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10008, б-р Старий, 7, м. Житомир, Україна
E-mail: info@sciencehorizon.com.ua
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Editors office address:

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

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Use of contraceptives in cats with ovarian and uterine pathology

Oleksandr Chekan*

PhD in Veterinary Sciences, Associate Professor

Sumy National Agrarian University

40021, 160 Herasym Kondratiev Str., Sumy, Ukraine

<https://orcid.org/0000-0002-5676-1947>

Artem Rokochyi

Postgraduate Student

Sumy National Agrarian University

40021, 160 Herasima Kondratieva Str., Sumy, Ukraine

<https://orcid.org/0000-0001-6340-8706>

Olesia Kysterna

PhD in Veterinary Sciences, Senior Teacher

Sumy National Agrarian University

40021, 160 Herasym Kondratiev Str., Sumy, Ukraine

<https://orcid.org/0000-0003-4010-6101>

Yurii Musiienko

PhD in Veterinary Sciences, Associate Professor

Sumy National Agrarian University

40021, 160 Herasym Kondratiev Str., Sumy, Ukraine

<https://orcid.org/0000-0002-9735-4758>

Iryna Levchenko

PhD in Agricultural Sciences, Associate Professor

Sumy National Agrarian University

40021, 160 Herasima Kondratieva Str., Sumy, Ukraine

<https://orcid.org/0009-0003-9117-4005>

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Abstract. Regulation of the reproductive capacity of cats using megestrol acetate (a gestagenic preparation) contributes to the development of pathological processes in the uterus, ovaries, and imbalance of gonadal hormones. Therefore, it is essential to investigate and scientifically substantiate the pathogenetic processes in the body of cats under medical contraception. The purpose of this study was to substantiate the pathomorphological changes and dynamics of certain blood parameters in the body of cats under the use of megestrol acetate. The study employed clinical (examination,

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*Corresponding author

palpation), laboratory (haematological, biochemical, hormonal), morphological (examination of the genital organs of cats amputated during ovariectomy) methods. A decrease in ovarian size in hypotrophic cats by $11.3 \times 9.05\%$ was found compared to the ovaries of healthy cats. The dependence of megestrol acetate administration on the occurrence of cystic formations in the ovaries was found, with an increase in ovaries in the experimental group by 1.7×2.47 times in follicular cysts and by 1.9×3.1 and 2.5×3.9 times in luteal cysts and polycystic cysts, respectively. It was found that the uterus in cats with megestrol acetate was increased by 3.16×1.93 times in the right horn and 2.84×2.1 times in the left horn compared to the uterus of healthy cats. The dynamics of progesterone (increase by 37.85%), follicle-stimulating hormone (decrease by 43.75%) and oestradiol (increase by 72.15%) were determined in comparison with intact animals. The increase in serum glucose levels in cats after megestrol administration by 34.31% , total protein by 25.0% and creatinine by 11.10% , aspartate aminotransferase by 43.84% (1.78 times) and alanine aminotransferase by 49.84% (1.99 times), and serum urea by 58.71% was substantiated. At the same time, haemoglobin levels decreased by 16.5% . The findings of this study can be used in the development of new methods and regimens of medical contraception with minimisation of risks of complications of the reproductive system, regulation of reproductive capacity, and prevention of infertility in cats

Keywords: contraceptives; gestagens; megestrol acetate; cysts; pyometra; endometritis

INTRODUCTION

The use of megestrol acetate-based drugs to correct sexual cyclicity in cats is widely used by pet owners. This leads to an increase in the number of gynaecological pathologies due to an imbalance of sex hormones. Uncontrolled use of megestrol acetate to interrupt sexual desire in cats causes both functional disorders of the reproductive system (hypotrophy and ovarian cysts) and inflammatory processes (metritis and pyometra). The scientific and practical substantiation of the negative impact of megestrol acetate will make it possible to prevent the occurrence of gynaecological pathology in cats.

The use of progestogenic contraceptives to prevent heat in cats with a 1-year interval leads to destructive changes in the genitals. S.S. Goericke-Pesch (2017) claims that after discontinuation of megestrol acetate-based preparations, fertility is restored. However, O.I. Shkromada and A.V. Rokochyi (2023) obtained data that the manifestation of gynaecological pathology in cats occurs due to the improper use of megestrol acetate-based preparations. This is what initially leads to disruption of the sexual cycles of cats, as noted by O. Burlakova and V. Stefanyk (2021), and subsequently to pathological inflammatory processes in the organs of the reproductive system: pyometra, endometritis. Furthermore, Z. Niewiadomska *et al.* (2023) point to the development of histological endometrial hyperplasia after the use of a megestrol acetate-based preparation, which further interferes with the physiological development of the placenta and contributes to early and subclinical abortions caused by impaired embryo implantation. G. Martini *et al.* (2023) point to the development of pyometra as a consequence of the increase in oestradiol $17\text{-}\beta$ content provoked by the use of megestrol acetate.

Therewith, R. Hagman (2022) notes the development of pyometra in cats as a consequence of hormone levels, specifically oestrogen and progesterone, during 4 months, which, according to C.V. Lindsay *et al.* (2023), is the cause of infertility in cats and is caused by

the prolonged use of megestrol acetate. In addition, C. Binder *et al.* (2021) pointed out the key role of progesterone block in the early stages of pregnancy in cats, noting that when progesterone levels decrease, either a habitual abortion or partial or complete foetal resorption occurs. S. Romagnoli and L. Ferre-Dolcet (2022) claim that the use of megestrol acetate-based preparations every 13 weeks can prevent ovulation, and longer-term use provokes the development of hypersecretory lobules and neoplastic areas in the mammary gland. In addition, S. Chotimanukul *et al.* (2023) notes that ovulation occurs at a 2:1 ratio of follicle-stimulating to luteinising hormone.

According to J. Liang *et al.* (2023), pathology of the ovaries and uterus in cats is quite common. As noted by A. Fontbonne *et al.* (2020), the most common pathological conditions are ovarian hypotrophy, ovarian cysts, and uterine inflammation. H. Moosavian *et al.* (2022) point out that the problem of feline reproduction has many issues that require a thorough analysis, namely: how to safely use medical contraceptives to prevent ovulation with the subsequent restoration of the fertility of cats, e.g., when resting between pregnancies in pedigree cats; what consequences can be expected when using megestrol acetate-based gestational agents and how to prevent them. E.A. McGlade *et al.* (2022) state that increased progesterone contributes to a decrease in local (tissue) defence responses and a decrease in the count of saprophytic (lactobacilli) bacteria in the vagina of cats, which contributes to an increase in the count of opportunistic pathogens such as streptococci and staphylococci, which subsequently provoke the development of endometritis.

Despite the considerable number of publications, the issue of using oestrus inhibitors in cats stays relevant for many areas of veterinary medicine, namely reproductive medicine – for the development of technological schemes in the professional breeding of purebred

animals, surgery – for the creation of surgery protocols in case of detected pathologies and risk reduction after surgery. This issue is also relevant in wartime. After all, in the current conditions in Ukraine, there is an acute problem of uncontrolled reproduction of abandoned and stray animals, hence the spread of dangerous infections in the absence of veterinary services for neutering dogs and cats and vaccination. Therefore, in dangerous and uncontrolled areas, alternative schemes should be sought to control animal reproduction with medicated contraception with minimal risk to animal health. That is why the purpose of this study was to establish and scientifically substantiate changes in the organs of the reproductive system of cats when using megestrol acetate-based preparations.

MATERIALS AND METHODS

The study was conducted in the clinical and diagnostic advisory centre “Vet camp” of the Faculty of Veterinary Medicine of Sumy National Agrarian University during 2023. The study was conducted in 2 stages. At the first stage, 2 groups of cats were formed: 1) control (no megestrol acetate was used), 2) experimental (megestrol acetate was used according to the guidelines). There were 15 animals in each group. Therewith, prevalence of gynaecological pathology, morphological characteristics of the ovaries and uterus, and the dynamics of progesterone, oestradiol, and follicle-stimulating hormone were determined. Morphometric parameters and cysts of the ovaries and uterus were examined after ovariectomy according to the generally accepted method of S. Bowen *et al.* (2007), according to which the normal size of the ovaries in cats is 10×6×4 mm. The concentration of progesterone, follicle-stimulating hormone and progesterone was determined using an ImmunoChem-2100 analyser (USA) according to the instructions. The obtained digital material was processed according to the methods of variation statistics using SPSS Data editor 17.0 version.

At the second stage, 30 experimental animals were divided into 2 groups based on the principle of analogues, with 15 cats in each group. The cats were divided according to the history of contraceptive use; the first group did not use such preparations, and the second group used preparations with the active ingredient megestrol acetate. Therewith, Group 1 was control (cats without signs of inflammatory processes in the reproductive system) and Group 2 was experimental (cats with signs of endometritis). Upon the study of blood serum of both groups of cats, the content of basic biochemical parameters such as glucose, haemoglobin, total protein, creatinine, aspartate aminotransferase, alanine aminotransferase, and urea were determined.

For the study of haematological parameters (number of red blood cells and white blood cells) and determination of hormonal status, blood was taken from the saphenous veins of the forearm or thigh, depending on the degree of their filling, using tubes with a blood clotting activator for biochemical studies and an anticoagulant ethylenediaminetetraacetate (EDTA K3) for clinical studies. Blood tests were performed using a semi-automatic analyser Mindray BA-88 (biochemical analysis) (China) and an automatic haematological analyser H360 (clinical analysis) (Erba Lachema s.r.o., Czech Republic). Animals were kept and all manipulations were carried out according to the Law of Ukraine No. 249 “Procedure for Conducting Research and Experiments on Animals by Scientific Institutions” (2012), European Convention for the Protection of Vertebrate Animals (1986).

RESULTS AND DISCUSSION

Analysing the prevalence of ovarian pathologies, it was found that in the group where megestrol acetate was not used, ovarian hypotrophy was diagnosed 3 times less frequently, which is associated with a violation of the rhythmicity of the cyclic peaks of the main sex hormones (Table 1).

Table 1. Prevalence of ovarian and uterine pathologies in cats depending on the use of contraceptives

Indicator	Group 1 – control (n=15)	Group 2 – with the use of megestrol acetate (n=15)
Ovarian hypotrophy	1	3
Follicular cyst	–	2
Luteal cyst	–	1
Polycystic disease	–	5
Pyometra	2	2
Endometritis	1	2

Source: compiled by the authors of this study

Follicular (n=2), luteal (n=1) and polycystic (n=1) cysts in cats were diagnosed only in the group treated with megestrol acetate, which emerged on the background of systematic disorders of sexual cycles, which led to a violation of the hormonal status of sex hormones and

the transformation of follicles into cysts. The morphological characteristics of the ovaries and uterus in cats of the experimental groups differ and can serve as differential parameters in the diagnosis and development of preventive measures (Table 2).

Table 2. Morphological characteristics of the ovaries and uterus

Indicator	Group 1 – control (n=15)		Group 2 – with the use of megestrol acetate (n=15)	
	ovary, hypotrophy			
x	right	left	right	left
Longitudinal diameter, cm	–	0.41	0.43±0.03	0.42±0.04
Transverse diameter, cm	–	0.22	0.21±0.02	0.25±0.03
	ovary, follicular cyst			
Longitudinal diameter, cm	–	–	0.91±0.01	0.93±0.02
Transverse diameter, cm	–	–	0.52±0.03	0.56±0.01
	ovary, luteal cyst			
Longitudinal diameter, cm	–	–	1.1±0.05	1.2±0.03
Transverse diameter, cm	–	–	0.61±0.04	0.65±0.06
	ovary, polycystic disease			
Longitudinal diameter, cm	–	–	1.4±0.07	1.6±0.08
Transverse diameter, cm	–	–	0.82±0.09	0.73±0.07
	uterus, pyometra			
	right horn	left horn	right horn	left horn
Length, cm	6.0±0.36	7.0±0.47	11.36±1.2	10.5±1.1
Transverse diameter, cm	2.6±0.02	2.7±0.5	3.1±0.1	3.3±0.1
	uterus, endometritis			
Length, cm	5.5	5.0	6.3±1.2	6.1±0.94
Transverse diameter, cm	1.1	1.2	0.97±0.06	0.87±0.12

Source: compiled by the authors of this study

In hypotrophy, the ovaries of cats of both groups were reduced, and therefore, the size of the ovary in the first group was 0.41×0.21 cm, while in cats of the Group 2 the size ranged from 0.42±0.04×0.25±0.03 – left to 0.43±0.03×0.21±0.02 cm – right. This is caused by excessively high levels of progestogen in the blood of cats, which blocks the formation of follicle-stimulating and luteinising hormones by blocking the release of releasing hormone. The conclusions coincide with those drawn by E.R.S. Maylem and L.J. Spicer (2022).

It was found that the occurrence of such pathologies as follicular and luteal cysts and polycystic ovaries is more correlated with the use of megestrol acetate. Thus, the development of cystic degeneration in cats that did not take megestrol acetate was not found. At the same time, in cats of the Group 2, the size of the ovaries in follicular cysts ranged from 0.91±0.01×0.52±0.03 to 0.93±0.02×0.56±0.01 cm, which is 1.7×2.47 times larger than in healthy cats ($p<0.05$). A comparable trend was observed in animals with luteal cysts and polycystic disease. The size of the ovary in cats with luteal cysts was 1.9×3.1 times ($p<0.001$) and in polycystic cysts – 2.5×3.9 times ($p<0.001$) greater than in healthy animals. The

formation of cysts is triggered by an imbalance of follicle-stimulating and luteinising hormones.

Under the influence of megestrol acetate, the release hormone is inhibited, which stimulates the formation of follicle-stimulating and luteinising hormones. In this case, releasing hormone has an α -isomer that promotes the formation of follicle-stimulating hormone and a β -isomer that affects the amount of luteinising hormone formation. Therefore, inhibition of the α -isomer of releasing hormone creates conditions for the development of luteal cysts, and reduction of the β -isomer creates conditions for the development of follicular cysts.

Pyometra is characterised by purulent inflammation of all layers of the feline uterus. Therewith, the size of the uterus increases significantly, it becomes pain-inducing and blood-filled. The uterus was found to be enlarged during pyometra in cats of Group 1 by 1.7×1.7 times ($p<0.001$) in the right horn (Fig. 1a) and 1.94×1.69 ($p<0.001$) in the left horn and cats of Group 2 – by 3.16×1.93 times ($p<0.001$) in the right horn and 2.84×2.1 ($p<0.001$) times in the left horn (Fig. 2b).

