeling was primarily used in the research. It was used to describe the mechanisms of increasing the competitiveness of Kazakhstan's sugar industry, the authors built a model of changes in the price and sales volumes of these products, depending on the selected mechanisms of redistribution of funds within the state budget. It is based on the standard interaction between supply and demand but considers the influence of international actors on the domestic market. In general, the model used in this work can be depicted as follows (Fig. 1).

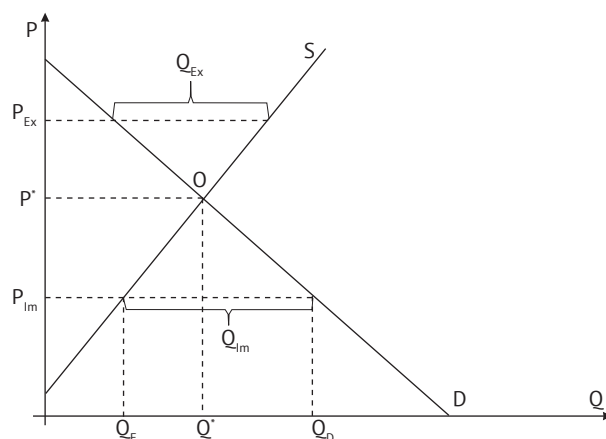


Figure 1. General view of the supply and demand model in the country's market

Note: P – price; P^* – equilibrium price; P_{Im} , P_{Ex} – domestic market price provided that the country is an importer/exporter; Q^* – volume produced by enterprises under conditions of no trade; Q_E – volume produced by domestic enterprises under free trade; Q_D – demand for sugar under conditions of free trade; Q_{Im} , Q_{Ex} – imported/exported volume of goods; S – demand curve; D – supply curve

Source: compiled by the authors based on the Sugar Market of the Republic of Kazakhstan (2022)

Figure 1 shows that the model used in the research is based on the typical interaction between supply and demand in the domestic market but considers the factor of the foreign sector (the outside world or abroad). It considers imports (if the international price is $P_{Im} < P^*$) or exports (if the international price is $P_{Ex} > P^*$) and their impact on the welfare of domestic producers, consumers, and the government budget. Thus, the country establishes an equilibrium not at the level of $S = D$ and with a price P^* , but at another, which is formed considering imported or exported goods. In this case, domestic demand is satisfied at the level of Q_D , and supply – Q_E . If the country is an importer, consumers of such products are at an advantage by consuming cheaper products, while producers are at a disadvantage because they produce fewer products at a lower price than they could (in the case of an exporter, the situation is the same). A similar situation is observed in the sugar market in Kazakhstan since the production of these products is in fact in any case less efficient than that

imported from abroad, which is discussed in more detail below.

RESULTS

The main entities directly producing sugar are the Burundai sugar factory, the Yeskeldy sugar factory, the Koksusugar factory, the Merken sugar factory, the Taraz sugar factory, and the Aksu sugar factory. At this time, it can be stated that the domestic capabilities of the country, as well as the production capacity of the above-mentioned plants, do not allow to fully provide the domestic market with sugar. In addition, the sugar industry in Kazakhstan is not in a better position compared to its competitors at least because only beets can be grown in the country, due to the existing peculiarities of the climate. Sugar production from cane is indeed more efficient than from beet. The main regions where beet is grown are Almaty and Zhambyl regions (beet is also grown in the Pavlodar region, but the volumes are too low, less than 1% of total production). Thus, it is possible to trace the volume of beet cultivation in the country (Fig. 2).

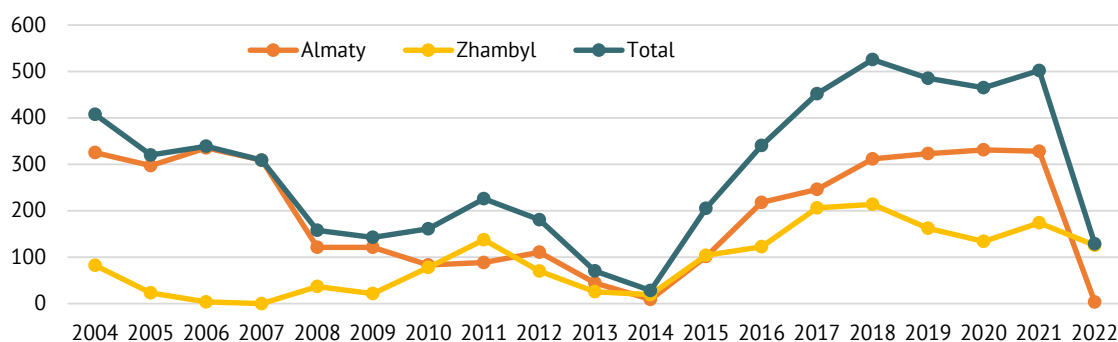


Figure 2. Volumes of the gross harvest of sugar beet in Almaty and Zhambyl regions in 2004-2022, thousand tons

Source: compiled by the authors based on the Sugar Market of the Republic of Kazakhstan (2022) and Bureau of National Statistics Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (2023 a, b)

As can be seen from Figure 2, the volume of sugar beet harvesting in the main regions of Kazakhstan was declining until 2014 and has now started to recover.

However, this is still not enough: according to approximate data, approximately 4 million tons of

sugar beet need to be grown to meet the demand for sugar in the RK in the amount of 450-500 thousand tons: thus, in 2020, only 10% of the desired result was grown in Kazakhstan (QazTrade, 2020). In addition, the area planted with sugar beets in the RK changes significantly from year to year (Fig. 3).

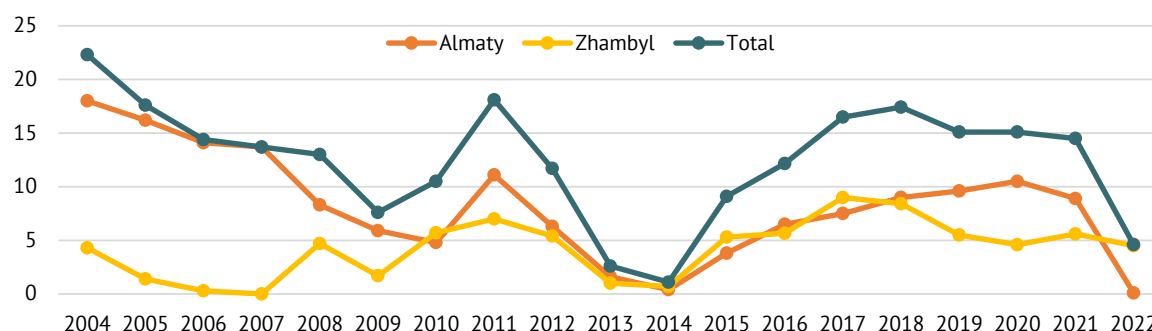


Figure 3. Dynamics of change in sugar beet sown areas in the main beet-growing regions of Kazakhstan in 2004-2022, thousand hectares

Source: compiled by the authors based on the Sugar Market of the Republic of Kazakhstan (2022) and Bureau of National Statistics Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (2023 a, b)

As can be seen from Figure 3, the dynamics of sugar beet acreage in Kazakhstan vary from year to year, similar to the graphs in Figure 2, on the grown volumes of

sugar beet in these regions. However, the most important data for us are the volumes of sugar beet cultivation per 1 ha of land (Fig. 4).

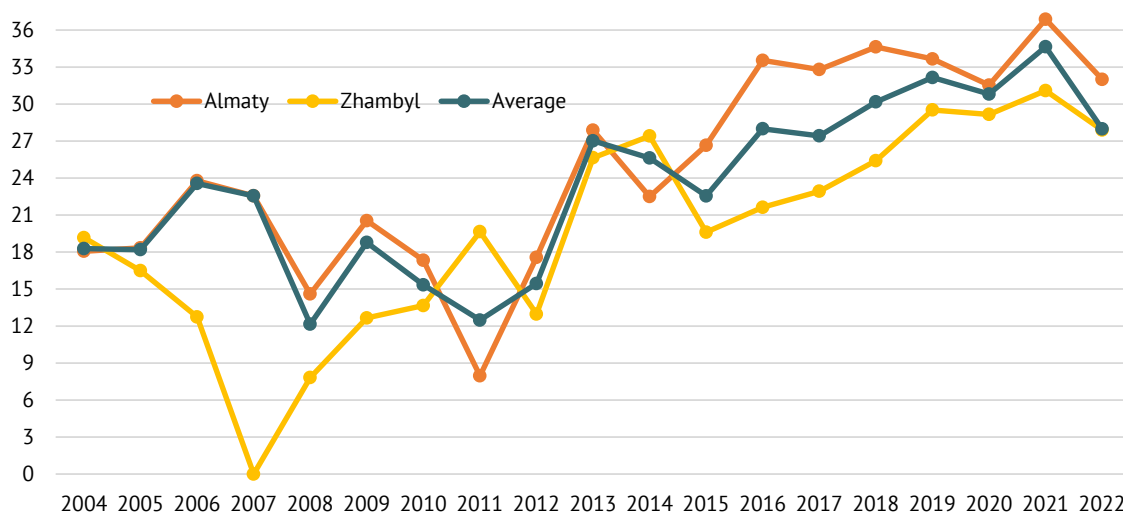


Figure 4. Dynamics of change in the average productivity of sown areas in Kazakhstan in 2004-2022, tons/ha

Source: compiled by the authors based on the Sugar Market of the Republic of Kazakhstan (2022) and Bureau of National Statistics Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (2023 a, b)

As can be seen from Figure 4, there has been an increase in crop productivity in the country: on average, 53% more sugar beets are being produced per hectare in Kazakhstan than in 2004. This can be seen from the trend line showing the trend in crop productivity. The territories of the Almaty region are more productive, and the Zhambyl region is less productive. This trend is positive, but such results are still not enough to fully provide the country with sugar. So, provided that the

amount of land on which beet will be grown stays at the same level as in 2021, enterprises in Kazakhstan will have to increase their output by 10 times to meet domestic demand. Although such results seem unrealistic, further work in this direction is important to increase the competitive ability of the industry.

It is worth noting that the formation of methods for improving the competitiveness of enterprises in the sugar industry can be considered from at least two points

of view: the state and the company. The reason for this is that the actions that a company can take to increase its abilities in the market and the state are significantly different. Thus, companies act selfishly and aim only to increase their position in the market, using the peculiar methods available to them, while the authorities look at

the situation of the industry globally and aim to increase its general well-being. It should be noted that the formation of public policy is prioritized. In order to describe the strategy for improving the competitiveness of the sugar industry in the country, it is necessary to first describe the main factors that will be used in its formation (Table 1).

Table 1. Description of the main factors affecting the competitive ability of enterprises from the company's point of view

Factor	Factor description
Economic	Financial policy
	Price policy
	Management
	Logistics
	Marketing
	Other
Technology-innovation	Newest production technology
	Digital technology
	Personnel training
Social	Achieving the goals of sustainable development and corporate social responsibility

Source: compiled by the authors based on research by A. Nowak and M. Rozanska-Boczula (2022)

In Table 1 the main factors for increasing the competitiveness of enterprises were provided. The actions available to improve position in the market are within the microenvironment. In Table 1, only 3 main groups of factors (levers) through which enterprises can directly manage their competitive advantages are noted. These are economic (change of financial or pricing policy, improvement of marketing campaign or basic processes of enterprise management), technological, and innovative (raising funds for the purchase or independent production of innovations to improve the process of production, recruitment, and training of highly skilled employees in enterprises) and social. The last factor is worth more detail: in modern economic science it is believed that if a company adheres to the principles of sustainable development and corporate social responsibility (CSR), it becomes more competitive in the eyes

of customers (because it improves the company's image among consumers).

In addition, such companies are often able to receive subsidies from the state budget or orders. Nevertheless, the consideration of this factor in our case is not important as the main reason for the low efficiency of the sugar industry in Kazakhstan compared to other countries is the inability to grow sugar cane on its territory. Thus, companies in the industry should, first of all, pay attention to how to improve the technology of production and reduce the cost of production. Achieving a higher level of efficiency can also be achieved by changing some improvements in the procedure of its production, and making decisions in the context of micromanagement. In turn, it is possible to show how the state can influence the competitive ability of enterprises with the help of Table 2.

Table 2. Description of the main factors affecting the competitive ability of enterprises from the government's influence point of view

Factor	Factor description
Economic support	Tax benefits
	Reduced lending rates
	Securing government contracts
	Providing guarantees to investors
Legal support	Providing a simple and clear regulatory framework
Technological	Formation of technological infrastructure for the development of innovative technologies
	Providing a high level of education for all kinds of professionals
Cooperation factor	Ensuring a high quality of interaction between the state and enterprises, understanding their basic needs and opportunities

Source: compiled by the authors based on the study by Z. Muhamamd et al. (2020)

Ensuring a high quality of interaction between the state and the enterprises, understanding their basic needs, and the need to develop the enterprises of the sector. As can be seen from Table 2, the factors affecting the development of the sector are both similar and different from those provided in Table 1. Thus, the state should provide economic assistance to selected companies (through tax incentives, lowering of credit rates, and other instruments), legal support (maximum simplification of the existing legal legislation), forming a positive influence on the technological development of companies (by providing appropriate conditions for innovation activities) and ensuring sustainable contact

between enterprises and the state. Actions of state support are more global and must be formed through specific budget programs with clear goals and objectives and performance parameters. And what exactly they can be, should be formed of the peculiarities of the industry and its enterprises. Taking into account the existing methods of state support for the industry and the peculiarities of its functioning, it is possible to form a model of support for sugar industry enterprises. In its framework, the duties received in one way or another for importing sugar into the country will be used as a support fund. The principle of its functioning is shown in Figure 5.