The use of contraceptives, specifically megestrol acetate, is based on changes in the content of sex hormones and their ratios (Table 3)

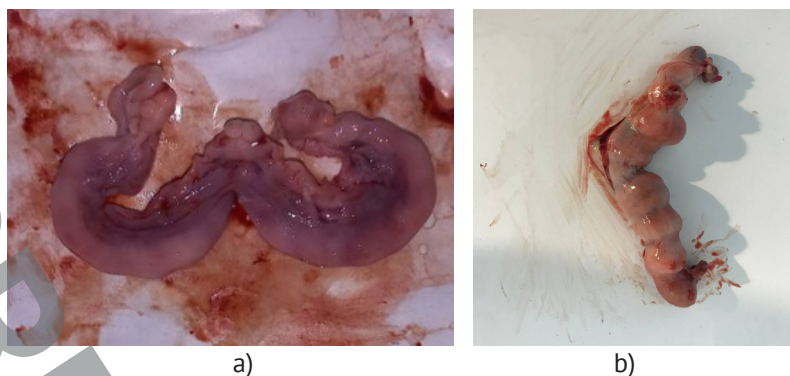


Figure 1. Feline uterus with pyometry

Notes: a) Uterus of a Group 1 cat with pyometry; b) Uterus of a Group 2 cat with pyometry

Table 3. Progesterone, oestradiol, and follicle-stimulating hormone levels ($M \pm m$)

Indicator	Group 1 – control (n=15)	Group 2 – with the use of megestrol acetate (n=15)
Progesterone, ng/ml	13.5±0.26	21.72±0.16
Oestradiol, pg/ml	61.11±5.37	105.2±1.97
Follicle-stimulating hormone, UI/l	0.16±0.045	0.09±0.008

Source: compiled by the authors of this study

It was found that the progesterone content in the blood serum of cats treated with megestrol acetate increased by 37.85% (1.61 times), oestradiol – by 72.15% (1.72 times), and follicle-stimulating hormone decreased by 43.75% (1.78 times). Therewith, 2 cases of follicular cysts formation were diagnosed in cats of Group 2, which was caused by an imbalance of sex hormones, namely, high (0.16±0.05 UI/l) follicle-stimulating hormone. This is also confirmed by an increase in the ratio of progesterone to oestradiol. Thus, the ratio

of progesterone to oestradiol in Group 1 was 4.5, while in Group 2, where megestrol acetate was used, this ratio was 4.84, which is 6.54% (1.07 times higher). Changes in the main biochemical parameters that are markers of homeostasis disorders, specifically the development of subclinical or chronic inflammatory processes, which subsequently lead to endometritis or pyometra, which in turn lead to persistent infertility of cats and the emergence of various pathologies of the reproductive system (Table 4).

Table 4. Dynamics of the main biochemical parameters

Indicator	Group 1 – control group (n=15)	Group 2 – with the use of megestrol acetate (n=15)
Glucose, mmol/l	4.48±0.12	6.82±0.16
Haemoglobin, g/l	90.25±3.47	75.36±0.32
Total protein, g/l	62.97±3.57	83.97±5.67
Creatinine, µmol/l	154.36±6.78	173.64±9.59
Aspartate aminotransferase, IU/l	18.91±1.37	33.64±2.53
Alate aminotransferase, UI/l	41.33±2.61	82.39±3.54
Urea, µmol/l	7.65±0.64	4.82±0.22

Source: compiled by the authors of this study

An increase in serum glucose levels in the megestrol-treated group by 34.31% (1.52 times) was found, which may indicate a compensated insufficiency of insulin content. At the same time, there was a decrease in haemoglobin levels by 16.5% (1.2 times). Therewith, the amount of total protein and creatinine increased by 25.0% (1.33 times) and 11.10% (1.12 times), indicating the development of subclinical inflammatory processes.

There was also an increase in liver enzymes: aspartate aminotransferase (AST) by 43.84% (1.78 times) and alanine aminotransferase (ALT) by 49.84% (1.99 times), serum urea by 58.71% (1.59 times), which also indicates the development of inflammatory processes. It is known that rapid changes in sex hormone levels cause changes in the dynamics of haematological parameters (Table 5).

Table 5. Dynamics of the main haematological parameters during the use of megestrol acetate

Indicator	Group 1 – control (n=15)	Group 2 – with the use of megestrol acetate (n=15)
Red blood cells, t/l	7.21±0.33	4.82±0.38
White blood cells, g/l	17.22±1.92	19.37±4.21
Banded, %	1.1±0.15	3.22±0.24
Segmented, %	43.22±3.29	54.91±4.65
Metamyelocytes, %	0.22±0.01	0.41±0.11

Source: compiled by the authors of this study

A 33.15% (1.5-fold) decrease in the count of red blood cells was found with megestrol acetate, while the count of leukocytes, especially different types of neutrophils, tended to increase and change their ratio. Thus, the total number of leukocytes increased by 12.49% (1.12 times) after megestrol acetate administration. Therewith, the percentage of banded neutrophils

increased by 192.73% (2.93 times), segmented neutrophils – by 27.05% (1.27 times), and metamyelocytes – by 86.36% (1.86 times).

The next stage of the study was to determine changes in the main biochemical parameters of the blood of cats with endometritis after the use of megestrol acetate (Table 6).

Table 6. Dynamics of the main biochemical parameters of the blood of cats with endometritis

Indicator	Group 1 – control (n=15)	Group 2 – with the use of megestrol acetate (n=15)
Glucose, mmol/l	4.48±0.12	8.24±0.22*
Haemoglobin, g/l	90.25±3.47	73.64±4.91
Total protein, g/l	62.97±3.57	81.33±4.29
Creatinine, µmol/l	154.36±6.78	192.61±7.02
AST, IU/l	18.91±1.37	62.27±3.32
ALT, IU/l	41.33±2.61	138.22±5.66
Urea, µmol/l	7.65±0.64	22.33±1.48

Source: compiled by the authors of this study

There was a significant increase in glucose by 83.93% (1.84 times), total protein by 29.16% (1.29 times), creatinine by 24.78% (1.25 times), AST by 229% (3.29 times), ALT by 234% (3.34 times), and urea by 191% (2.92 times). At the same time, the haemoglobin level tended to decrease, amounting to 18.40% (1.23 times). Therewith, comparing analogous biochemical parameters of the blood of animals that did not have inflammatory processes (endometritis) of the reproductive system (Table 4), the study found a tendency

to increase glucose levels by 20.82% (1.21 times), creatinine by 10.92% (1.11 times) and a significant increase in AST by 85.11% (1.85 times), ALT by 67.76% (1.68 times), and urea by 363.28% (4.63 times). This change is caused by the development of inflammatory processes against the background of an increase in progesterone levels by 37.85% compared to intact animals. Significant changes in the morphological parameters of blood in the manifestation of endometritis in cats after the use of megestrol acetate were found (Table 7).

Table 7. Dynamics of the main haematological parameters of blood in cats with endometritis

Indicator	Group 1 – control (n=15)	Group 2 – with the use of megestrol acetate (n=15)
Red blood cells, t/l	7.21±0.33	4.81±0.53
White blood cells, g/l	17.22±1.92	23.22±2.62
Banded, %	0.22±0.01	9.41±1.02
Segmented, %	43.22±3.29	64.31±2.37
Metamyelocytes, %	1.1±0.15	0.73±0.11

Source: compiled by the authors of this study

Thus, the count of red blood cells decreased by 33.29% (1.5 times) compared to intact cats. Therewith, the count of leukocytes increased by 34.84%

(1.35 times), including the count of banded neutrophils by 755.45% (8.55 times), segmented neutrophils – by 48% (1.49 times) and metamyelocytes – by 231.82%

(3.32 times). It is also important to compare analogous blood parameters in cats treated with megestrol acetate but without endometritis (Table 5). Thus, the count of the red blood cells was not statistically different, but the count of leukocytes in the blood of cats with endometritis was 19.88% higher (1.2 times), the count of banded neutrophils was 192.24% higher (2.92 times), segmented neutrophils – 17.12% higher (1.17 times), and metamyelocytes – 78.05% higher (1.78 times).

The use of megestrol acetate in cats to prevent signs of heat increases the incidence of gynaecological pathology such as ovarian atrophy and cysts, endometritis, and pyometra. Therewith, the content of oestradiol and progesterone increases, while follicle-stimulating hormone decreases. A decrease in glucose, haemoglobin, and urea, and an increase in total protein, creatinine, AST, ALT in the blood serum of cats treated with megestrol acetate were also found.

The use of contraceptives that contain substances that affect the course of the sexual cycle of cats by changing the synthesis of both hypothalamic-pituitary hormones and sex hormones, specifically megestrol acetate, contributes to the more frequent manifestation of pathologies such as ovarian hypofunction, follicular cysts, luteal cysts, and polycystic disease, which is also confirmed by publications by foreign authors (Brändli *et al.*, 2021). Particular attention should be paid to the manifestation of pyometra and endometritis after megestrol acetate use. An increase in the number of cases of acute inflammatory processes in the organs of the reproductive system of cats, such as endometritis and pyometra, has been established. Thus, S. Romagnoli *et al.* (2023) report a more intense uterine inflammatory process in cats and an increase in the number of complications after systematic use of megestrol acetate, noting that short-term and especially long-term administration of progesterone derivatives disrupts cell receptors in the endometrial epithelium, allowing pathogens to break through this first natural barrier and thus increase the risk of developing pyometra.

The findings of the study by C. Binder *et al.* (2019) confirmed a 1.9×3.1 to 2.5×3.9-fold increase in ovarian size in cystic degeneration, indicating that the incidence increased with the age of the cats and was complicated by purulent endometritis and hyperplastic endometrial lesions. The determining role of persistent corpus luteum in the development of cystic degeneration was also noted. Therewith, as noted by L. Schirrer *et al.* (2021), cysts occurred when megestrol acetate was used to prevent sexual desire, and polycystic disease occurred when sexual desire was interrupted, which is fully consistent with the findings of the present study. Data were also obtained on the formation of both follicular and luteal cysts in cats treated with megestrol acetate. The reason for this phenomenon is the dysfunction of the hypothalamic and pituitary hormones. H.J.J. Kim *et al.* (2023) indicate that the use of contraceptives disrupts the

functioning of cannabinoid receptors, which is characterised by changes in their expression in relation to oestrogen and progesterone, which coincides with the data obtained. At the same time, the development of luteal cysts is triggered by a violation of follicle-stimulating hormone production or an increase in luteinising hormone secretion (Xia *et al.*, 2020).

Research found destructive changes in the uterine mucosa, manifested in the form of endometritis and pyometra in cats previously treated with megestrol acetate to suppress reproductive capacity. Violation of oestrogen synthesis is accompanied by pathological processes in the organs of the reproductive system. Z. Niewiadomska *et al.* (2023) note that most cases of infertility in cats after megestrol acetate administration are caused by hyperplastic endometrial degeneration, which prevents embryo implantation and placental development.

Data was obtained on the increase in serum glucose by 34.31%, total protein by 11.10%, and creatinine by 25.0% in cats treated with megestrol acetate, which coincides with the opinion of R. Hagman (2018), who notes that pyometra, which develops within 4 months after oestrus or contraceptive use, is caused by the influence of microflora, in the development of which high progesterone levels play a leading role. At the same time, pyometra and endometritis complications are reported in cats older than 5-7 years. The use of megestrol acetate has a negative impact on the reproductive capacity of cats, creating conditions for the formation of ovarian cysts, development of endometritis and pyometra, accompanied by hypertrophic degeneration of the endometrium, and disruption of the main indicators of homeostasis.

CONCLUSIONS

The study substantiated the negative effect of megestrol acetate on the reproductive system of cats and revealed morphological changes in the ovaries and uterus, as well as complications in the form of endometritis and pyometra, which was confirmed by haematological and biochemical blood tests. An increase in the manifestation of ovarian hypotrophy by 333.33%, endometritis by 100.0% in cats treated with megestrol acetate compared to intact animals and the occurrence of follicular cysts and pyometra by 13.33% of polycystic disease – 30.0% of the total number of affected cats, indicating the systemic nature of the effect of megestrol acetate on both the organs of the reproductive system and the body of cats as a whole. The study found an increase in the size of ovaries with polycystic ovaries by 2.5×3.9 times, in luteal cysts – by 1.9×3.1 times, and in follicular cysts – by 1.7×2.47 times compared to the ovaries of healthy cats, which may indicate destructive changes in the form of accelerated apoptosis and sclerotic processes in the reproductive system. An increase in the progesterone content in the blood serum of cats

treated with megestrol acetate by 37.85%, oestradiol – by 72.15%, and follicle-stimulating hormone – by 43.75% characterises persistent irreversible changes in the secretory capacity of the feline body, which leads to a violation of both the general reproductive capacity and the rhythm of sexual cycles specifically.

Analysing biochemical parameters, the study found a decrease in haemoglobin levels by 16.5% and an increase in glucose by 34.31%, total protein – by 25.0%, creatinine – by 11.10%, AST – by 43.84%, ALT – by 49.84%, and serum urea – by 58.71%, which also indicates the development of inflammatory processes in cats after prolonged use of megestrol acetate, indicating a violation of both metabolic processes, inherent in the onset of oxidative stress, and the toxic effects of the contraceptive on the feline body. A decrease in the count of red blood cells by 33.15% was recorded with the use of megestrol acetate, while the count of

leukocytes increased by 12.49%, the percentage of banded neutrophils increased by 192.73%, segmented neutrophils – by 27.05%, and metamyelocytes – by 86.36%, which suggests the development of inflammatory processes, such as endometritis and pyometra, specifically, which have an acute course and are accompanied by erythropoiesis disorders and an increase in the white blood cell fraction. In the future, it is planned to investigate cytological changes in the tissues of the reproductive system organs and develop methods for diagnosing and preventing the development of pathologies of the reproductive organs of cats.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Застосування протизаплідних засобів кішкам із патологією яєчників та матки**Олександр Миколайович Чекан**

Кандидат ветеринарних наук, доцент
Сумський національний аграрний університет
40021, вул. Г. Кондратьєва, 160, м. Суми, Україна
<https://orcid.org/0000-0002-5676-1947>

Артем Володимирович Рокочий

Аспірант
Сумський національний аграрний університет
40021, вул. Г. Кондратьєва, 160, м. Суми, Україна
<https://orcid.org/0000-0001-6340-8706>

Олеся Сергіївна Кистерна

Кандидат ветеринарних наук, старший викладач
Сумський національний аграрний університет
40021, вул. Г. Кондратьєва, 160, м. Суми, Україна
<https://orcid.org/0000-0003-4010-6101>

Юрій Володимирович Мусянко

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

Ірина Володимирівна Левченко

Кандидат сільськогосподарських наук, доцент
Сумський національний аграрний університет
40021, вул. Г. Кондратьєва, 160, м. Суми, Україна
<https://orcid.org/0009-0003-9117-4005>

Анотація. Регуляція репродуктивної здатності кішок з використанням мегестролу ацетату (гестагенний препарат) сприяє розвитку патологічних процесів у матці, яєчниках, дисбалансу гонадальних гормонів. Тому важливим є дослідження та наукове обґрунтування патогенетичних процесів в організмі кішок за медикаментозної контрацепції. Метою роботи було обґрунтування патоморфологічних змін та динаміки окремих показників крові в організмі кішок, при застосуванні мегестролу ацетату. Під час проведення досліджень використані клінічні (огляд, пальпація), лабораторні (гематологічні, біохімічні, гормональні), морфологічні (дослідження органів статеві системи кішок, ампутованих під час овариогістеректомії) методи. Встановлено зменшення величини яєчників при гіпотрофії кішок на 11,3×9,05 % порівняно із яєчниками здорових кішок. Виявлено залежність застосування мегестролу ацетату із виникненням кістозних утворень в яєчниках із збільшенням яєчників у кішок дослідної групи у 1,7×2,47 рази при фолікулярній кісті та у 1,9×3,1 та 2,5×3,9 рази при лютеїновій кісті та полікістозі, відповідно. Встановлено збільшення матки за піометрію у кішок при застосуванні мегестролу ацетату в 3,16×1,93 рази правого рогу і 2,84×2,1 рази лівого рогу порівняно із маткою здорових кішок. З'ясовано динаміку прогестерону (підвищення на 37,85 %), фолікулостимулюючого гормону (зниження на 43,75 %) та естрадіолу (підвищення на 72,15 %) порівняно із інтактними тваринами. Обґрунтовано підвищення рівня глюкози у сироватці крові у кішок після застосування мегестролу на 34,31 %, загального білку на 25,0 % та креатиніну на 11,10 %, аспартатамінотрансферази на 43,84 % (1,78 раза) та алатамінотрансферази на 49,84 % (1,99 раза), сечовини сироватки крові на 58,71 %. В той же час зниження рівня гемоглобіну на 16,5 %. Результати досліджень можуть бути використані при розробці нових методів та схем медикаментозної контрацепції з мінімізацією ризиків щодо ускладнень з боку репродуктивної системи, регулювання репродуктивної здатності та профілактиці неплідності у кішок

Ключові слова: контрацептиви; гестагени; мегестролу ацетат; кісти; піометра; ендометрит



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Efficacy of treatment of small animals with triamcinolone-based medications for atopic dermatitis

Anatoliy Paliy

Doctor of Veterinary Science, Professor
National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine"
61023, 83 Pushkinska Str., Kharkiv, Ukraine
State Biotechnological University
61002, 44 Alchevskikh Str., Kharkiv, Ukraine
<https://orcid.org/0000-0002-9193-3548>

Kateryna Rodionova*

PhD in Veterinary Science, Associate Professor
Odesa State Agrarian University
65012, 13 Panteleimonivska Str., Odesa, Ukraine
<https://orcid.org/0000-0002-7245-4525>

Olena Pavlichenko

PhD in Veterinary Sciences, Doctor of Juridical Sciences, Associate Professor
State Biotechnological University
61002, 44 Alchevskikh Str., Kharkiv, Ukraine
<https://orcid.org/0000-0002-6577-6577>

Andrey Telyatnikov

Doctor of Veterinary Science, Associate Professor
Odesa State Agrarian University
65012, 13 Panteleimonivska Str., Odesa, Ukraine
<https://orcid.org/0000-0002-7772-8679>

Mariia Khimych

PhD in Veterinary Sciences, Associate Professor
Odesa State Agrarian University
65012, 13 Panteleimonivska Str., Odesa, Ukraine
<https://orcid.org/0000-0003-2646-3196>

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Abstract. Among the diseases of small pets, skin diseases are one of the leading ones. Atopic dermatitis is a common skin condition in small pets, which, according to veterinarians' estimates, affects 10-15% of dogs and 7-18% of cats. Depending on the allergens involved, clinical signs can be seasonal or non-seasonal, and the disease

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*Corresponding author

usually requires lifelong treatment. The purpose of this study was to investigate the therapeutic efficacy of treating atopy in dogs and cats with the use of triamcinolone-based veterinary medications (oral suspension). Standard clinical, haematological, biochemical, and immunological methods were used in the study. The increased content of total immunoglobulin E (Ig E) in the blood of experimental animals before treatment (Group I – 3.4 times, and Group II – 3.9 times compared to the control) indicates the presence of an allergic response in the body. It was found that the tested veterinary medications do not substantially affect the haematological parameters of dogs and cats when administered orally, and in therapeutic doses do not have a toxic effect on the functional state of the liver and kidneys. It was proved that on Day 4 of use of oral suspension No. 1 and No. 2 based on triamcinolone (1%) acetonide and a complex of B vitamins in a therapeutic dosage, an improvement in the general clinical condition of animals was recorded. On Day 8 of treatment, dogs and cats in experimental Groups I and II showed improvement in skin condition: a reduction in the lesion area, absence of redness, scratching, and exfoliation. The general clinical condition of the skin of the experimental animals stayed unchanged from Day 8 to Day 12. The developed oral suspensions have a pronounced anti-allergic effect and improve the skin condition of small pets (dogs and cats) with atopic dermatitis

Keywords: atopy; immunoglobulin; treatment; suspension; blood plasma; research; dogs; cats

INTRODUCTION

The issue of treating dermatological diseases in cats and dogs is a highly relevant issue in veterinary medicine. The principal causes of skin diseases in animals are ectoparasites (fleas, ticks), insect bites, household chemicals, feed components, plant and tree pollen, and mould spores. Dermatitis can also be associated with the development of autoimmune processes, dysfunction of the endocrine glands, or a lack of vitamins and minerals. A. Paliy *et al.* (2019, 2020) and V.L. Kovalenko *et al.* (2020) noted that a vital role in ensuring animal health is played by the conditions of their keeping, including the level of sanitation, which must be constantly maintained at a high level to prevent animal diseases.

Atopic dermatitis is a systemic disease that occurs because of hypersensitivity to specific (allergens) and non-specific stimuli (Marchegiani *et al.*, 2020; Franco *et al.*, 2021). Typical clinical manifestations of atopy are inflammation of the skin, which manifests itself in the form of eczematous and lichenoid rashes and is accompanied by itching (Case & Burgess, 2018; Jaffey *et al.*, 2020). The disease is progressive and has a recurrent course, which has an extremely negative impact on the quality of life of the animal and can be very unpleasant for its owners (Bensignor & Videmont, 2021). According to S. Segarra *et al.* (2023), the development of atopy is the result of a complex interaction of genetic and environmental factors that shape the immune response and skin barrier function. The concept of atopic dermatitis proposed by L.M. Tomich and J.B. Pieper (2019) explains the hyperproduction of Ig E from the standpoint of congenital immune deficiency and impaired T-suppressor function, which is manifested in the weakening of the predominant effect on the B-cell clone with which Ig E synthesis is associated.

Recently, in the pathogenesis of atopic dermatitis, great importance has been attached to disorders of fatty acid metabolism in the formative elements,

blood plasma, and adipose tissue. According to A.K. Masters *et al.* (2018), it is the lack of certain fatty acids that leads to a deficiency of the components of the prostaglandin cascade, which have regulatory functions primarily in relation to cellular synthesis of Ig E and contribute to the reduction of inflammatory phenomena. According to A.R. Vaughn *et al.* (2019), a lack of B vitamins in cats and dogs can contribute to the development of dermatitis and seborrhoea. B. Hesselmar *et al.* (2018) proved the effectiveness of glucocorticoids in atopic dermatitis due to their haemodynamic effect: they help to normalise blood pressure, counteract the development of vasodilation; have an anti-oedematous effect on cells, including neurons of the central nervous system (CNS); reduce the aggregation of polymorphonuclear white blood cells and prevent the occurrence of leukostasis in the vascular bed; inhibit the formation of substances that act on the reticuloendothelial system; and inhibit the release of histamine. I. Dávila *et al.* (2018) indicate the effectiveness of synthetic glucocorticoids in the regimen of complex therapy for faster and more reliable control of clinical manifestations of dermatitis on the skin of animals.

J. Altamirano-Vallejo *et al.* (2018) and M. Langworthy *et al.* (2019) prove the high efficiency of using a medication based on the synthetic glucocorticoid triamcinolone acetonide for the treatment of inflammatory, allergic, and autoimmune diseases. The authors recommend the medication as a safer alternative to injections of other corticosteroid medications. Data from N.K.Y. Gedon and R.S. Mueller (2018) indicate a high (70-80%) clinical efficacy of injectable and topical forms of glucocorticoids in the general anti-inflammatory and antipruritic therapy of atopic dermatitis in dogs and cats. The authors note that the addition of essential fatty acids and vitamins to the diet of sick animals can be beneficial: improving the quality of the coat, strengthening

the skin barrier, reducing transepidermal water loss, which reduces the doses of glucocorticoids required to control the disease. The purpose of this study was to scientifically substantiate the effectiveness of triamcinolone-based veterinary medications on cats and dogs of different breeds and sexes.

MATERIALS AND METHODS

The study was conducted in 2020-2022 at the Laboratory of Veterinary Sanitation, Parasitology, and Bee Diseases of the National Research Centre "Institute of Experimental and Clinical Veterinary Medicine", at an animal shelter (Balakliia, Kharkiv region) and a multidisciplinary laboratory of Odesa State Agrarian University. The research programme was reviewed and approved following the current procedure by the Bioethics Committee of the National Research Centre "Institute of Experimental and Clinical Veterinary Medicine".

The following medicinal products (oral suspensions) were used in this study:

- Product No. 1 – a bright yellow suspension with a specific smell of its components. The product contains (1 ml): triamcinolone acetonide – 1 mg; vitamin B₁ – 2 mg; vitamin B₂ – 4 mg; vitamin B₃ – 10 mg; vitamin B₆ – 2 mg; excipients (xanthan gum, potassium sorbate, bentonite, glycerine, purified water).

- Product No. 2 – a light yellow to dark yellow suspension. The product contains (1 ml): triamcinolone – 1 mg; vitamin B₆ – 3 mg; vitamin B₂ – 5 mg; vitamin B₃ – 10 mg; excipients (xanthan gum, potassium sorbate, bentonite, glycerine, purified water).

The medications were administered to animals individually orally, once a day, in the morning feeding hours with a small amount of feed or injected into the root of the tongue using a syringe-dispenser in therapeutic doses (Table 1).

Table 1. Dosage of the medications under study

Animal species	Animal body weight, kg	Medication dosage per animal, ml
Dogs	up to 10	0.5
	10-20	1.0
	20-30	1.5
	30-40	2.0
	over 40	2.0
Cats	up to 3	0.25
	3 or more	0.5

Source: compiled by the authors of this study

For the first 4 days, the medications were administered at a therapeutic dose, and for the next 8 days, the dose was halved. The vials with medications were thoroughly shaken before use. Clinical studies were conducted on cats and dogs of different breeds, ages, and body weights. The study used laboratory glassware, spectrophotometer, thermostat, dispensers, reagent kits for haematological and biochemical studies (manufactured by the Scientific and Production Enterprise "Filis-it-Diagnostics" (Ukraine)); a kit for the quantitative determination of the concentration of immunoglobulin E (Ig E) in blood serum by enzyme-linked immunosorbent assay (ELISA) (Monobind (USA)); automatic single- and multi-channel pipettes of fixed or variable volume of 5-1000 µl. General laboratory equipment. Immunoassay analyser with a wavelength of 450 nm.

At the first stage of the study, the efficacy of veterinary medications was investigated on outbred cats of different ages and sexes. To conduct the study, a control and two experimental groups were formed based on the principle of analogues, considering the body weight, age, and type of animal constitution. The number of animals in each group was 5. Animals of the control and experimental groups were kept in enclosures on a standard balanced diet with free access to water, according to physiological needs. Animals in the

control group were clinically healthy outbred cats aged 1 to 5 years, weighing 2.7-4.8 kg, vaccinated against calicivirus, rhinotracheitis, and panleukopenia. Before the experiment, the cats were dewormed.

Experimental Group I – cats with clinical signs of allergic dermatitis, which were administered with product No. 1. Experimental Group II – cats with clinical signs of allergic dermatitis, which were administered with product No. 2. Clinical studies of cats in the experimental groups included a detailed anamnesis, study of skin lesions, considering the course of pathological changes. Animals in the control group received dry feed (Ukraine) and clean drinking water without medications. During the experimental period, blood samples were taken from cats for haematological, biochemical, and immunological studies: before medication administration, on Day 1, Day 4, and Day 8 of the experiment. Before and during the experiment, cats were clinically examined, including inspection, palpation, thermometry, examination of respiratory and heart rate. The study recorded the nutritional state, condition of the ears, and visible mucous membranes. The study was conducted using general and special diagnostic methods.

At the second stage of experimental research, the efficacy of veterinary medications was investigated on

outbred dogs of different ages and sexes. To conduct the study, a control and two experimental groups were formed based on the principle of analogues, considering the body weight, age, and type of animal constitution. The number of animals in each group was 5. Animals of the control and experimental groups were kept in enclosures on a standard balanced diet with free access to water, according to physiological needs.

The animals of the control group were clinically healthy dogs aged 1 to 5 years, weighing 7.5-12 kg, vaccinated against canine distemper, adenovirus type II, canine parvovirus, and canine parainfluenza. Prior to the experiment, the dogs were dewormed and underwent coprological tests to rule out the presence of canine endoparasitic diseases. Experimental Group I – dogs with clinical signs of atopic dermatitis, which were administered with product No. 1. Experimental Group II – dogs with clinical signs of atopic dermatitis, which were administered with product No. 2.

Clinical examination of the hair coat of the dogs in the experimental groups included registration of alopecia. The skin thickness, elasticity, dryness, and oiliness were determined. Attention was paid to the potential sites of primary changes in dermatitis of parasitic origin (interdigital area, ventral surface of the body in the abdomen, skin of the ears and external auditory meatus, areas of the head, specifically around the mouth, brow arches, between the auricles; detection of primary skin lesions (papules, pustules), assessment of their location and number.

Dogs in the control group received dry feed and clean drinking water without medications. During the experimental period, blood samples were taken from dogs for haematological, biochemical, and immunological studies: before medication administration, on Day 1, Day 4, and Day 8 of the experiment. Blood for research in dogs and cats was taken from the *vena cephalica antebrachii*. Blood was collected following the rules of asepsis and antisepsis.

To obtain serum, the tubes with blood samples were incubated in a thermostat for 15 min. To separate the blood serum from the clot, a stainless metal stick was used to draw along the inner wall of the tube. The sample tubes were centrifuged at 3,000 rpm for 15 min. Serum was collected using a pipette dispenser into sterile Eppendorf tubes. Haematological tests included determination of total haemoglobin (HGB), haematocrit (HCT), red blood cell (RBC), and white blood cell (WBC) counts. The study of the functional state of the liver and kidneys of cats and dogs included the determination of total protein according to the turbidimetric method, albumin with bromocresol green, urea according to the diacetyl monoxide method, and creatinine with picric acid. The activity levels of the following enzymes were found in the blood serum of cats and dogs: alanine aminotransferase (ALT; GCF 2.6.1.2) and aspartate aminotransferase (AST; GCF 2.6.1.1) according to the

Reitman-Frankel method, gamma-glutamyl transpeptidase (GGTP; GCF 2.3.2.2).

Immunological methods included the determination of the level of total allergen-specific serum Ig E in the blood serum of cats and dogs using the ELISA test system "Granum" (Ukraine). The specified test system uses a sandwich method based on the principle of a two-site enzyme-linked immunosorbent assay. The test sample was introduced into the well of the plate with immobilised antigen (specific Ig E antibodies). Ig E total from the sample binds to antibodies on the surface of the well. Unbound material is removed by washing. Conjugate (second anti-Ig E antibody labelled with peroxidase) is added to the well. Then, the washing was repeated. The activity of the enzyme (bound on the surface of the plate well) was evaluated after substrate addition and measurement at 450 nm. The intensity of the colour reaction was directly proportional to the amount of total Ig E in the sample.

Statistical processing of the research results was carried out using statistical methods (STATISTICA 10.0 for Windows). The results obtained were processed using standard methods of variation statistics with non-parametric and parametric criteria. Numerical data were processed using one-factor analysis of variance (ANOVA). In case of a parametric distribution, the quantitative characteristics of the traits were presented in the form of the arithmetic mean of the sample and the standard deviation ($M \pm m$). Differences between samples were considered significant at $P < 0.05$. Comparison of three or more groups on a quantitative basis with the correct distribution of values was carried out using the Tukey test for subsequent pairwise comparisons of groups, considering the Bonferroni correction (Xu *et al.*, 2010).

All experimental studies were conducted following the modern methodological approaches and corresponding 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

Results of cats' treatment. According to the results of observations, no changes in the clinical condition were detected in the cats of the control group during the experiment. The cats were active, eagerly consumed feed and water, and their nutritional state was average. Visible mucous membranes are pale pink, moderately shiny, without damage, pigmented. Skin was smooth, medium thickness, elastic, undamaged. Coat was thick, shiny, tight against the body. Prior to the experiment, cats in experimental Groups I and II showed clinical signs of allergic dermatitis: the skin on the limbs, neck, and back

was dry, not elastic, the coat was thin, dull, tousled, and not tight against the body. Animals showed constant itching, redness, exfoliation, and soreness of the skin. Two of the cats in the group had cracks, bleeding, and changes in skin pigmentation. The cats' appetite deteriorated compared to the control group. Animals were lethargic and apathetic.