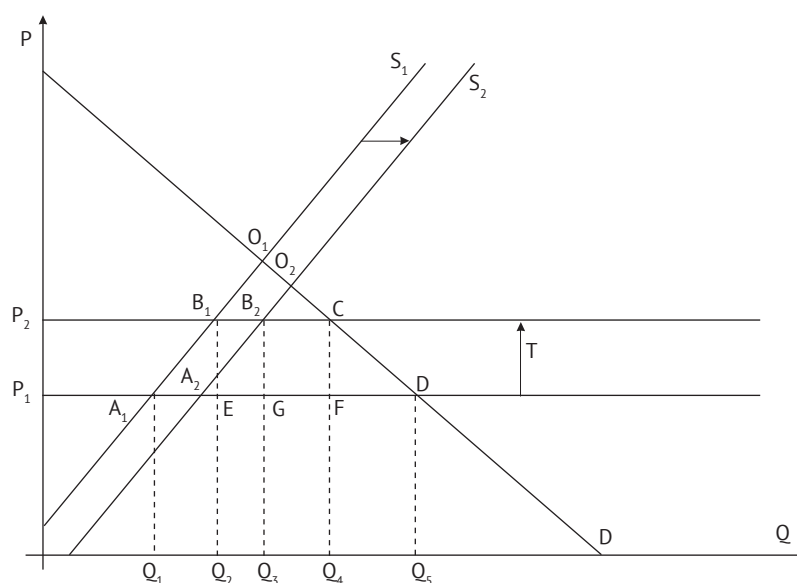


Figure 5. Model of state support for companies producing sugar industry in the Republic of Kazakhstan

Note: P_1 – world sugar price; $P_2 (P_1 + T)$ – the price of sugar in Kazakhstan including customs duties; T – level of duty rate; Q_1, Q_2, Q_3, Q_4, Q_5 – volumes of product sales in the country; S_1, S_2 – direct supply; D – direct demand

Source: compiled by the authors

Figure 5 shows schematic interaction between sugar beet production by Kazakh producers, consumers, and imported products. Equilibrium in consumption and production is established at point O_1 , however, due to more competitive imported goods, the real price is set at the level of P_1 . Under the influence of tax duties, the price rises to the level of $P_2 (P_1 + T)$, and the revenue to the budget will be equal to the area of rectangle B_1CFE . In case the state will use these funds to support producers, they will be able to increase their production capacity next year. This is shown in the graph as a movement of the line S_1 to S_2 . This in turn will cause the equilibrium price to move to the O_2 level, and the volume of production by exporters will increase from the Q_2 level to Q_3 , which will reduce tax revenues to the B_2CFG level. This trend will continue until S_2 crosses line D at point C when imports will stop (although in practice such a situation can never be achieved).

Although Figure 5 describes a mechanism for supporting sugar producers in Kazakhstan with state support, it is still not enough to make the industry sufficiently efficient and effective. For this purpose, the state authorities need to ensure technological development in Kazakhstan, in which enterprises will be able to increase the productivity of their cultivated areas, thereby producing more sugar. Connecting this statement with Figure 5, it can be said that technological development is one of the main forces, which moves the straight line S_1 to S_2 , that is, it allows companies to increase their production capacity. Thus, without the development of the technological base, it will not be possible to significantly increase the output of companies in the sugar industry, which is the separate role of this indicator. Thus, the mechanism to increase the competitiveness of the sugar industry in Kazakhstan is shown in Figure 6.

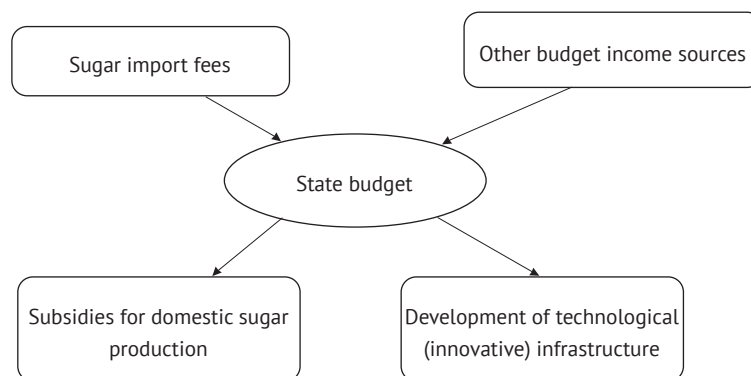


Figure 6. The main mechanisms of support for producers of the sugar industry in Kazakhstan

Source: compiled by the authors

As can be seen from Figure 6, support for sugar producers in Kazakhstan should consist of two main components. These are economic support (provision of subsidies for national sugar producers to increase their competitiveness against imported products) and technological development of the state (arrangement of innovation infrastructure, personnel training, and allocation of funds to promote innovative projects).

Thus, the sugar industry in Kazakhstan faces difficulties in fully satisfying domestic demand due to insufficient domestic capacity and climatic constraints that allow only beet cultivation. Although beet production in the country has increased to date, it remains insufficient and requires even more growth to meet demand. The tools that can be undertaken by both the government and enterprises to improve the development of the sector have been described in detail above. In case of their active and competent application, it is possible to really achieve significant success in terms of increasing the output of sugar in the country, and thus improve the competitive position in the international arena.

DISCUSSION

A. Nowak and M. Rozanska-Boczula (2022) analyze and consider the state of competitiveness of agricultural enterprises in the European Union (EU) within their research and form their model of comparing the level of their competitive advantages. It is worth noting that scientists within the framework of the study focus on the factor of human capital (human resources), pointing out that they have the greatest influence on the competitiveness of an enterprise. In general, it is possible to agree with this statement, since it is the company's employees who formulate its development strategy, implement technologies, manage economic processes, etc. Therefore, the efficiency of the company's activities will depend on how competently employees will perform their main tasks. Thus, in the realities of Kazakhstan, directors of sugar companies should pay special attention to the training and recruitment of highly qualified personnel.

T. Galovic and H. Bezi (2019) also considered factors of competitiveness of sugar industry enterprises in their

research, but they made their analysis in the context of European countries. Researchers note the existing relationship between the competitiveness of enterprises in the industry with economic, political, and so-called natural factors of the position of companies. It can be concluded that political stability plays an important role in the stable development of such enterprises, as well as the institutions of the country. This is indeed true, but the research does not focus on this, since the state is unable to influence these factors, and changes in the context of these indicators are only possible over rather large time intervals of dozens of years. There is also a widespread belief in Kazakhstan that beet is not a competitive raw material for sugar production compared to imported cane sugar, which was also mentioned several times in the study. Because of this, companies are forced to save resources, since the final financial result of their production and financial activities depends in full; these problems can also be solved by achieving a higher quality level of sugar beet seeds and root crops. In other words, sugar producers in Kazakhstan are forced to save money to be able to function.

Some methods to increase the competitive ability of the sugar industry are proposed in their work by A. Yeginbayeva *et al.* (2022). During the study, they conclude that a higher level of competitive ability of enterprises can be achieved by diversification of innovation, technical re-equipment of industrial enterprises, and the use of technological innovation as an incentive for the strategic development of the industrial enterprise. Thus, the scientists propose the following methods of increasing competitiveness: diversification of production, technical re-equipment, and reconstruction with a focus on expanding the range of products; modernization of beet harvesting equipment and technologies used; resource-saving and integrated processing of by-products (recycling of waste); environmentalization of products (optimization of the removal of the fertile layer of land from agricultural fields, prevention of environmental pollution from the plants' emissions). Although such activities related to an increase in the technological effectiveness of sugar plants in Kazakhstan

will indeed be able to reduce the cost of production and make them more competitive, in the international arena sugar cane will still be more efficient in comparison with sugar beet. However, even to increase the share of domestic sugar inside the country, such activities seem to be extremely effective. It is worth noting that stimulation of such activities can be carried out with the help of the state, especially given the strategic role of this industry for the economic well-being of the country.