On Day 4 of the experiment, after administration of the experimental medications to cats of Groups I and II, respectively, an improvement in the general clinical

condition of the animals was recorded. The area of the lesion and skin itching decreased in cats. An improvement in appetite was noted. On Day 8, the skin condition of cats in experimental Groups I and II improved. No redness, scratching, exfoliation, or cracking of the skin was observed. Visible mucous membranes were moderately moist, pale pink. Animals' activity increased, they eagerly consumed feed and water. Therewith, a range of morphological parameters of the blood of cats of the control and experimental groups were determined (Table 2).

Table 2. Morphological and physiological parameters of cat blood under the influence of veterinary medications ($M \pm m$, $n=5$)

Experimental groups	Duration of the study, days			
	before administration	Day 1	Day 4	Day 8
Haemoglobin (HGB), g/dm ³				
Group I	120.18±1.21 ^a	121.26±1.48 ^a	122.13±2.14 ^a	122.15±1.58 ^b
Group II	119.15±1.34 ^a	120.14±2.07 ^a	121.17±1.26 ^a	122.06±1.47 ^b
control	124.23±1.11 ^a	125.73±1.13 ^a	125.06±1.41 ^a	125.26±1.31 ^a
Haematocrit (HCT), %				
Group I	40.23±1.19 ^a	37.12±0.45 ^b	37.08±1.19 ^b	36.99±1.43 ^b
Group II	39.32±1.24 ^a	37.96±0.99 ^b	35.87±1.28 ^c	38.25±1.71 ^b
control	37.65±1.23 ^a	36.25±1.32 ^a	37.12±1.11 ^a	38.15±1.18 ^a
Erythrocytes (RBC), 10 ¹² /dm ³				
Group I	8.21±0.68 ^a	8.83±0.45 ^a	8.97±0.18 ^a	9.11±0.21 ^a
Group II	8.27±0.43 ^a	9.11±0.32 ^a	8.58±0.12 ^a	8.94±0.57 ^a
control	8.16±0.23 ^a	8.16±0.13 ^a	8.46±0.98 ^a	9.25±0.67 ^a
White blood cells (WBC), 10 ⁹ /dm ³				
Group I	13.36±0.62 ^a	11.17±0.48 ^b	9.64±0.53 ^c	9.27±0.22 ^c
Group II	11.97±0.24 ^a	10.36±0.62 ^a	9.98±1.12 ^b	8.76±0.74 ^b
control	9.63±0.54 ^a	9.61±0.21 ^a	9.87±0.24 ^a	9.54±0.15 ^a

Notes: different letters indicate values significantly different from each other within one line of Table 3 based on the results of comparison using the Tukey test ($P < 0.05$) with Bonferroni correction

Source: compiled by the authors of this study

Table 2 shows that the veterinary medications under study cause significant changes in haematological parameters in cats when administered orally. A significant improvement in the level of haemoglobin in the blood of cats on Day 8 of the experiment was found (in Group I by 2.03 g/dm³, and in Group II – by 2.91 g/dm³), compared

with this indicator before the administration of medications. On Day 8 of treatment, the count of white blood cells decreased to the normalised level: in Group I – by $4.09 \times 10^9/\text{dm}^3$, and in Group II – by $3.21 \times 10^9/\text{dm}^3$. The study also investigated the dynamics of biochemical parameters in the blood serum of cats (Table 3).

Table 3. Biochemical parameters in the blood serum of cats under the influence of veterinary medications ($M \pm m$, $n=5$)

Experimental groups	Duration of the study, days			
	before administration	Day 1	Day 4	Day 8
Total protein, g/l				
I experimental	76.64±1.21 ^a	75.48±0.46 ^a	76.48±0.94 ^a	77.19±1.02 ^b
II experimental	73.89±0.59 ^a	79.03±0.32 ^b	77.13±0.93 ^b	75.36±1.27 ^b
control	77.65±0.43 ^a	78.64±0.87 ^a	77.24±1.03 ^a	78.51±1.74 ^a
Albumin, g/l				
I experimental	37.9±1.07 ^a	37.16±0.52 ^a	38.25±0.17 ^b	39.24±1.44 ^b
II experimental	36.13±1.21 ^a	38.92±0.37 ^b	38.43±0.5 ^b	39.88±1.72 ^b
control	35.67±1.11 ^a	38.41±0.12 ^b	39.05±0.14 ^b	37.69±0.85 ^a
ALT, IU/l				
I experimental	13.48±1.14 ^a	12.94±0.12 ^a	13.19±1.11 ^a	12.17±0.31 ^a

Table 3. Continued

Experimental groups	Duration of the study, days			
	before administration	Day 1	Day 4	Day 8
II experimental	13.88±0.27 ^a	14.01±0.24 ^a	13.56±0.15 ^a	13.54±1.05 ^a
control	12.17±0.71 ^a	13.92±0.15 ^a	14.17±0.18 ^b	13.25±0.78 ^a
AST, IU/l				
I experimental	29.26±1.48 ^a	28.91±1.53 ^a	29.15±0.51 ^a	30.78±1.47 ^a
II experimental	29.94±0.62 ^a	29.17±0.51 ^a	30.92±1.16 ^a	29.78±0.52 ^a
control	28.14±0.23 ^a	29.56±0.37 ^a	30.24±0.28 ^a	31.74±0.45 ^a
Gamma-glutamyl transpeptidase (GGT), IU/l				
I experimental	5.42±0.83 ^a	5.73±0.12 ^a	5.37±0.22 ^a	6.67±0.29 ^a
II experimental	5.99±0.17 ^a	6.67±0.18 ^a	6.55±0.11 ^a	6.15±0.17 ^a
Control	4.58±0.31 ^a	5.12±0.16 ^a	6.49±0.12 ^b	6.28±0.18 ^b
Creatinine, µmol/l				
I experimental	74.14±1.05 ^a	74.85±0.17 ^a	75.26±0.21 ^a	76.18±1.09 ^b
II experimental	74.97±0.53 ^a	76.01±0.12 ^b	75.17±0.28 ^a	75.53±1.42 ^a
control	73.58±1.06 ^a	75.39±1.25 ^b	74.57±0.45 ^a	74.31±0.75 ^a
Urea, mmol/l				
I experimental	5.12±0.94 ^a	5.19±0.31 ^a	5.63±0.21 ^a	4.18±0.26 ^a
II experimental	5.46±0.17 ^a	5.71±0.19 ^a	6.11±0.37 ^b	5.58±0.15 ^a
control	4.74±0.29 ^a	4.88±0.12 ^a	5.94±0.18 ^a	6.23±0.15 ^b

Notes: different letters indicate values significantly different from each other within one line of Table 3 based on the results of comparison using the Tukey test ($P<0.05$) with Bonferroni correction

Source: compiled by the authors of this study

Table 3 shows that the veterinary medications under study improve the functional state of the liver and kidneys of experimental animals when administered orally. The study found a significant improvement in the level of creatinine in the blood serum of cats on Day 8 of the experiment (in Group I – by 2.04 µmol/l, and in Group II – by 0.56 µmol/l) and albumin (in Group I – by 1.34 g/l, and in Group II – by 3.75 g/l) compared with this indicator before the administration of the medications.

The next step was to conduct immunological studies to determine the concentration of total Ig E in the blood serum of cats that had been administered veterinary medications. It was found that the concentration of total Ig E in the blood serum of cats of Groups I and II was significantly higher than the control values at all study periods: before administration – 3.4 times and 3.9 times, on Day 1 – 3.8 times and 4.3 times, on Day 4 – 2.9 times and 3.1 times, on Day 8 – 2.2 times and 2.1 times, respectively (Fig. 1).

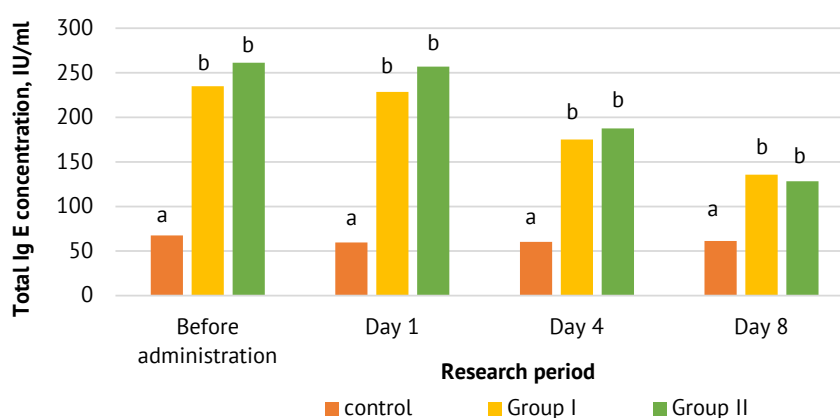


Figure 1. The content of total Ig E in the blood serum of cats after the use of veterinary medications ($M\pm m, n=5$)

Source: compiled by the authors of this study

The increased content of total Ig E in the blood indicates the presence of an allergic reaction in the body of experimental animals. Notably, on Days 4 and 8 after administration of medication No. 1 to cats, the level of Ig E

in the blood of cats of the experimental groups was significantly lower compared to the previous study period.

Results of treatment of dogs. Therewith, clinical trials were conducted on outbred dogs of different ages

and sexes to establish the effectiveness of veterinary medications. The general clinical condition of dogs was investigated; haematological, biochemical, and immunological studies of dogs' blood were performed under conditions of oral administration of a suspension of experimental medications. The clinical condition of the dogs in the control and experimental groups was determined daily throughout the study. Thermometry, examination of visible mucous membranes, examination of the skin and coat were carried out. No changes in clinical condition were observed in the dogs of the control group during the experiment. The animals were active, eagerly consumed feed and water, and their nutritional state was average. Visible mucous membranes are pale pink, moderately shiny, without damage, pigmented. Skin was smooth, medium thickness, elastic, undamaged. Coat was thick, shiny, tight against the body.

Prior to the experiment, dogs in experimental Groups I and II showed clinical signs of atopic dermatitis: hair loss, scabs, itchy skin, which was manifested by scratching, licking, and biting their skin, irritability and changes in habitual behaviour (anorexia, aggressiveness). In the dogs of the experimental groups, the

affected skin areas were recorded in the muzzle, interdigital areas, and the outer surface of the elbow joints. The skin on the limbs, neck, and back was dry, not elastic, the coat was thin, dull, tousled, and not tight against the body. Animals showed constant itching, redness, exfoliation, and soreness of the skin. Two of the dogs in the group had cracks, bleeding, and changes in skin pigmentation. The dogs' appetite deteriorated compared to the control. Animals were lethargic and apathetic.

On Day 4 of the experiment, after administration of the experimental veterinary medications to dogs of Groups I and II in a therapeutic dose, an improvement in the general clinical condition of the animals was recorded. The area of the lesion and skin itching decreased in dogs. There was an improvement in appetite. On Day 8, the skin condition of dogs in experimental Groups I and II improved. No redness, scratching, exfoliation, or cracking of the skin was observed. Visible mucous membranes were moderately moist, pale pink. Animals' activity increased, they eagerly consumed feed and water. In addition, a range of morphological parameters of the blood of dogs of the control and experimental groups were determined (Table 4).

Table 4. Morphological and physiological parameters of dog blood under the influence of veterinary medications ($M \pm m$, $n=5$)

Experimental groups	Duration of the study, days			
	before administration	Day 1	Day 4	Day 8
Haemoglobin (HGB), g/dm ³				
Group I	140.37±3.14 ^a	141.19±3.31 ^a	143.17±1.36 ^a	150.15±2.11 ^b
Group II	139.96±2.31 ^a	141.27±3.14 ^a	142.65±3.01 ^a	148.44±1.26 ^b
control	149.66±1.18 ^a	148.16±4.52 ^a	147.99±2.16 ^a	149.18±1.17 ^a
Haematocrit (HCT), %				
Group I	58.61±1.85 ^a	57.55±1.24 ^b	50.51±1.49 ^b	50.11±1.21 ^b
Group II	56.36±2.87 ^a	54.17±2.91 ^a	51.91±1.83 ^b	49.67±2.31 ^b
control	49.19±2.16 ^a	49.16±1.26 ^a	49.56±1.27 ^a	51.24±1.13 ^a
Erythrocytes (RBC), 10 ¹² /dm ³				
Group I	8.22±0.24 ^a	8.56±0.24 ^a	8.63±0.26 ^a	7.99±0.25 ^a
Group II	9.01±0.31 ^a	8.17±0.23 ^a	8.42±0.19 ^a	8.51±1.23 ^a
control	8.41±0.52 ^a	8.92±0.16 ^a	7.89±0.76 ^a	7.91±1.12 ^a
White blood cells (WBC), 10 ⁹ /dm ³				
Group I	16.28±1.72 ^a	14.83±1.23 ^a	12.93±1.52 ^b	10.47±1.65 ^b
Group II	14.08±1.33 ^a	13.32±1.12 ^a	12.43±0.98 ^a	11.64±1.22 ^b
control	11.12±0.14 ^a	11.52±1.14 ^a	10.15±1.76 ^a	11.33±1.21 ^a

Notes: different letters indicate values significantly different from each other within one line of Table 4 based on the results of comparison using the Tukey test ($P<0.05$) with Bonferroni correction

Source: compiled by the authors of this study

Table 4 shows that the veterinary medications under study, when administered orally, have a positive effect on the haematological parameters of dogs in the experimental groups. On Days 1 and 4 of the experiment, a significant ($P<0.05$) decrease in haemoglobin content was recorded. On Days 4 and 8, this indicator did not differ significantly from the control. In dogs of experimental

Groups I and II, a significant increase in haematocrit content was found before the administration of medications and on Day 1 of the experiment compared to the control. On Days 4 and 8, a significant ($P<0.05$) decrease in this indicator was recorded in dogs of the experimental groups. The dynamics of biochemical parameters in the blood serum of dogs was investigated (Table 5).

Table 5. Biochemical parameters in the blood serum of dogs under the influence of veterinary medications ($M \pm m$, $n=5$)

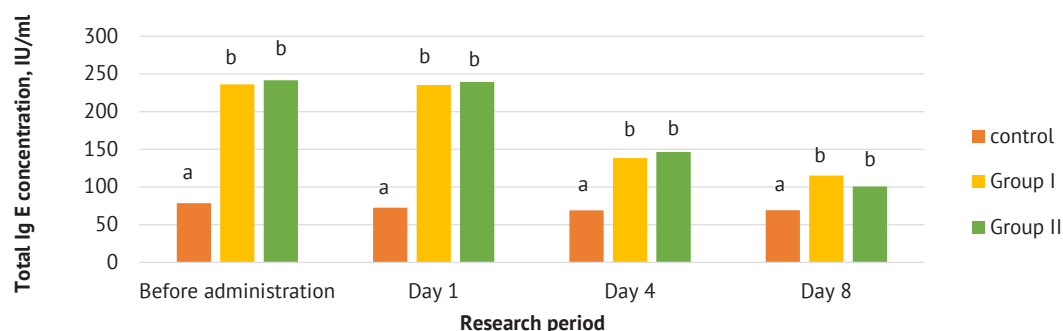
Experimental groups	Duration of the study, days			
	before administration	Day 1	Day 4	Day 8
Total protein, g/l				
I experimental	64.23 $\pm$ 1.96 ^a	63.18 $\pm$ 0.68 ^a	62.94 $\pm$ 0.15 ^a	61.30 $\pm$ 1.53 ^b
II experimental	67.75 $\pm$ 0.14 ^a	67.28 $\pm$ 0.43 ^a	64.54 $\pm$ 1.06 ^b	60.18 $\pm$ 1.19 ^b
control	60.22 $\pm$ 0.17 ^a	61.69 $\pm$ 0.15 ^a	61.95 $\pm$ 1.03 ^a	59.75 $\pm$ 1.16 ^a
Albumin, g/l				
I experimental	41.57 $\pm$ 1.21 ^a	41.99 $\pm$ 0.35 ^a	37.23 $\pm$ 0.18 ^b	36.48 $\pm$ 1.05 ^b
II experimental	39.44 $\pm$ 1.52 ^a	37.41 $\pm$ 0.44 ^a	37.42 $\pm$ 0.21 ^a	36.12 $\pm$ 0.31 ^a
control	35.52 $\pm$ 1.12 ^a	35.48 $\pm$ 0.52 ^a	35.06 $\pm$ 0.57 ^a	36.51 $\pm$ 0.16 ^a
ALT, IU/l				
I experimental	10.55 $\pm$ 1.24 ^a	11.57 $\pm$ 0.52 ^a	10.48 $\pm$ 1.27 ^a	10.28 $\pm$ 0.33 ^a
II experimental	11.22 $\pm$ 0.81 ^a	10.83 $\pm$ 0.45 ^a	11.59 $\pm$ 0.13 ^a	11.87 $\pm$ 1.12 ^a
control	11.12 $\pm$ 0.17 ^a	10.54 $\pm$ 0.15 ^a	11.52 $\pm$ 0.16 ^a	11.27 $\pm$ 0.47 ^a
AST, IU/l				
I experimental	21.16 $\pm$ 1.22 ^a	20.95 $\pm$ 1.47 ^a	19.99 $\pm$ 0.61 ^a	20.55 $\pm$ 1.25 ^a
II experimental	19.89 $\pm$ 0.53 ^a	21.74 $\pm$ 0.63 ^a	21.91 $\pm$ 1.44 ^a	21.44 $\pm$ 0.53 ^a
control	20.14 $\pm$ 0.13 ^a	19.57 $\pm$ 0.29 ^a	20.71 $\pm$ 0.54 ^a	20.73 $\pm$ 0.12 ^a
Gamma-glutamyl transpeptidase (GGT), IU/l				
I experimental	9.58 $\pm$ 0.53 ^a	10.78 $\pm$ 0.21 ^a	8.69 $\pm$ 0.52 ^b	7.32 $\pm$ 0.28 ^b
II experimental	8.18 $\pm$ 0.23 ^a	9.55 $\pm$ 0.42 ^a	6.78 $\pm$ 0.19 ^a	6.96 $\pm$ 0.35 ^a
control	7.28 $\pm$ 0.12 ^a	7.98 $\pm$ 0.15 ^a	7.25 $\pm$ 0.23 ^a	7.28 $\pm$ 0.27 ^a
Creatinine, μ mol/l				
I experimental	74.21 $\pm$ 1.19 ^a	75.15 $\pm$ 0.78 ^a	74.78 $\pm$ 0.37 ^a	75.46 $\pm$ 1.21 ^a
II experimental	74.98 $\pm$ 0.15 ^a	74.21 $\pm$ 0.99 ^a	75.28 $\pm$ 0.69 ^a	74.88 $\pm$ 1.62 ^a
control	73.75 $\pm$ 1.12 ^a	74.36 $\pm$ 1.37 ^a	73.62 $\pm$ 0.73 ^a	74.16 $\pm$ 0.25 ^a
Urea, mmol/l				
I experimental	5.19 $\pm$ 0.32 ^a	6.12 $\pm$ 0.11 ^a	6.75 $\pm$ 0.21 ^a	5.33 $\pm$ 0.12 ^a
II experimental	5.87 $\pm$ 0.23 ^a	5.57 $\pm$ 0.18 ^a	5.78 $\pm$ 0.39 ^a	6.05 $\pm$ 0.67 ^a
control	5.76 $\pm$ 0.28 ^a	5.81 $\pm$ 0.29 ^a	5.65 $\pm$ 0.23 ^a	5.44 $\pm$ 0.15 ^a

Notes: different letters indicate values significantly different from each other within one line of Table 5 based on the results of comparison using the Tukey test ($P < 0.05$) with Bonferroni correction

Source: compiled by the authors of this study

Table 5 shows that the administration of the experimental medications to dogs in therapeutic doses does not have a toxic effect on the functional state of the liver and kidneys. Thus, before administration and on Day 1 of the experiment, a significant ($P < 0.05$) increase in the level of total protein and albumin in the blood serum of dogs was observed. However, on Days 4 and 8

of the experiment, a decrease in these indicators to the control was observed. No significant changes in enzyme activity were recorded in dogs of the experimental groups. Along with this, a study was conducted to determine the concentration of total Ig E in the blood serum of dogs that had been administered veterinary medications (Fig. 2).

**Figure 2.** The content of total Ig E in the blood serum of dogs after the use of veterinary medications ($M \pm m$, $n=5$)

Source: compiled by the authors of this study

Figure 2 shows that the concentration of total Ig E in the blood serum of dogs of Groups I and II was significantly higher than the control values at all study periods: before administration – 3.0 times and 3.1 times; on Day 1 – 3.2 times and 3.3 times; on Day 4 – 2.0 times and 2.1 times; on Day 8 – 1.7 times and 1.5 times, respectively. On Days 4 and 8 of treatment with medication No. 1, the level of Ig E in the blood of the experimental animals was significantly lower compared to the previous study period. Thus, the detection of high concentrations of total Ig E (up to 240...245 IU/ml) in the serum of cats and dogs is a vital indicator that allows differentiating atopy from other pathological conditions clinically manifested by dermatitis.

The clinical manifestations of atopic dermatitis (itching and erythema) can be reliably controlled using complex topical medications containing corticosteroids (triamcinolone acetonide) and B vitamins. As a result of the studies, it was found that the use of veterinary medications (oral suspension) in a dose of 1 mg of triamcinolone acetonide (active ingredient) in cats and dogs has a pronounced anti-allergic effect and improves the condition of the animals' skin already on Day 8 of use.

Ö. Barili and D. Pekmezci (2019) show that in atopic dermatitis in dogs, specific Ig E levels are above the threshold. They note that genetic factors affect the level of Ig E in dogs. Based on clinical studies, it was proved that the concentration of total Ig E in the blood serum of dogs over 130 IU/ml indicates atopic dermatitis. D.T. Morena *et al.* (2021), in their study to identify the most relevant environmental allergens in a population of atopic dogs living in Northern Italy, found that the concentration of total Ig E in the serum of affected dogs was more than 186 IU/ml. These data correlate with the findings obtained in the present study. However, in the studies conducted, the concentration of total Ig E in the blood serum of dogs was 230...245 IU/ml, while in cats – 227...240 IU/ml. It is believed that this is caused by the prolonged absence of treatment of the animals under study in the shelter against the background of a chronic course of the disease.

The publicly available literature data indicate the effectiveness of corticosteroid medications in the treatment of atopic dermatitis, which is the reason for the choice of the active ingredient for the proposed oral suspension. According to P. Chauhan *et al.* (2017), corticosteroid medications in small pets (cats and dogs) have a pronounced local anti-inflammatory and anti-allergic effect, which significantly reduces itching and helps to accelerate the completion of the pathological process in the skin. T.H. Yang *et al.* (2018) shows the effectiveness of using external dosage forms containing fluorinated corticosteroids, specifically triamcinolone. Notably, fluorinated compounds are safer than their non-fluorinated counterparts due to slower resorption through the skin surface. T.J. Nuttall *et al.* (2019) note

that triamcinolone can be used orally, by inhalation, injection (intramuscular, intra-articular, intravitreal), and topically (in the form of ointments, liniments). However, T. Weitzer and R. Mueller (2023) warn that, when administered orally, the medication has a stimulating effect on the secretion of hydrochloric acid and pepsin in the stomach, which may contribute to the development of peptic ulcer disease. In the present study, no adverse reactions to the use of oral suspension from the gastrointestinal tract were found.

M. Fuller (2021) notes the high efficacy of topical use of 0.015% triamcinolone acetonide (GENESIS Topical Spray, Virbac) in atopic dermatitis in dogs. Adverse events of polyuria and polyphagia with this treatment were reported in <6% of dogs. T. Olivry and R. Mueller (2020) noted the efficacy of triamcinolone acetonide for the treatment of feline atopic skin syndrome (FASS) at a dose of 0.18 mg/kg once daily for ≤14 days to achieve remission. V. Bruet *et al.* (2022) reported that topical treatment with a 0.015% triamcinolone acetonide solution applied to the affected skin for 28 days was efficacious and clinical improvement was observed in 67.3% of treated dogs. D.Z. Telci *et al.* (2023) also reported that topical application of 0.015% triamcinolone spray for 28 days was efficacious in the treatment of canine atopic dermatitis. The findings presented in the current study correlate with the data of scientists, however, upon the use of the developed oral suppressants, a stable remission occurs ≤8 days of treatment. It is believed that this is achieved by enriching the study medications (oral suspensions) with B vitamins: vitamin B₁ – 2 mg; vitamin B₂ – 4 mg; vitamin B₃ – 10 mg, and vitamin B₆ – 2 mg were added to the composition of medication No. 1, and vitamin B₆ – 3 mg; vitamin B₂ – 5 mg, and vitamin B₃ – 10 mg were added to medication No. 2. N. Suwannasom *et al.* (2020) have scientifically proven that a lack of B vitamins in cats and dogs leads to the development of dermatitis and seborrhoea. The use of a combination of B vitamins (B₁, B₂, B₃, B₆) in the developed medications was chosen based on the findings of scientific research by P.H. Kook *et al.* (2018), who note that B vitamins affect a range of processes in the body: they are part of enzymes that catalyse the metabolism of carbohydrates, lipids, and proteins, contribute to the normalisation of the metabolism of autonomic centres, the processes of formation, impulse transmission, and neurocyte trophism, and improve the functioning of nerve cells and subcortical centres.

According to scientists, triamcinolone is the main component of the treatment regimen for chronic atopy in small pets, which is a moderately potent corticosteroid according to the European Classification of Corticosteroids, which does not have a negative effect on the animal's body. The developed oral suspension based on triamcinolone (1%) acetonide and enriched with a complex of B vitamins for the treatment of atopy in dogs (0.5...2.0 ml per day) and cats

(0.25...0.5 ml per day) contributed to the improvement of the general clinical condition of animals within a week (on Day 8 of treatment).

CONCLUSIONS

The findings of morphological and physiological parameters of the blood of experimental animals before treatment indicate the presence of an allergic reaction in the body of experimental animals: the increased content of total Ig E in the blood of experimental cats by 3.4....3.9 times, and in dogs – by 3.0....3.1 times compared to the control groups. According to the results of experimental studies of triamcinolone veterinary medications, they are well tolerated by cats and dogs and do not cause changes in the clinical condition of animals. A significant improvement in haemoglobin levels in the blood of experimental animals on Day 8 of the experiment was found: in cats – by 2.47 ± 0.44 g/dm³, and in dogs – by 9.13 ± 0.65 g/dm³.

It was found that the administration of the experimental veterinary medications (oral suspension) to cats and dogs at a dose of 1 mg of triamcinolone acetonide (active ingredient) does not have a toxic effect on the functional state of the liver and kidneys: on Day 8 of treatment, the level of total protein and albumin in the

blood serum decreased to the control level. No significant changes in enzyme activity were recorded in the animals of the experimental groups. It was proved that the medications under study in doses for dogs within 0.5-2.0 ml and for cats within 0.25-0.5 ml per animal, depending on its body weight, exhibit pronounced anti-inflammatory, anti-allergic, and desensitising activity. The prospect of further research is to investigate the recurrence of atopy in the follow-up control using the developed medication.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Ефективність лікування дрібних тварин препаратами на основі триамцинолону за atopічного дерматиту

Анатолій Павлович Палій

Доктор ветеринарних наук, професор
Національний науковий центр «Інститут експериментальної і клінічної ветеринарної медицини»
61023, вул. Пушкінська, 83, м. Харків, Україна
Державний біотехнологічний університет
61002, вул. Алчевських, 44, м. Харків, Україна
<https://orcid.org/0000-0002-9193-3548>

Катерина Олександрівна Родіонова

Кандидат ветеринарних наук, доцент
Одеський державний аграрний університет
65012, вул. Пантелеймонівська, 13, м. Одеса, Україна
<https://orcid.org/0000-0002-7245-4525>

Олена Володимирівна Павліченко

Кандидат ветеринарних наук, доктор юридичних наук, доцент
Державний біотехнологічний університет
61002, вул. Алчевських, 44, м. Харків, Україна
<https://orcid.org/0000-0002-6577-6577>

Андрій Володимирович Телятніков

Доктор ветеринарних наук, доцент
Одеський державний аграрний університет
65012, вул. Пантелеймонівська, 13, м. Одеса, Україна
<https://orcid.org/0000-0002-7772-8679>

Марія Сергіївна Хіміч

Кандидат ветеринарних наук, доцент
Одеський державний аграрний університет
65012, вул. Пантелеймонівська, 13, м. Одеса, Україна
<https://orcid.org/0000-0003-2646-3196>

Анотація. Серед хвороб дрібних домашніх тварин одне з провідних місць займають захворювання шкіри. Атопічний дерматит є поширеним захворюванням шкіри дрібних домашніх тварин, яке, за оцінками ветеринарних лікарів, вражає від 10 до 15 % собак та від 7 до 18 % котів. Залежно від залучених алергенів, клінічні ознаки можуть бути сезонними або несезонними, і захворювання, як правило, вимагає довгочасного лікування. Метою роботи було вивчити терапевтичну ефективність лікування atopії у собак та котів за застосування ветеринарних препаратів (пероральна суспензія) на основі триамцинолону. В роботі використано стандартні клінічні, гематологічні, біохімічні та імунологічні методи дослідження. Підвищений вміст загального імуноглобуліну Е (Ig E) в крові дослідних тварин до початку лікування (I група – у 3,4, а II група – у 3,9 рази у порівнянні з контрольною) свідчить про наявність алергічного процесу в організмі. Встановлено, що апробовані ветеринарні препарати суттєво не впливають на гематологічні показники собак та котів за перорального застосування, у терапевтичних дозах не чинять токсичного впливу на функціональний стан печінки та нирок. Доведено, що на 4-ту добу використання оральної суспензії № 1 та № 2 на основі триамцинолону (1 %) ацетоніду та комплексом вітамінів групи В у терапевтичній дозі реєстрували покращення загального клінічного стану тварин. На 8-му добу лікування у собак та котів I та II дослідної групи встановили покращення стану шкіри: зменшення площі ураження, відсутність почервоніння, розчосів та лущення. Загальний клінічний стан шкіри дослідних тварин з 8-ї до 12-ї доби залишався без змін. Розроблені оральні суспензії проявляють виражену протиалергічну дію та покращують стан шкіри дрібних домашніх тварин (собак та котів) за atopічного дерматиту.