Some scientists have considered methods and needs for the support of sugar producers in Kazakhstan as part of their research. Thus, A. Erseitova *et al.* (2017) described the main measure of state support as the formation of subsidies for various purposes (spring field and harvest work, the expertise of seeds, interest rates on leasing, preferential lending, reducing the cost of fertilizers and herbicides). Obviously, this activity will significantly reduce the price of these products in the domestic market, which will increase demand for sugar domestic production and allow the sector enterprises to develop. In the work above, the authors also note the economic support of enterprises as one of the main conditions for their well-being in the future. However, this is not enough to secure the long-term and sustainable development of the industry. Therefore, the role of state influence on technological and innovative development, which will increase the amount of sugar beet grown from one hectare of land, is also noted in the model built.

In turn, G. Zhaxygulova *et al.* (2020) describes how the introduction of innovations in sugar production processes affects its quality and output. Researchers show that the use of the latest technologies can improve the performance of enterprises, as well as reduce waste and increase environmental sustainability. In addition, they are the main reason for creating new jobs and organizing new types of production. Thus, scientists say that the greening of manufactured products plays an important role in the overall efficiency of the enterprise. This, in general, can be argued, at least in the context of the sugar industry, for which, according to the authors, it is now more relevant to develop technology to increase their productivity, rather than to achieve the goals of sustainable development.

The study of innovative development in the sugar industry was also worked on by G. Kashakova *et al.* (2022). Researchers note that the latest technology plays a special role in the welfare of sugar producers, as it allows for increasing the number of grown products, as well as the efficiency of sugar beet processing. Describing the most common options for innovative development in the field of sugar production technologies, the researchers note selective genetic research (discovery and implementation of new varieties of sugar beet, as well as hybrids); development and implementation of technical, technological, and production capacity of the enterprise; changes in the structure of organization and management of the company; work

on the influence of the company on the external environment and the society. Note that although all these components of the development of innovativeness and technology in the context of the sugar industry of Kazakhstan are relevant, the most important among them should be considered in the first-place selection and genetic research and development of their preferential capacity for the possibility of providing as much as possible domestic demand for sugar.

CONCLUSIONS

The paper considers some indicators that characterise the development of the industry over the past 16 years, namely the amount of sugar beet grown, the area planted with this product, and the productivity of the sown areas. It was shown that there are positive trends in the country, as the area used for sugar beet cultivation is decreasing, but the productivity and the total amount of sugar produced in the country are increasing.

In addition, an analysis of all the main opportunities for the development and support of the sugar industry in Kazakhstan was carried out. It was shown that the most effective assistance for this industry will be carried out if the state will take actions affecting both the short-term and long-term development of the industry. In the first case, economic support of enterprises is implied, which includes tax benefits, subsidies, and other methods of support. They will reduce the price of the products produced by the companies, which will make them more competitive compared to imported products, which means that they will receive more funds and develop their production capacities faster. In the second case, the authors imply support for technological development through the creation of innovative infrastructure, personnel training, support for newly created technological projects, and other similar actions. These conclusions were argued using the model built by the author, which characterizes the changes in the volume of production, imports, and prices in the sugar market in the conditions of state support.

Thus, the research formed some tips on how to increase the competitiveness of the sugar industry in the Republic of Kazakhstan. The novelty of the conducted research consists directly in the author's vision of mechanisms to improve the state of the industry enterprises and increase the efficiency of their subsequent development. Prospective for further research is to find other methods of improving the competitiveness of the sugar industry in Kazakhstan, as well as a comparative analysis of the development of this industry with other countries to determine its main strengths and weaknesses.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

None.

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Формування механізму підвищення конкурентоспроможності цукрової галузі в Республіці Казахстан

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Анотація. Сільське господарство відіграє важливу роль у розвитку будь-якої країни, що зумовлює необхідність вивчення та врахування особливостей його розвитку. Однією зі складових аграрного сектору є цукрова галузь, що робить її аналіз актуальним. Таким чином, метою дослідження є формування методів підвищення рівня конкурентоспроможності продукції цукрової галузі. Дослідження проводилося в рамках Республіки Казахстан з урахуванням особливостей, що характеризують розвиток галузі в країні. Основним методом дослідження можна вважати моделювання. Крім того, варто відзначити аналіз, історичний метод, індукцію та інші. Таким чином, в ході дослідження були розглянуті основні тенденції обсягів вирощування буряків в Казахстані. Показано, що кількість тонн продукції на ділянці 1 га в країні з часом збільшується, що є гарним показником розвитку галузі. Також було показано та обґрунтовано механізм підвищення конкурентоспроможності цукрової продукції. Автори дійшли висновку, що досягти кращих результатів для підприємств галузі можна за умови більш активного впливу держави на діяльність компаній в аспекті економічної допомоги, а також за умови розвитку технологічної інфраструктури, підготовки кадрів, збільшення виробничих можливостей, використання нових сортів буряків для підвищення рівня родючості тощо. Таким чином, дане дослідження створює нові знання в контексті вивчення особливостей державної стимулюючої політики, а також дає можливість по-іншому поглянути на ринок цукрової промисловості в Казахстані та особливості його розвитку

Ключові слова: підприємництво; управління; макроекономіка; маркетингові дослідження; сільське господарство



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Technological innovations in agriculture: Impact on production efficiency

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Abstract. The necessity to meet the food needs of Azerbaijan requires modernisation of the agricultural industry navigating the challenges associated with an innovative mode of progress. This process is accompanied by technical and technological optimisation of most of the production processes in agriculture. The aim of this research was to examine and assess the integration of technological innovations in Azerbaijan's agricultural sector. The investigation employed fundamental scientific methods, including system analysis, synthetic method, method of abstraction, deductive method, and methods of summarisation, and concretisation. During the investigation, the main objectives of technological modernisation of the agrarian sector were analysed, the dynamics of their impact on the efficiency of production depending on different factors was investigated, measures for optimisation of the situation with regard to the priority of innovative solutions were developed. Additionally, the research explored the potential application of contemporary management and technological strategies

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to mitigate the adverse environmental effects of agricultural production. This involved integrating environmental safety and economic efficiency through the adoption of cutting-edge innovative technological approaches. Their effectiveness as a viable tool for the transformation of the agrarian sector following the current social and economic requirements for the intensification of its development was determined. Implementing the ecosystem approach and adopting integrated agro-landscape management can pave the way for a renewed development of the agronomy industry in Azerbaijan. The results of this study carry significant practical implications for advancing and modernising the country's agricultural economy system, contributing to the formulation of targeted programs for technological innovations

Keywords: modernisation; agricultural sector; sustainable development; intensification; organic production

INTRODUCTION

Global processes of transformation of agriculture with orientation on innovative technologies, organic production, and minimisation of destructive impact on the environment in accordance with the tenets of sustainable development form new challenges for the agricultural sector of Azerbaijan. The prospective growth in demand for agricultural products with limited resources can be met only through effective technological modernisation of agricultural projects. Currently, human activities are actively shaping a distinct altered natural backdrop characterised by a considerable pollution index. Frequently, the upper limits surpass the officially designated maximum permissible concentrations of pollutants in the natural environment. Intensive farming in past historical periods has left a substantial footprint in the form of soil chemisation and salinity, erosion, and minimisation of organic matter. Under such conditions, there is a need to modernise the agricultural sector and develop a new conceptual approach to farming.