Ключові слова: atopія; імуноглобулін; лікування; суспензія; плазма крові; дослідження; собаки; коти



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Spectrophotometric method for determining the quantity and quality of DNA in animal breeding

Vita Antane

Doctor of Veterinary Medicine, Senior Lecturer
Latvia University of Life Sciences and Technologies
LV-3001, 2 Liela Str., Jelgava, Latvia
<https://orcid.org/0000-0002-9357-9562>

Yktyar Sarybayev*

Doctoral Student
Kazakh National Agrarian Research University
050010, 8 Abay Ave., Almaty, Republic of Kazakhstan
<https://orcid.org/0000-0002-7907-2757>

Askar Osserbay

PhD in Agricultural Sciences, Senior Lecturer
Mukhtar Auezov South Kazakhstan University
160012, 5 Tauke Khan Ave., Shymkent, Republic of Kazakhstan
<https://orcid.org/0000-0003-2885-4940>

Kudratulla Shatmanov

Master of Science, Senior Lecturer
Mukhtar Auezov South Kazakhstan University
160012, 5 Tauke Khan Ave., Shymkent, Republic of Kazakhstan
<https://orcid.org/0009-0002-7644-3310>

Tansyk Baltakhozhayev

Master of Science, Lecturer
Mukhtar Auezov South Kazakhstan University
160012, 5 Tauke Khan Ave., Shymkent, Republic of Kazakhstan
<https://orcid.org/0009-0009-7953-9183>

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Abstract. In animal breeding, genetic methods have become the basis of breeding work and veterinary diagnostics. Therefore, their development and improvement is an actual direction of modern science. The aim of the presented work was to study the concentration and quality of nucleic acids obtained from venous blood of cattle for further genetic studies. For this purpose, a modified method of phenol-chloroform extraction, adapted for DNA extraction from blood, with subsequent

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*Corresponding author

spectrometric determination of DNA concentration and assessment of its quality were applied. As a result of this research, it was found that the average concentration of genetic material isolated from animal blood was 146.5 ± 14.98 ng/ μ L. The main part of samples – more than 93% contained concentration of nucleic acids in the range from 50 to 200 ng/ μ L. At the same time, the time interval between DNA extraction and its spectrometric determination of concentration and quality of genetic material by the ratio of optical density at A260/A280 wavelengths during a year did not have significant changes on its parameters. The used method of nucleic acid extraction in 94% allowed obtaining samples of good quality suitable for further genetic studies. A correlation of 43% ($P < 0.001$) was obtained between the concentration of genetic material and its quality. The coefficients of repetition of intra-laboratory studies of the results of extraction and spectrometric analysis were at the level of 97% ($P < 0.001$), which indicates that this method of obtaining nucleic acids is adapted for its use in animal husbandry. The use of this method of DNA extraction allows obtaining quality material from animals with minimal economic costs for its further use in genetic research

Keywords: genetic material; nucleic acids; optical density of the solution; correlation; repeatability; nucleic acid extraction

INTRODUCTION

Genetic methods have recently gained popularity in animal breeding. Being the most informative and accurate diagnostic methods in determining infectious diseases, they are often used in veterinary practice, as well as in breeding programmes using genomic selection. But for this purpose, only high-quality nucleic acid samples with known concentration are needed, which allows high accuracy of the research. For most medical laboratories, the work of quantifying and characterizing genetic material has already become routine. While in animal breeding, this direction is just developing and therefore needs both research of theoretical bases and practical work on adaptation of this method to physiological parameters of different types of agricultural and domestic animals.

In Kazakhstan, such studies are conducted mainly only at the level of nucleic acid extraction from animals of certain species and determination of concentration and quality of the obtained DNA. Since these parameters determine the further use of such samples for genotyping (Kulibaba *et al.*, 2023). Thus, A. Ibadullayeva *et al.* (2022) conducted experiments to study different methods for the isolation of genetic material from sheep of Kazakhstani Edilbai breed from tissue preparations (pieces of auricle). The use of classical and commercial methods for DNA extraction showed that the salt method allowed obtaining the most suitable material for further studies.

D.I. Kabybekova *et al.* (2023) conducted similar studies. They analysed the quality and concentration of nucleic acids suitable for genetic studies of the equine population in Kazakhstan. Commercial kits were used in these studies, which proved to be effective in isolating genetic material from this animal species. Similar studies were carried out by V.A. Ulyanov *et al.* (2023). The object of the study was a wild saiga species. In this work, only commercial kits were used for the isolation of nucleic acids from skin and its derivatives, with subsequent assessment of efficiency by

electrophoresis. Similar work has also been carried out by K.B. Adyrbekova and K.B. Isbekov (2022) on isolation of DNA-material of fish for subsequent genetic study. In this work, a comparative analysis of DNA obtained by two methods adapted for aquaculture was also carried out.

Considering that in the Republic of Kazakhstan there is quite a large population of cattle, accordingly, and with this kind of animals were carried out similar work. Sh.D. Orkara *et al.* (2022) investigated the effect of two commercial foreign kits for DNA extraction from cow hair follicles and the phenol-chloroform method for subsequent genotyping by nucleotide polymorphisms markers (SNPs). As a result of this study, the authors concluded that both methods provided genetic material suitable for further work. Unlike the other method, the use of the PureLink™ Genomic DNA Mini Kit provided high quality nucleic acids, which contributed to a more accurate result. The only disadvantage of this study was the limited number of animals involved in the study – only 8 animals. Thus, experiments on isolation of genetic material from cattle in Kazakhstan are rather limited and need further research. This is facilitated by significant selection work in cattle breeding, aimed at studying and evaluating productive qualities of dairy animals to determine breeding value.

In this work, more in-depth studies were conducted on the influence of several conditions in the isolation of DNA from blood cells from cows, covering a significant population and considering laboratory errors. In this connection, the aim of the work was the spectrophotometric determination of qualitative and quantitative characteristics of genetic material isolated from the blood of cattle using the phenol-chloroform method.

LITERATURE REVIEW

Nucleic acid extraction is an important component of genetic analysis. According to M.N. Emaus *et al.* (2020), insufficient purification of nucleic acids during their

extraction inhibits amplification of genetic material and reduces the sensitivity of the method. Therefore, numerous methods have been developed to simultaneously obtain and purify genetic material. The most commonly used chemical methods for extraction of nucleic acids from tissues in laboratories are the centrifugation in caesium chloride gradient with ethidium bromide (Nasir *et al.*, 2017); phenol-chloroform extraction and its modifications (Piskata *et al.*, 2019; Roy *et al.*, 2020); cetyltrimethylammonium bromide (CTAB) extraction (Kouakou *et al.*, 2022), chelate extraction using Chelex resin (Simon *et al.*, 2020; Gautam, 2022); alkaline extraction (Dairawan & Shetty, 2020), and one of the most recent developments is the purification method using cellulose chromatography (Baierdörfer *et al.*, 2019). Each of the above methods has advantages and disadvantages.

According to the information of A. Ibadullayeva *et al.* (2022), the advantage of chemical methods is the relatively inexpensive price of reagents, but their significant toxicity and low efficiency compared to commercial kits gradually reduces their use. To reduce the negative impact of reagents and to improve the quality of extracted genetic material, some authors carry out several modifications. Thus, K.P. Bojang *et al.* (2021) indicated that of the four modifications of the CTAB protocol, the simplest method was the most effective. Not the last factor in using different methods is the labour intensity of their application.

Almost all the above methods are quite labour-intensive and unsafe for the health of the personnel who perform them. Therefore, recently, most laboratories and genetic companies use ready-made commercial kits to obtain and purify native nucleic acids (Yevstafieva *et al.*, 2019). But even commercial kits differ in the amount of DNA isolated from the sample and its quality. Thus, in the work of C. Butler *et al.* (2022), authors extracted nucleic acids from milk using four kits, followed by spectrophotometric control. This work confirmed differences in DNA amounts between kits. The Norgen Biotek test kit provided significantly higher DNA concentration measured at ratios of 260/280 and 260/230. Therefore, it is better to use kits from reputable genetic companies to obtain a higher result. Thus, in the work of S. Tsuji *et al.* (2019) the DNeasy Blood and Tissue DNA extraction reagent kits DNeasy Blood and Tissue DNA extraction Kit and PowerWater DNA Extraction Kit from the German company Qiagen were the most efficient for animal DNA extraction.

In addition, commercial kits contain the most novel and effective approaches that allow obtaining the highest quality samples of genetic material (Shahsavari *et al.*, 2022). Thus, the use of magnetic beads in modern kits, according to P. Oberacker *et al.* (2019), provide high performance in obtaining plasmid, genomic and total DNA and RNA regardless of the origin of the material. In a study by V. Ooi *et al.* (2023),

the authors produced amplification results from low cellular material comparable to DNA extraction from skin using a novel eluent "High Volume-Cetyltrimethyl Ammonium Bromide-Phenol-Chloroform-Isoamyl Alcohol" (HV-CTAB-PCI).

Given the considerable variability between methods in the isolation, sequencing and bioinformatic approaches to assessing genetic material, K. Greathouse *et al.* (2019) found that it was the extraction methods that made the most noticeable differences. This influenced the work to investigate phenol-chloroform extraction of DNA from ruminant blood and to determine the laboratory bias in the studies.

MATERIALS AND METHODS

The experimental part of the work was carried out in 2021-2022. For this purpose, laboratory determination of quality and concentration of DNA isolated by phenol-chloroform extraction method from 137 samples from cattle blood was performed. Blood samples for the study were taken from productive dairy cows in "Baiserke-Agro" LLP, Talgar district, Almaty region, Republic of Kazakhstan. Blood was collected from the tail vein into vacuum tubes "Acti-Fine®" with anticoagulant. Ethylenediamine tetraacetic acid (EDTA), which is recommended in protocols of work with nucleic acids as a specific anticoagulant to reduce the error in the quantitative determination of DNA and RNA, was used as a blood clotting agent.

The extraction of genetic material was carried out using the phenol-chloroform extraction method with slight modifications. For this purpose, 300 µl of lysing buffer based on Tris-HCl and proteinase K from Quai-gen Company were added to a microtube with 100 µl of whole blood. Subsequently, it was thermostated for 3 hours at 56-58°C. A Thermal Mixer from Thermo Scientific (USA) was used for this purpose. After incubation, 400 µl of phenol-chloroform-isoamyl alcohol mixture in the ratio of 1:1:1 was added to the solution and stirred. After 15-20 minutes, centrifugation was carried out at 6000 rpm for 10 minutes using mySPIN 12 centrifuge from Thermo Scientific (USA). Using a micropipette, the supernatant was collected and transferred to a new tube. An equal volume of phenol-chloroform mixture with isoamyl alcohol was added, and the above procedure was repeated 3-4 times.

Cold (-20°C) 96% ethanol alcohol was added to the obtained supernatant and the tube was left at -20°C for 20 minutes in the freezer, partial precipitation of DNA was observed. To accelerate this process, centrifugation at 6000 rpm for 10 minutes was performed. The supernatant was removed using a micropipette and the precipitate was washed several times with 400-500 µl of 70% ethyl alcohol solution. After completion of washing, ethanol was removed as much as possible using a micropipette, and its residue in the tube was allowed to volatilize by thermostating with the lid open at 37°C.

The resulting genetic material was further diluted using DNA dilution buffer from Evrogen.

DNA quality and concentration were checked and evaluated in the laboratory of Green Biotechnology and Cell Engineering of the Kazakhstan-Japan Innovation Centre at the Kazakh National Agrarian University. For this purpose, spectrophotometric method of DNA concentration determination by the level of absorption of certain wavelengths using Nanodrop 2000 spectrophotometer according to the instrument operation manual was used (Thermo Fisher Scientific, 2009). Qualitative and quantitative evaluation of DNA isolated was carried out in a 2 μ l sample at 20°C at 230, 260 and 280 nm. The studies were carried out on the next day after the isolation of genetic material, two months later and one year later. The results of measurements from the instrument database were transferred to a spreadsheet format for further statistical processing in Tibco Statistica 14.0.1 software. For this purpose, using the

methods of mathematical-statistical analysis and descriptive statistics, the influence of the optical density of samples with the concentration and quality of nucleic acids for further generalization, preparation of conclusions and recommendations for subsequent works were calculated.

RESULTS

For the study, 155 venous blood samples were collected from clinically healthy, productive dairy cows. As a result of extraction, nucleic acids were obtained from only 137 samples. Part of samples, through blood coagulation, which occurred during transport and storage of samples did not allow extraction of genetic material from them, so they were excluded from further work. As a result of this research, an average of 146.5 ± 14.98 ng/ μ l of total nucleic acid concentration (DNA and RNA combined) was extracted from cattle blood. The limits ranged from 31.5 to 1114.4 ng/ μ l (Fig. 1).

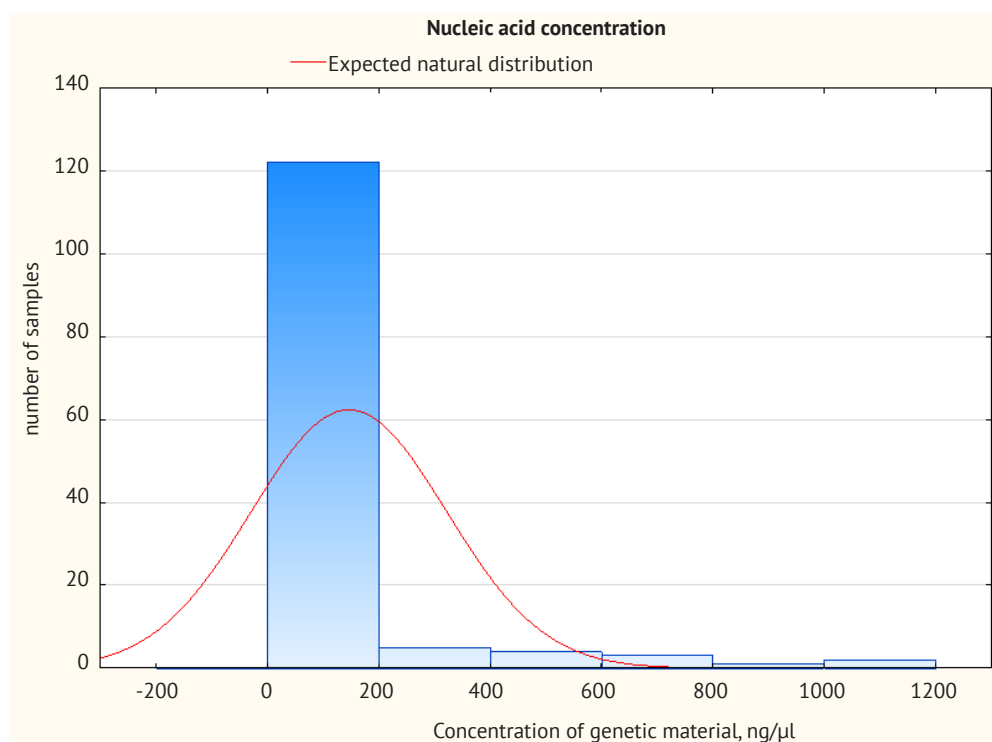


Figure 1. Graph of distribution of blood samples depending on the isolated concentration of nucleic acids

Source: compiled by the authors

The obtained graph, considering the grouping of sample results by DNA concentration, corresponds to a normal distribution. Considering that nucleic acids in blood cells are contained only in leukocytes, and their content in blood varies widely among animals even within a species, it is worth expecting certain fluctuations in DNA concentration in samples, which should correlate with the amount of this fraction of formational elements in controlled animals. In addition, the

question of the maximum possible DNA yield and DNA loss during isolation by different methods remains open. Most samples had nucleic acid concentrations between 50 and 250 ng/ μ l. When the initially non-natural results were discarded and only averaged values within three sigma were left, this graph allowed the determination of the modal class and associated classes indicating the nucleic acid content of bovine blood (Fig. 2).

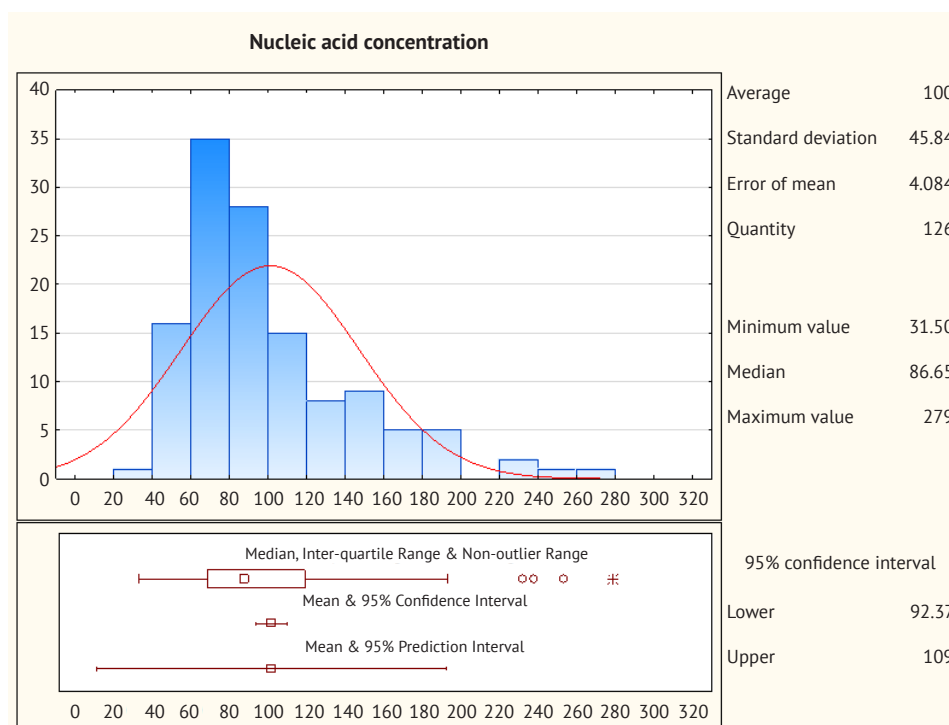


Figure 2. Graph of the distribution of the number of samples depending on the concentration of nucleic acids
Source: compiled by the authors

The modal class was samples with a concentration of genetic material between 50 and 100 ng/μl, which corresponded to 59.5% of the results of all studies. Furthermore, samples with concentrations of 100 to 150 ng/μl and 150 to 200 ng/μl were classified as high percentage classes, which accounted for 20.6% and 12.7%, respectively. These three classes totalled about 93%. Therefore, it can be stated that for clinically healthy cattle, the value of natural nucleic acid concentration will be between 50 and 200 ng/μl. Measurement of the optical density of solutions with extracted nucleic acids was carried out in several

steps. The first study was carried out the day after the extraction of genetic material, for this purpose, only a minor part of samples was analysed, all the rest were stored in a freezer at -20°C. Most samples (n=82) were analysed two months after extraction from biological material. This was due to the recommendations of some researchers to keep the solution with nucleic acids after extraction for some time. And the third stage of the analysis was already connected with determining how long storage affects the preservation of genetic material. The graphical results of this study are presented in Figure 3.

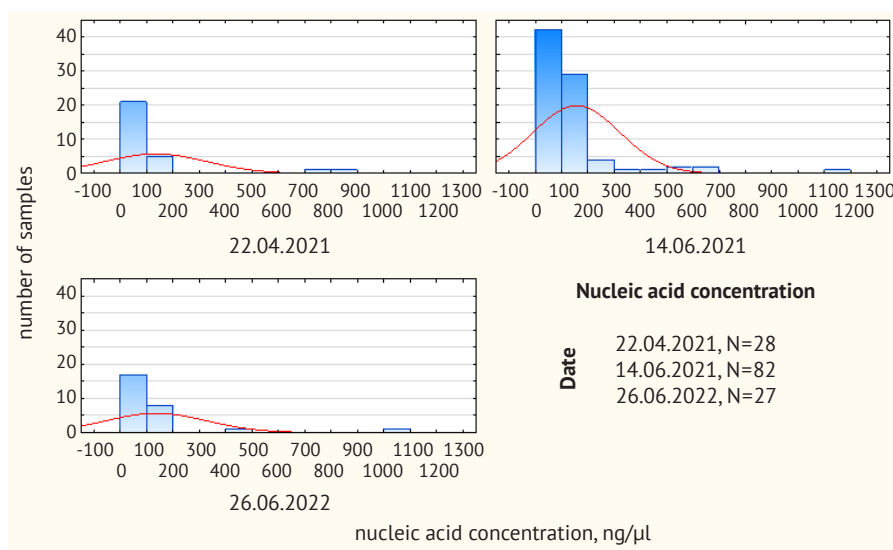


Figure 3. Concentration of nucleic acids in samples depending on storage time after extraction
Source: compiled by the authors

As follows from the presented graphical material, regardless of storage time, the profile of nucleic acid content remained practically the same and corresponded to

a normal distribution. It is noteworthy that in all studies, the bulk of samples contained the concentration of genetic material at the level of 120-150 ng/ μ l (Table 1).

Table 1. Results of determining DNA concentration depending on sample storage time

Date of the study	N, samples	M $\pm$ m, ng/ μ l	Min, ng/ μ l	Max, ng/ μ l
22.04.21	28	133.6 $\pm$ 36.78	31.5	830
14.06.21	82	154.8 $\pm$ 18.15	48	1114.4
26.06.22	27	134.8 $\pm$ 36.92	44.3	1019.1

Note: Min – minimum value; Max – maximum value

Source: compiled by the authors

As a result of the work, there was a slight increase in DNA concentration two months after its isolation, but this could be the result of a simple increase in the number of measurements (more than 3 times) due to the averaging of results. But despite this increase in DNA values, the difference between the groups was not significant ($P>0.1$). Nevertheless, this nuance needs to be further investigated and tested on a larger sample. The conducted studies indicate, during the year, there was almost no change in the concentration of nucleic acids during its storage. This insignificant difference between the results of their measurement in samples allows using extracted DNA in this interval, without loss of informativeness, for genetic research in animal breeding.

To check the quality of the genetic material extracted from blood, already proven methods that are based on the ratio of the optical density of the solution at wavelengths of 260, 280 and 230 nm were used. This approach is related to the properties of nucleic

acid molecules to absorb and reflect waves of different intensities. The most recommended method is the difference of indices at wavelengths 260 and 280. The A260/A280 ratio for pure nucleic acids is in the range of 1.8-2. The absorption optimums are about 1.8 for DNA and about 2 for RNA. Values less than 1.8 indicate incomplete purification of the preparation and residual concentrations of protein molecules. The presence of samples with a ratio higher than 2 indicates inadequacy of the reagents used or excessive physical strain, which causes DNA degradation and, as a consequence, the presence of free nucleotides in the solution. Since in the research the determination of not pure fractions of nucleic acids, but their mixture was carried out, the optimal difference was set between 1.7 and 1.9. All samples with the value of the absorbance difference falling within this range were considered suitable for further genetic study. All other values were grouped in the ratio less than 1.7 and greater than 1.9 (Fig. 4).

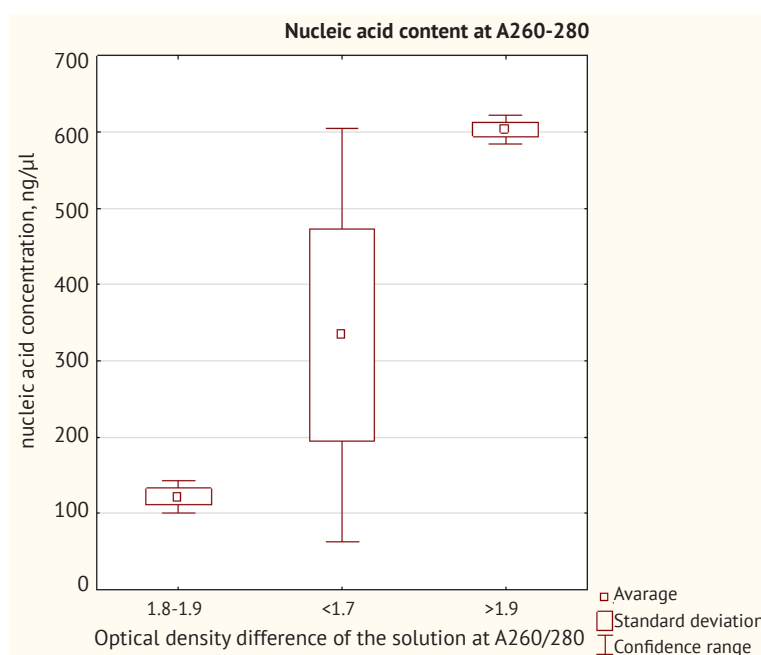


Figure 4. Graphic display of the distribution of nucleic acid samples depending on the difference in optical density at wavelength A260/A280

Source: compiled by the authors

As can be seen from the presented graphs, the amount of nucleic acids varied greatly in samples with a density difference of less than 1.7 A260/A280, which

may have been a marker of impaired extraction of genetic material. Whereas in other groups no such variation was observed (Table 2).

Table 2. Indicators of the concentration of nucleic acids in samples with different optical densities at wavelength A260/A280

Optical density at A260/A280	n, samples, (%)	M±m, ng/μl	Standard deviation	Minimum value, ng/μl	Maximum value, ng/μl
Less than 1.7	9 (7%)	334.1±138.5	415.5	31.5	1114.4
1.7-1.9	125 (91%)	122.1±10.7	119.8	44.3	412.3
More than 1.9	3 (2%)	603.8±9.6	16.7	588.8	621.8

Source: compiled by the authors

At values of optical density A260/A280 above 1.7, a direct correlation between optical density and DNA concentration in the sample was clearly observed, with the correlation coefficient between these parameters being 0.43 ($P<0.001$). Whereas in samples with optical density lower than 1.7, the variation of the concentration of genetic material ranged from 31 to 1114 ng/μl, and these were the borderline values from the array of all studies conducted. Therefore, samples with optical density below 1.7 are not recommended for use in further genetic studies.

Based on the rather high statistical informativeness of the A260/A280 ratio indicator in the evaluation system of extracted genetic material, analysis of variance was performed to clarify the strength of the effect of the amount of nucleic acids in solution on its optical difference at A260/A280 wavelengths. To obtain a more reliable value, samples that, initially, were considered not logical were also excluded. It is worth noting that this group did not include all samples with an optical difference below 1.7. As a result of the single-factor analysis of variance, a reliable influence of the factor of nucleic acid concentration and quality on the indicator of its optical density was found, with an influence power of 24.4% ($P<0.001$; $F=21.6$). Another indicator used in the analysed

literature to characterize the quality of nucleic acids is also the ratio of the optical density of the solution at A260/A230 wavelengths. In this study, the variation of this ratio was between 0.65 and 1.83, while the average ratio was 1.2 ± 0.02 . This result was significantly different from that reported in the literature, where the value of this ratio is between 1.8 and 2.2. The analysis of this situation may be related to the use of a new DNA dilution buffer from Evrogen. Therefore, it is planned to compare the difference of solution optical density at A260/A230 using different buffers for nucleic acid dilution at the last extraction step in the next studies.

One more area of work was to investigate the repeatability of nucleic acid concentration results when extracting nucleic acids from the same sample. This was made possible through coagulation of blood in several tubes during transport of samples to the laboratory. Therefore, this portion of the samples was replaced with samples from tubes where there was sufficient biological material to duplicate the extraction process of genetic material. This approach resulted in the extraction of nucleic acids from 5 samples. The extraction of genetic material was carried out in parallel at the same time to avoid errors in the procedure. The results of such work are presented in Table 3.

Table 3. Results of parallel extraction of nucleic acids from samples of biological material

Sample number	Ind. animal number	First sample		Second sample	
		Concentration of n.a.	A260/A280	Concentration of n.a.	A260/A280
83	147	50.8	1.79	54.3	1.78
60	723	59.1	1.76	62.2	1.82
107	758	127.2	1.85	80.7	1.83
66	856	125	1.84	72.3	1.83
81	498	83.4	1.79	152.1	1.81

Note: n.a. – nucleic acids

Source: compiled by the authors

The presented tabular material indicates that there was no significant difference between the nucleic acid concentration indices. At the same time, the genetic material, according to the results of optical density difference at A260/A280 wavelengths,

was of very high quality in both variants. Therefore, the repeatability of results between parallel studies was determined by correlation analysis. As a result, a correlation coefficient of 0.97 ($P<0.001$) was obtained (Fig. 5).

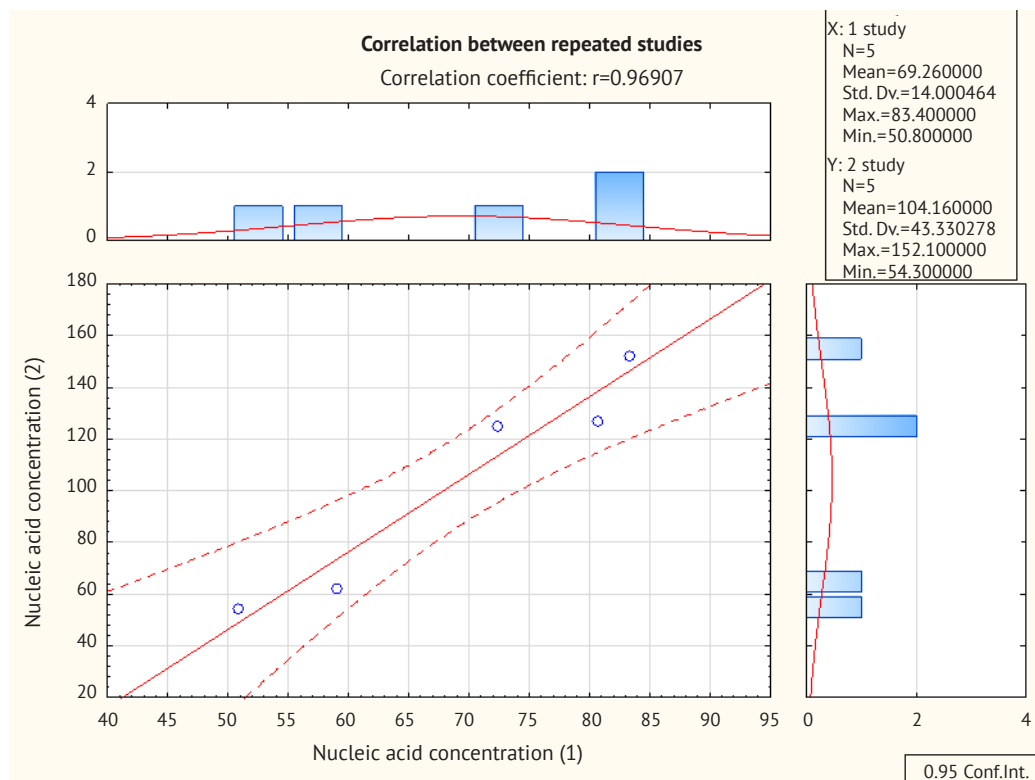


Figure 5. Results of correlation analysis of repeated studies of nucleic acid extraction from blood samples

Source: compiled by the authors

The above studies indicate that the method of phenol-chloroform extraction of nucleic acids can be successfully used to work with biological material from animals. The obtained material has the necessary concentration and quality for subsequent genetic studies.

DISCUSSION

Modern livestock breeding increasingly uses genetic methods in breeding work and veterinary diagnostics. And, in this regard, the isolation of genetic material is a priority task in genetic research. Venous blood was chosen as an object for isolation of nucleic acids. This was due to the versatility of this body system for carrying out diagnostics of the animal's condition. It is used for clinical diagnostics of the physiological state of the organism, immunological testing and other examinations. Any tissues of the organism containing cells are suitable for DNA isolation. In most of the analysed studies, skin derivatives (hair follicles, skin biopsy pieces) or tissue pieces left after breeding manipulations (ear plucking) were used to isolate genetic material. The use of blood is associated with a more labour-intensive process of its collection, but considering that such manipulations are carried out at least twice a year (according to veterinary and sanitary rules of research of productive cows, whose products are used for food preparation) such material for genetic research becomes the most universal object in animal breeding, well, or in dairy cattle breeding at least (Króliczewska *et al.*, 2023).