Numerous scientific articles and studies have delved into the issues of modernisation of the agricultural sector in Azerbaijan, which would allow increasing the efficiency of production while minimising the anthropogenic load on the environment. M. Aliyeva (2022) investigated the main theoretical aspects of the technological modernisation of the agrarian sector. The scholar concentrates on enhancing both the infrastructure and competitiveness of agricultural products. Recent findings by contemporary scientists A.A. Hasanov (2019) and Sh. Abdullayeva (2019) suggest that substantial accumulation of toxic substances in soils resulting from industrial processes is a key factor triggering numerous irreversible changes in agroecosystems. Researchers are convinced of the need for a gradual transition from conventional land use to an organic system that is gentle on the environment and allows for the regeneration of degraded landscapes.

The findings of the scientists complement the results of the studies by V. Rzayev *et al.* (2021) and A. Nasibov (2023), who investigate the innovative enhancing the industry of agriculture of Azerbaijan's economy and the role of technological transfers in the innovative development of the agricultural sector. Researchers study the impact of the growth of financing

of innovation processes on the economic effectiveness of production of agricultural industry, as well as the mechanism of implementation of technological developments in agricultural processes. The scientists substantiated the function of innovation providers in catalysing technology transfer and implementing innovations, along with an examination of the facets influencing the economic processes resulting from the technological transformation of the agricultural sector.

In the pursuit of strategies to optimise conditions and alleviate adverse effects, researchers E. Gulieva (2021) and A. Gurbanova (2021) assert that the most effective approach to agricultural production, minimising negative environmental impacts, involves the modernisation of existing technological solutions and the development of new environmentally friendly alternatives. They emphasize the importance of implementing an efficient monitoring system. Simultaneously, A.D. Ibragimov (2023) highlights that agricultural modernisation primarily entails updating fundamental technical assets while concurrently developing new infrastructure. This approach enhances industry competitiveness and elevates social well-being.

Despite the current significance of the studied issue, numerous aspects still necessitate detailed examination, analytical processing, and the exploration of optimal solutions, specifically the introduction of precision and organic farming systems, the use of optimisation solutions at the regulatory level, the use of breeding opportunities, innovative approach to intensification of agricultural production, and cost reduction through technological modernisation. The aim of this investigation was to research the main areas of technological modernisation of the agrarian industry in the aspect of innovation activity.

MATERIALS AND METHODS

This study utilized various general scientific methods of inquiry. The deduction method was employed to ascertain the significance of the impact of technological innovation in agricultural production on performance indicators as a multifactorial process. Representative economic indicators for the industry under study were considered. The use of the method of analysis allowed performing

a comparative study of various modern approaches to technological modernisation of agriculture, the possibility of their application in the practice of agro-industrial production in Azerbaijan, as well as the impact on productivity and production efficiency indicators.

Using the generalisation method, the investigation unveiled distinct features and patterns related to the examined issues, as well as the potential for optimising the situation and identifying the most favourable solutions. The focus was on the most recent and credible research on the issue under study. Furthermore, the method of rising from abstract to concrete was applied in this study in a sequential progression, moving from general abstract data regarding the directions of innovative technological modernisation of the agricultural industry to concrete data on the impact of their introduction in the agriculture of Azerbaijan in the aspects of productivity growth, economic efficiency, and reduction of destructive impact on the environment.

A systematic methodology was employed to assess the effectiveness, characteristics, and advantages of specific measures within the studied process. Through this approach, the investigation aimed to uncover the overall structure of the subject, conduct a comprehensive exploration for optimising risk management, and devise a suitable strategy for the technological modernisation of agricultural production, aligning with the principles of sustainable development and a green economy. Special emphasis was placed on acknowledging the diversity of approaches and anticipating potential implementation challenges within the economic contexts of developing nations. The synthesis method was utilised to evaluate the viability of contemporary managerial decisions within the realm of innovation in the agro-industrial sector, both at regional and national levels. This evaluation was grounded in the principles of public management.

The abstraction method was utilised to construct a representation of the monitoring, management, and control processes concerning the impact of technological innovations on the quantitative and qualitative aspects, as well as the productivity of production processes in agriculture. This extended to the assessment of the anthropogenic load on the environment. In parallel, the concretization method was employed to identify the effectiveness and economic feasibility factors of innovative measures. This involved forecasting and modelling potential consequences and performance indicators. Notably, this approach underscored the pivotal factors in selecting viable management and production-technological solutions to mitigate adverse environmental impacts, adhering to the principles of sustainable agricultural production. The potential for an effective integration of innovative technological solutions with the development of a monitoring system for the innovative optimisation of production processes in the agro-industrial complex was explored.

Additionally, the formalisation method was employed to pinpoint the conclusive prospects of technological modernisation in Azerbaijan's agriculture and to shape the outcomes of this study.

RESULTS

In the dynamic evolution of economic processes and the increasing globalisation emphasising sustainable development, the primary focus for advancing Azerbaijan's economy lies in the modernisation of key production processes. This strategic approach guarantees the efficiency of a step-by-step shift towards a novel form of economic development grounded in innovation (Hamidova, 2020). Prolonged extensive use of land resources has led to an uncompromising need to replace conventional production processes with intensive methods and innovative technologies that uphold the principle of sustainable enhancement of agriculture (Kaldiyarov *et al.*, 2019). Thus, the wide utilisation of modern technological innovations in the agro-industrial complex is currently the main way to ensure food security and minimise the destructive impact on the environment. The priority of the task of reforming the traditional agricultural system is also conditioned by the need for sustainable use of agro-landscapes and bringing resource use in line with global climate change trends, and increasing food production (Nasibov, 2023; Strategic road map..., 2023).

Notably, one of the priority areas of modernisation of the agro-industrial complex in Azerbaijan is a proficient government strategy for fostering innovation in the agricultural industry. Its purpose should be the development of mechanisms and methods of stimulating innovation activity, renewable resource use, use of technological developments (Kizi, 2020). The concept of modern agricultural modernisation is based on integration and diversification processes, cost minimisation through increased production efficiency, the use of biotechnology to ensure competitiveness, and organic production within the framework of green economic development. It also involves the introduction of multi-operator, multi-tasking technological equipment and ways to stimulate yield and productivity indicators while maintaining a sustainable environment (Glover *et al.*, 2019; Kovalenko, 2021; Domaratskiy *et al.*, 2023).

In 2016, "Strategic road map for the production and processing of agricultural products in the Republic of Azerbaijan" (2023) was adopted, which is a forward-looking approach to the development of the sector. The main objectives formed in the document include increasing agricultural production capacity, strengthening institutional capacity, preservation of the environment, responsible utilisation of natural resources, and improving the efficiency of management processes in the industry (Clapp & Ruder, 2020). Therewith, the development of research areas in the agricultural industry within the context of the worldwide trend of wide introduction of innovative production technologies is an

essential resource for influencing the level of competitiveness and production efficiency.

Modernisation of the agricultural sector mainly implies the introduction of innovative agro-technologies, improvement of agricultural machinery, optimisation of the fertiliser and chemicalisation system with priority given to biological methods of plant protection and organic fertilisers, selection of high-performance varieties (Kizi, 2020; Herrero *et al.*, 2020). The concept of effective modernisation of agriculture is based on the principles of alternativeness and comprehensiveness, as well as preventive insurance against possible risks, which determines the increase in the efficiency of agricultural production. It allows agriculture to develop in an intensive way, encouraging investment in modernised machinery and the adoption of sustainable agricultural production (Hasanov, 2019). The implementation of modernisation also encompasses the practical application of research and development (R&D) capabilities, exerting a direct influence on the expansion of the export potential within the agro-industrial complex.

The process of innovation in the agriculture industry takes place in stages: scientific solution – technology – production – consumption (Curry *et al.*, 2021). Therewith, this process is influenced by a set of specific features of agrarian production, including functional, regional, managerial, and other factors (Ibragimov, 2023). Some of these factors determine the pace of agricultural modernisation. Specifically, they include the level of integration (availability of innovation infrastructure), readiness to absorb functional change, resource capacity (managerial, material, information, human resources) (Aldieri *et al.*, 2021).

Notably, modernisation and innovation are inextricably linked processes, with innovation involving the creation of novel technologies and modernisation involving the introduction of the most progressive and efficient ones of their totality (Clapp & Ruder, 2020). Currently, there is a significant focus on advancing agrarian science in Azerbaijan. Under the auspices of the Azerbaijan State Agrarian University and the Centre for Agrarian Science, 13 research institutes are functioning, where research projects are being carried out, oriented towards effective practical implementation: selection of resistant varieties and crops, improvement of agricultural technologies, development of optimal sparing methods of plant protection and stimulation of production performance (Rose *et al.*, 2021). Modernisation of the industry of agriculture is inextricably linked to the optimisation of logistics infrastructure. A good-working logistics system can substantially influence competitiveness and good governance (Rzayev *et al.*, 2021). It implies the use of effective methods of rational use of resources, including information resources.

The ongoing phase of economic advancement in Azerbaijan is marked by an escalating demand from domestic consumers and favourable trends in investment

processes. These factors, coupled with the distinctive elements of the current global situation, underscore the imperative of prioritising the modernization of the agricultural sector. The goal is to foster synergistic development in technology, innovation, and management processes within the framework of sustainable agricultural development. This strategy ensures a substantial enhancement in overall factor productivity within the industry, consequently leading to an upturn in agricultural production (Steinke *et al.*, 2021). For Azerbaijan, such performance is particularly desirable due to the considerable depletion of the main production factors and resource potential. Furthermore, modernisation of agriculture, based on the above principles, can, simultaneously with productivity improvements, stimulate an increase in the level of resilience of the agricultural sector to negative global climate trends. Therewith, the main principles of modernisation of agricultural production still include ensuring economic and production security, ecological and economic balance, priority of innovation, state support, and compliance with the principles of sustainable development (Fleming *et al.*, 2021).

Using modern technological opportunities for modernising agriculture, it is possible to increase the competitiveness of exported products substantially, as well as improve their quality while minimising costs. Moreover, it is crucial to identify the unique characteristics of the agricultural sector within the context of regions, which will allow using the synergy of the resource base and technological modernisation most effectively (Abdullayeva, 2019; Gulieva, 2021). For instance, Azerbaijan has specific types of soil that are best for growing pomegranate, which is in considerable export demand, and modern farmers are making effective use of the situation to increase production efficiency by investing in technological modernisation of production. Along with the specific climatic features of Azerbaijan, the innovative technological approach allows for harvesting in modernised greenhouses 3-4 times a year.

Modernisation of the agricultural industry through technological innovations makes it possible to switch from exporting raw materials to exporting finished products, which considerably increases the indicators of economic efficiency of production processes (Kaldiyarov *et al.*, 2019). Furthermore, certification to international standards is becoming available, increasing access to markets on a global scale (Kizi, 2020). Significantly, technological progress in the agricultural sector entails an enhancement of energy efficiency in the use of the main types of resources. Such a goal can be achieved through basic strategic solutions in the form of innovative optimisation of irrigation equipment and systems, genetic breeding, precision farming concepts, and organic chemicalisation of agricultural processes (Rolandi *et al.*, 2021; Ibragimov, 2023). Bioenergy strategies are also becoming popular as an additional aspect of technological modernisation, due to the emphasis on

the principles of sustainable development takes precedence across all sectors of production, including agricultural production.

In Azerbaijan, organic farming is a highly promising avenue for the development of the agro-industrial sector. This trend is associated with increased demand for organic food and growing public awareness of the benefits of organic products (Rzayev *et al.*, 2021; Gurbanova, 2021). Organic farming technology utilises natural resources and methods that are environmentally tolerant and preserve biodiversity. Unlike conventional industrial farming, organic farming does not actually use chemical fertilisers and pesticides, nor does it use genetic modification (Kaldiayarov *et al.*, 2019). This makes it possible to produce quality and environmentally friendly food products that do not pollute the environment and are essential for people's health. To ensure a systematic approach to the study of organic farming processes in Azerbaijan, it is necessary to consider its sectoral characteristics and guidelines that form a model of effective biological land use (Strategic road map..., 2023). Thus, organic farming is a complex production complex, the effective management of which requires considering the specifics of sectoral functioning in terms of resource potential indicators, as well as ensuring its reproduction at the suitable level (Clapp, 2021). Compliance with the principles of sustainable development requires ensuring a technological closed cycle of production in an agricultural enterprise. That is, organic farming should be implemented under the influence of reproduction algorithms using available factors of production – organisational, economic, production, material and technical, ecological, and social.

Long-term intensive farming has led to substantial environmental problems in the agricultural sector of Azerbaijan. Agro-ecosystems, by their nature, are extremely energy intensive, with poor adaptive capacity, and potentially high levels of soil fertility depletion. The situation is particularly difficult in the field of

irrigated vegetable production. Organic land use technologies, which make provision for covering the soil cover with vegetation for as long as possible, are a relevant alternative. Innovative technological methods and algorithms that allow implementing a step-by-step transition from an over-intensified farming system to a science-based biologisation of agrarian processes will help translate the European standards of sustainable development into practical reality. In the agricultural sector, these standards are positioned by the world community as alternative or renewable farming.

Innovative precision farming technology involves determining agrochemical and agrophysical characteristics of fields, drafting electronic maps, and applying agrochemicals considering the different needs of crops in certain parts of the field. The application of this technological approach implies the use of GPS system, based on which the system of mechanisation of soil cultivation, fertilisation, sowing, plant protection is developed without direct involvement of the operator. Precision farming involves inseparable monitoring of crop and soil conditions to promptly plan a set of actions to optimise the condition of problem areas (Raihan *et al.*, 2022). The characteristic trait of contemporary technologies in production of agricultural industry is the active influence on plants in different phases of their vegetation and soil fertility by performing an algorithm of biological-technological techniques, which unfailingly affects the yield, productivity, and efficiency of farming (Kernecker *et al.*, 2021).

The adoption of precision farming technologies within the Azerbaijan's agricultural domain involves measures to increase production efficiency, improve product quality, rational use of chemicals, prevention of destructive impact on environmental elements implies an algorithm of data collection and analytical processes, compilation of electronic maps of fields, effective system of management decision-making, development of technology for agricultural production processes (Fig. 1).

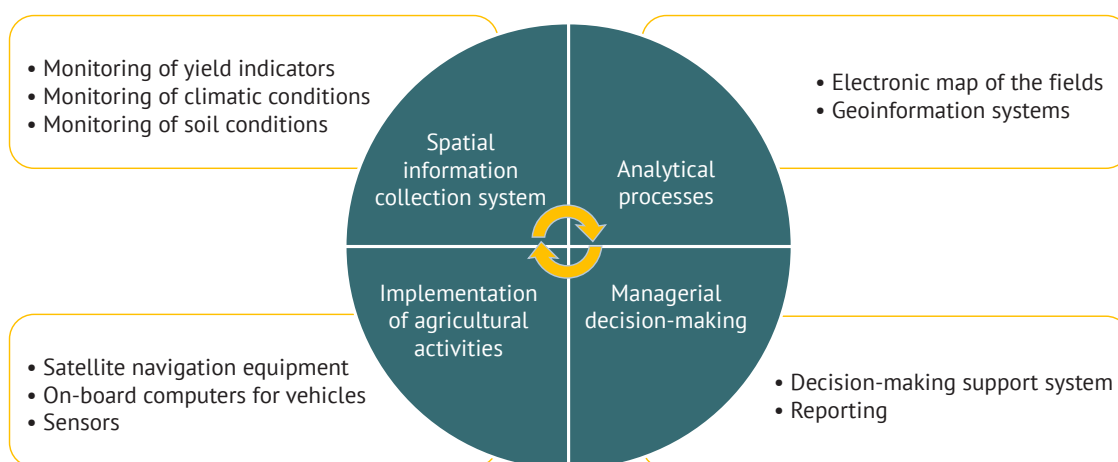


Figure 1. Components of precision farming technology

Source: compiled by the authors

Evaluating the economic effectiveness of incorporating modern technologies involves examining indicators such as yield growth and enhanced productivity, cost recovery and profitability level, annual economic effect per unit area, determined based on informative data on qualitative and quantitative indicators of yield are used (Lacoste *et al.*, 2022). Analysing the statistical data regarding the indicators of economic efficiency of agricultural enterprises (Table 1), which fully reflect the efficiency of technological modernisation of the industry, we can state a significant positive effect of innovative technological optimisation of agro-industrial

processes. The concept of increasing total profit and profitability according to the gradual transition from extensive to intensive agricultural activities reflects the positive dynamics of the impact of innovative technological approaches in crop and livestock production. Particularly indicative of the nature of changes is the ratio between enterprises operating at a loss and at a profit, which is characterised by pivotal dynamics in the 2005-2022 period (Table 1). It can be argued that, in the majority, there are still enterprises actively practising an innovative approach to farming technologies in the market.

Table 1. Financial results of agricultural enterprises in Azerbaijan

	2005	2010	2015	2018	2019	2020	2021	2022	2023
Number of operating enterprises, units	1,782	2,043	1,659	1,641	1,471	1,024	1,035	1,047	1,055
Including:									
Those working for profit	1,579	1,869	1,530	1,560	1,376	880	880	836	853
Those working at a loss	203	174	129	81	95	61	70	48	43
Total profit, thousand AZN	5,892	27,658	39,763	96,917	92,696	104,016	171,197	310,012	375,015
Total profitability, %	9.1	16.7	11.3	20.1	17.2	19.4	27.2	37.9	38.8
Profit (loss (-)) in crop production, thousand AZN	2,769	8,855	20,241	56,577	68,795	69,321	127,159	170,174	155,120
Profit (loss (-)) in livestock breeding, thousand AZN	3,117	13,021	19,993	19,048	10,481	27,180	32,557	113,751	108,745
Agricultural products Return on sales, %	9	13.4	11.3	16.2	15.3	19	26.4	35	38.5
Including:									
In crop production	41.5	30	36	40.4	35.7	29.2	51.7	51.9	52.5
In livestock production	5.4	10.5	7.1	5.9	3.4	9.5	8.5	23.6	24.7

Source: G. Curry *et al.* (2021)

Therefore, the technological modernisation of Azerbaijan's agricultural sector is oriented towards systematically transitioning from extensive to intensive methods of agricultural production. This shift aligns with the principles of sustainable development and the green economy. These benchmarks advocate for the widespread adoption of innovative technological systems that integrate technical, informational, and intellectual resources.

DISCUSSION

Modern technological modernisation of agriculture accumulates vectors of innovative optimisation of spheres of agrarian activity, including material and technical support, production organisation, innovation management, information flows, and technological processes. Political support for agrarian reforms, diversification, preventive protection of the ecological state of agro-landscapes, along with active technological modernisation of the agricultural sector, can provide favourable environment for optimal development of Azerbaijan's sustainable agriculture. The synergy of technological modernisation and innovative solutions

are the factors of intensive growth in the aspect of agro-industrial complex development, as well as the basis for guaranteeing food security of the state and leading positions in the export segment of the market.

Numerous scientific studies substantiate the economic impracticality and profoundly adverse effects associated with extensive methods of agricultural production on the environment in modern conditions. L. Rocchi *et al.* (2020) believe that agricultural modernisation is a gradual transition from traditional production to technological production and should be in line with the priorities of green economic development. This standpoint encapsulates the core scientific concept of the present study, making it challenging to dispute the researchers' conclusions. The acquired results align with the outcomes of various scientific investigations, reinforcing the notion that the degree of modernisation acts as a significant gauge for the practical application of research and development (R&D) advancements. Furthermore, this process is oriented towards enhancing the export potential of the agricultural sector within the economy.

According to the research of S. Ruzzante *et al.* (2021), innovative technological modernisation of agricultural

production contributes to the development of a new organisational and management mechanism in the agricultural industry. F. Farrokhi and H. Pellegrina (2023), however, contend that the enduring outcomes of such processes include an increased local demand for skilled labour, the commercialisation of scientific processes, and increased rates of urbanisation. The findings of the present study have demonstrated that the innovative framework of agricultural knowledge, in synergy with other links of the production process, reveal new opportunities for the implementation of methods of technological modernisation of agriculture, which was confirmed by E. Sadik-Zada (2021). According to scientists, the main principles of the innovative approach to technological modernisation of agriculture include rationality, competent economic planning, transformation of public opinion, and scientific approaches to agricultural production. This fact is also emphasised by modern scientists A. de Janvry and E. Sadoulet (2020), arguing the need for a radical reorientation of strategic vectors of economic activity.

The findings of I. Cisternas *et al.* (2020) suggest the need to intensify agricultural production through organic technologies, biological sparing methods of plant protection, minimising the use of aggressive chemicals. Scientists are considering ways of levelling the negative effects on agro-landscapes by introducing protective buffer zones within agricultural areas, imposing restrictions on the use of heavy agricultural machinery, and breeding sustainable species. Such conclusions of scientists align with the results of this investigation, emphasising the expectation of a considerable productivity surge and improved quality of agricultural products in case of consistent and full replacement of conventional agricultural technologies with more sparing organic ones.

X. Yang *et al.* (2021) follow up on this issue by highlighting the priority of closed-loop technologies that minimise the environmental impact of agro-industry. Scientists are convinced that the development of sustainable agriculture requires large-scale support in the form of investments and lending to support natural landscape systems, preserve biodiversity, and reduce greenhouse gas emissions. Furthermore, sustainable agricultural production contributes to optimising the main components of food security: availability, utilisation, access, stability. This is what G.K. Kurmanova *et al.* (2022) are convinced of in their research. They also assert that numerous studies overlook information pertaining to the three dimensions of sustainability – social, economic, and environmental. According to the authors, a more extensive adoption of modern precision farming methods, particularly electronic field maps and geographic information systems, will enable the proactive safeguarding of agro-landscapes and positively influence the efficiency of agricultural production.

Practical aspects of technological modernisation of agriculture in Azerbaijan in the conditions of transition

economy require further research. Most scientists, whose studies are discussed above, are united in the system of requirements for effective innovative modernisation of the agricultural sector. In their opinion, it should be carried out systematically, stemming from the introduction of innovative solutions, while being directed at increasing competitiveness, increasing export potential, and developing the structural elements supporting the agricultural part of the economy. Utilising contemporary environmental monitoring capabilities is essential for an impartial evaluation, formulation of preventive measures, and implementation of regeneration actions to mitigate environmental impact. Achieving this objective necessitates the modernisation of the functionalities embedded in agricultural production management tools and streamlining their operations in alignment with the digital economy's prerequisites. C. Gras and D. Cáceres (2020) share a similar perspective, highlighting the inadequacies in the current monitoring system within the agricultural sector.

Y. Gao *et al.* (2020) advocate for a future paradigm in which biological technologies take precedence in agricultural production. This approach relies on understanding the regularities of physiological and biochemical processes that facilitate ecosystem self-purification. The research, including the present study, suggests that it is prudent to incrementally embrace closed-loop technologies. The primary focus should be on gradually halting soil degradation and maximising the separation of economic ties within agro-industry from natural ones. Integrated management in agricultural production is crucial for effectively addressing strategic objectives, including optimising resource utilisation, enhancing the sustainability of agro-ecological systems, monitoring quality, and responding effectively to environmental risks. To improve the situation, it is imperative to curtail activities leading to chemical and other hazardous forms of natural resource pollution and destruction, while also regulating the impact of agricultural production processes.

Summarising the findings of modern scientists and conclusions obtained in the current study, we can highlight the primary features of technological modernisation of agriculture: modernisation increases the competitiveness of agricultural production; introduction of technological innovations stimulates the growth of productivity and efficiency indicators of agricultural processes; modernisation creates a need for qualified personnel and development of scientific potential; the successful optimisation of the agricultural sector yields an expansion of export opportunities for the industry and attracts investment.

Therewith, a pivotal factor contributing to the successful technological modernisation of the agricultural sector is the establishment of a mechanism for state support in agricultural innovation. This involves the creation of a unified investment system and the global

integration of a network of agricultural innovation transfer centres. Thus, enhancing the effectiveness of the agro-industrial complex and ensuring food security become possible only based on technological renewal of basic production processes, a balanced approach to mechanisation and automation, considering the principles of sustainable agricultural development. Optimal technological modernisation makes it possible to transition to an innovative development model, increase production efficiency, and minimise destructive processes in agro-landscapes.

CONCLUSIONS

During this study, a comprehensive analysis of the efficient operation of agrolandscape complexes was conducted through a multifactorial approach grounded in the principles of sustainable development. The study successfully delved into the present state of technological modernisation in Azerbaijan's agriculture, examining existing resources, and identifying potential opportunities in this domain. The statistical data of indicators of economic efficiency of agricultural enterprises of Azerbaijan, which are representative of the efficiency of technological modernisation of the industry, were analysed. Considerable positive effect of innovative optimisation of technological processes of agro-industrial processes was recorded.

The study substantiated the necessity of introducing innovative solutions in the agrarian sector of the Republic, identifies the main features of gradual transition from extensive to intensive, organic and biological technologies. It was found that the development of biological renewable land use and precision farming systems is a considerable factor in increasing economic performance in crop production, strengthening the priorities of ecologization of agriculture, and optimising the state of agro-ecosystems in the aspect of restoration and recreation. It was substantiated that the technological modernisation of the agricultural sector of the economy of Azerbaijan should be oriented towards the gradual

replacement of extensive methods of agricultural production with intensive ones in accordance with the principles of sustainable development and green economy. The study also found that the main approaches to the modernisation of the agricultural industry are based on the principles of diversification and rationalisation of production processes. It was established that significant reduction in environmental impact and improvement in both quantitative and qualitative aspects of agricultural production can only be achieved through the combined implementation of technological and managerial measures, alongside the adoption of innovative control methods. The information gleaned from this study can be effectively applied in practical scenarios.

Adherence to stringent environmental load regulations, aligning current norms of agricultural production with those of developed nations, and the incorporation of modern innovative technological solutions rooted in a closed resource utilisation cycle will play a pivotal role in optimising Azerbaijan's agricultural sector. This optimization, coupled with efficient management strategies and the implementation of an innovative monitoring system, is anticipated to contribute positively to the country's economic landscape. The potential of wide introduction of geoinformation technologies, satellite navigation devices and electronic mapping into the processes of agricultural activities requires further research. Active implementation of a modern management decision support system is also expected to be promising. In combination, such measures will substantially improve the efficiency of agricultural production in the country, expand export opportunities, ensure food security and stimulate economic development towards a green sustainable economy.

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Технологічні інновації у сільському господарстві: вплив на ефективність виробництва

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Анотація. Необхідність забезпечення продовольчих потреб Азербайджану потребує модернізації аграрного сектора у напрямі інноваційного типу розвитку. Такий процес супроводжується технічною та технологічною оптимізацією більшості виробничих процесів у сільському господарстві. Мета роботи – дослідження та аналіз запровадження технологічних інновацій в аграрній галузі Азербайджану. Дослідження проводилися з використанням загальнонаукових методів пізнання, зокрема системного аналізу, синтезу, абстрагування, дедукції, узагальнення, конкретизації. У ході дослідження проаналізовано основні завдання технологічної модернізації аграрної галузі, вивчено динаміку їх впливу на ефективність виробництва залежно від різних факторів, розроблено заходи для оптимізації ситуації з урахуванням пріоритетності інноваційних рішень. Крім того, розглянуто можливості застосування сучасних управлінських та технологічних заходів щодо мінімізації деструктивного впливу сільськогосподарського виробництва на навколишнє середовище, що ґрунтуються на синергії екологічної безпеки та економічної ефективності, з впровадженням сучасних інноваційних технологічних підходів. Визначено їх ефективність як дієвий інструмент для трансформації аграрної галузі відповідно до актуальних соціально-економічних вимог для інтенсифікації її розвитку. Встановлено, що застосування екосистемного підходу, а також запровадження інтегрованого управління агроландшафтами здатні забезпечити сталий розвиток агрономічної галузі в Азербайджані. Результати дослідження мають суттєву практичну значущість для розвитку та модернізації системи сільського господарства у країні, створення векторних програм технологічних інновацій.

Ключові слова: модернізація; аграрний сектор економіки; сталий розвиток; інтенсифікація; органічне виробництво

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