The phenol-chloroform method for nucleic acid extraction was used in the research because it is reported by researchers such as S.A. Sakyi *et al.* (2023) to be much cheaper than other commercial methods. And in addition, it allows maximizing the yield of DNA from the sample. This is especially relevant given the relatively low concentration of leukocytes in the blood, almost the only mature cells with nuclei, which are the carriers of DNA. When nucleic acid extraction was performed from cow blood, an average of 146.5 ± 14.98 ng/ μ l DNA per sample was isolated. This result was significantly higher than in the study of M. Akinwale and A. Babarinde (2019), A. Ibadullayeva *et al.* (2022), in which the amount of genetic material was only 43.4–48.5 ng/ μ l when DNA was extracted from ear pluckings by the same method. This difference is quite significant and can be related only to the number of cells that contain nucleic acids. Since in the ear pluck a significant part of the tissue is represented by cartilaginous base, in which DNA is practically absent, the concentration of genetic material was reduced. This could be the explanation for the different concentration of genetic material extracted using the same method. Moreover, that when nucleic acids were extracted from ear pluck by other methods, the concentration of DNA was found to be about the same level as with the phenol-chloroform method, namely 50–61.2 ng/ μ l.

But in the studies of J. Carvajal-Agudelo *et al.* (2021) on the extraction of nucleic acids from blood of wild an-

imal species using the phenol-chloroform method and other commercial kits, lower levels of nucleic acids up to 25 ng/ μ L were also obtained. In these studies, dried blood samples from dogs were used for analysis, which could significantly affect the number of cells with nuclei. By drying the blood, some leukocytes may have broken down, and this could have affected the isolated DNA concentration in the dried blood (Mazur *et al.*, 2022). This assumption was partially confirmed in the study of H. Mayta *et al.* (2019) who performed DNA extraction from whole blood and clotted blood samples. As a result of such work, a greater concentration of genetic material was extracted from blood clotted blood as the concentration of cells passed in them, but the cost of extraction also increased significantly. It follows from this that, regardless of the state of blood tissues, it can be used for isolation of genetic material, but the highest yield of nucleic acids with the lowest financial costs can be obtained from whole or stabilized blood (Irgashev *et al.*, 2020).

Very different results for DNA extraction from animal blood were obtained in the work of L. Le Clercq *et al.* (2023). In their study, using a commercial Extract-Now™ Blood DNA Mini Kit, the concentration of nucleic acids from blood was obtained at the level of 4 ng/ μ L, which corresponded to 4000 ng/ μ L, which is an order of magnitude higher than the results obtained using the phenol-chloroform method. Therefore, such differences in the concentration of genetic material obtained from the studies of the above authors and those obtained in this article remain unresolved and need to be investigated more thoroughly. In particular, this will be facilitated by studying the influence of the leucocyte content in blood on the concentration of nucleic acids during its extraction. This direction will be a priority in future works.

But no less important factor than the quantity of genetic material obtained from a biological sample is its quality. In the conducted studies, the only factor that could testify to the quality of the obtained nucleic acids was the difference in the optical density of the solution at wavelengths 260, 280 and 230 nm. As a result of the analysis, the quality control of the isolated DNA was carried out by the A260/A280 ratio. In this case, 93% of samples had quality parameters that meet the requirements for genetic testing. This result indicates that the method used for DNA extraction from such a biological object as blood is quite suitable. In similar studies conducted by Z. Piskata *et al.* (2019) indicate that when the phenol-chloroform method was used, the number of samples having the criterion as qualitative by A260/A280 ratio was also more than 90%. Whereas, when commercial nucleic acid extraction kits were used, this control method was not effective for several kits. Therefore, the optical density at A260/A230 was also used to control the quality of the extracted genetic material. This method, which is also

used for spectrometric quality control in the conducted studies, was at the level of 1.2 ± 0.02 , while the norm is 1.8-2 (as an indicator of solution purity without protein molecules admixture). In the works, where the method of phenol-chloroform extraction was also used by G. Koetsier and E. Cantor (2019), A. Sophian (2021) the optical density ratio at A260/A230 was also below the recommended level of 1.24 and 0.81-0.87, respectively. Therefore, it is worth revisiting the compliance rates for the quality of extracted DNA in spectrometric analysis.

The laboratory reproducibility of the results is also an important factor. In this work, a correlation analysis method was applied between adjacent samples from the same biological sample. Such work resulted in a very high repeatability coefficient of 0.96 and statistically significant ($P < 0.001$). Unfortunately, there were no similar works that studied the reproducibility of DNA extraction results from animal blood, but this assumption is confirmed in the work of A.J. Sepulveda *et al.* (2020), where very high repeatability rates of 91 and 92% were also obtained for DNA isolation from molluscs using a commercial Qiagen kit. But in contrast to such optimistic results, D. Burke *et al.* (2022) point out that when the same methods and test kits were used in two different laboratories, the results of quantitative isolation of genetic material differed significantly. Therefore, works on the unification of methods and their protocols are still quite relevant in genetic research not only in animal breeding, but also from the biological perspective in general.

In conclusion, it should be noted that the use of phenol-chloroform extraction of nucleic acids from animal blood is an effective, inexpensive, and reproducible method within the laboratory, which is reasonable to use in genetic studies in animal breeding.

CONCLUSIONS

The results of the application of phenol-chloroform method of extraction of genetic material from cattle blood and its further spectrometric analysis allow drawing the following conclusions and developing several directions for further works. The use of phenol-chloroform method of extraction of nucleic acids from such biological object as venous blood of cattle allowed isolating DNA in a concentration 146.5 ± 14.98 ng/ μ L. At the same time, 93% of samples had concentration from 50 to 200 ng/ μ L.

Spectroscopic analysis with such time intervals as one day, two months and one year after extraction allowed stating that there were no significant deviations in the concentration of genetic material and its qualitative characteristics. No significant difference was observed between the groups. This indicates that the genetic material can be used for one year without loss of informativeness. The quality of the isolated genetic material determined by measuring the ratio of optical absorption density at A260/A280 wavelengths

indicates that more than 90% of the isolated samples were qualified as qualitative and suitable for genetic studies. The correlation between DNA concentration and quality was 43% ($P < 0.001$). Intra-laboratory repeatability of the results of genetic material extraction and its spectroscopic study carried out from the same biological samples indicated a high correlation of 97% ($P < 0.001$). Such repeatability of results indicates the efficiency of the method for its use in animal husbandry when working with blood.

The most promising directions for future work are to determine the influence of leukocyte concentration

in blood sample on the results of nucleic acid extraction and to study the influence of different buffers for DNA dilution on optical density at wavelengths of 260 and 230 nm for further quality control of genetic material. These areas of research will increase the informativeness and reliability of the method in animal breeding.

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CONFLICT OF INTEREST

None.

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Спектрофотометричний метод для визначення кількості та якості ДНК у тваринництві

Віта Вітаутівна Антане

Доктор ветеринарної медицини, старший викладач

Латвійський університет біонаук та технологій

LV-3001, вул. Лієла, 2, м. Єлгава, Латвія

<https://orcid.org/0000-0002-9357-9562>

Іктіяр Уалібекович Сарibaєв

Докторант

Казахський національний аграрний дослідницький університет

050010, просп. Абая, 8, м. Алмати, Республіка Казахстан

<https://orcid.org/0000-0002-7907-2757>

Аскар Жанабайули Оссербай

Кандидат сільськогосподарських наук, старший викладач

Південно-Казахстанський університет імені Мухтара Ауезова

160012, просп. Тауке Хана, 5, м. Шимкент, Республіка Казахстан

<https://orcid.org/0000-0003-2885-4940>

Кудратулла Кочкарович Шатманов

Магістр, старший викладач

Південно-Казахстанський університет імені Мухтара Ауезова

160012, просп. Тауке Хана, 5, м. Шимкент, Республіка Казахстан

<https://orcid.org/0009-0002-7644-3310>

Тансик Рамаданович Балтахожаєв

Магістр, викладач

Південно-Казахстанський університет імені Мухтара Ауезова

160012, просп. Тауке Хана, 5, м. Шимкент, Республіка Казахстан

<https://orcid.org/0009-0009-7953-9183>

Анотація. У тваринництві генетичні методи стали основою селекційної роботи та ветеринарної діагностики. Тому їх розвиток та удосконалення актуальний напрямок сучасної науки. Метою представленої роботи стало вивчення концентрації та якості нуклеїнових кислот отриманих з венозної крові великої рогатої худоби для подальших генетичних досліджень. Для цього було застосовано модифікований метод фенол-хлороформної екстракції, адаптований для виділення ДНК із крові, з подальшим спектрометричним визначенням концентрації ДНК та оцінкою її якості. У результаті проведених досліджень було встановлено, що середня концентрація генетичного матеріалу, виділеного з крові тварин, становила 146.5 ± 14.98 нг/мкл. Основна частина зразків – понад 93 % містила концентрацію нуклеїнових кислот в інтервалі від 50 до 200 нг/мкл. При цьому часовий інтервал між екстракцією ДНК та її спектрометричним визначенням концентрації та якості генетичного матеріалу за співвідношенням оптичної густини за хвиль A260/A280 упродовж року не спричиняв суттєвих змін на її показники. Використаний метод виділення нуклеїнових кислот у 94 % дав змогу одержати зразки доброї якості, придатні для подальших генетичних досліджень. Між концентрацією генетичного матеріалу та його якістю отримано кореляційний зв'язок на рівні 43 % ($P < 0,001$). Коефіцієнти повторення внутрішньо лабораторних досліджень результатів екстракції та спектрометричного аналізу виявилися на рівні 97 % ($P < 0,001$), що вказує на адаптованість цього методу отримання нуклеїнових кислот для його використання у тваринництві. Застосування цього способу екстракції ДНК дало змогу з мінімальними економічними витратами отримати якісний матеріал від тварин, придатний для його подальшого використання в генетичних дослідженнях

Ключові слова: генетичний матеріал; нуклеїнові кислоти; оптична густина розчину; кореляція; повторюваність; екстракція нуклеїнових кислот



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Ecological plasticity and sustainability of cotton in the Southern Steppe of Ukraine

Raisa Vozhehova

Doctor of Agricultural Sciences, Professor, Academician
of the National Academy of Agrarian Sciences of Ukraine

Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine
67666, 24 Mayatska Doroga Str., Khlybodarske Village, Ukraine
<https://orcid.org/0000-0002-3895-5633>

Vira Borovyk*

PhD in Agricultural Sciences, Senior Researcher

Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine
67666, 24 Mayatska Doroga Str., Khlybodarske Village, Ukraine
<https://orcid.org/0000-0003-0705-2105>

Sergii Zaiets

Doctor of Agricultural Sciences, Senior Researcher

Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine
67666, 24 Mayatska Doroga Str., Khlybodarske Village, Ukraine
<https://orcid.org/0000-0002-3895-5633>

Leonid Serhieiev

PhD in Agricultural Sciences

Odesa State Agricultural Research Station

Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine
67666, 24 Mayatska Doroga Str., Khlybodarske Village, Ukraine
[https:// orcid.org/0000-0003-4169-8938](https://orcid.org/0000-0003-4169-8938)

Inna Kohut

PhD in Agricultural Sciences, Associate Professor

Odesa State Agricultural Research Station

Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine
67666, 24 Mayatska Doroga Str., Khlybodarske Village, Ukraine
<https://orcid.org/0000-0002-4418-5954>

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Abstract. Depending on the length of the cotton growing season, the area of cultivation and its highest productivity potential are determined, and the prompt ripening of raw cotton allows for home-grown harvesting and high-quality soil preparation. The purpose of this study was to identify samples adapted to the conditions of the Southern

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*Corresponding author

Steppe of Ukraine from the cotton gene pool. The study used methods of plant variety expertise: phenological observations, morphological studies, and evaluation of breeding material for valuable traits. Based on the study results, parental components were selected to expand the process of forming early-ripening forms and productivity of raw cotton. The ability of cotton plants to grow in extreme conditions of the Southern Steppe of Ukraine was assessed and its homogeneity or stability, the index of growing conditions, the coefficient of variation – the difference in the numerical values of the trait duration of the period “germination – beginning of flowering” of plants in varieties with different growing season and their fluctuations around the average value were determined. It was found that according to the determined plasticity (b_i), the variance of stability (S_i^2), and the coefficient of variation of the duration of “germination – the beginning of flowering” higher tolerance to environmental factors was characterised by ultra-early ripening varieties. The average regression coefficient (b_i) was -0.36; the variation in the duration of the period by year was 10.1%, which is significantly lower compared to early-ripening varieties – -0.77% and 12.9%, mid-ripening varieties – -0.80% and 15.0%, and late-ripening varieties – -1.30% and 16.6%, respectively. The maximum average productivity to frosty raw cotton of 49.4 g/plant was formed by the sample Pidzerskyi 4 (UF0800003), the duration of the period “germination – beginning of flowering” was 47 days, and “germination – full ripening” – <103 days. The lowest raw cotton productivity of 21.2 g/plant was demonstrated by the late-ripening sample Joloten 32 (IU14056549) of Turkmen selection, the duration of the growing season of which was 145 days on average over the years of research. The findings of this study will be used in further breeding to create high-yielding cotton varieties with increased environmental plasticity and stability and high-quality fibre

Keywords: cotton samples; duration of the growing season; ripeness groups; plasticity; stability; coefficient of variation; productivity

INTRODUCTION

The length of the growing season depends on a range of properties that affect the formation of the variety's yield, its adaptive response to adverse environmental factors, the manifestation of disease symptoms, pest damage, and product quality. In this regard, it is necessary to create crop varieties, and cotton specifically, such that the length of the growing season determines the area of introduction of the variety and contributes to its maximum productivity.

The findings of long-term research by V. Borovyk *et al.* (2022) of the Institute of Climate-Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine report that only early-ripening forms of cotton ripen under low temperature conditions. Thus, the extremely cold year of 1993, with a sum of effective temperatures above 10°C of 1397°C, had a positive impact on the differential strict culling of the collection material. In fact, only 30% of Bulgarian cotton varieties have been recorded as ripening before frost. They believe that the lack of heat in cotton cultivation in the Southern Steppe of Ukraine is one of the factors that affects the maturation of long-growing cotton. A. Hamid *et al.* (2020) and S. Majeed *et al.* (2021) suggest growing early-ripening varieties in such areas as well as in mountainous terrain.

The early ripeness of a variety, i.e., its ability to produce the bulk of the crop in a short time, is considered to be a valuable feature that determines the quantity and quality of the raw cotton harvest. O. Skripka *et al.* (2021) found that the onset of growth and development phases and their duration is determined by the biological characteristics of the variety

and the agroclimatic conditions of the growing area. However, I. Njouenwet *et al.* (2021) note that high rainfall prolongs the duration of cotton developmental stages and shortens them under dry conditions.

J.L. Snider *et al.* (2022) proved that low temperature during the late growth period not only affects the time of ripening, but also leads to a decrease in yield. M. Yaşar (2023) points out that the length of the cotton growing season is an economic trait. According to M. Yan *et al.* (2021), it determines the degree to which plants adequately perceive changes in environmental conditions and depends on both it and genetic characteristics. One of the principal tasks of cotton breeding in the Southern Steppe of Ukraine is to increase the resistance of varieties to extreme environmental conditions, the level of genotype response to changing factors of growing conditions, and the selection of the best combinations of source material. M. Mubarik *et al.* (2020) argue that the attention of scientists is constantly drawn to the genotype-environment interaction, which is determined by plasticity and stability.

Ecological plasticity is the degree to which a trait responds to changes in environmental conditions, which is manifested in phenotypic variability; and ecological stability is the ability to form high productivity even under the influence of abiotic and biotic stresses, when environmental conditions change from optimal. According to S.I. Zandalinas *et al.* (2018), the “variety” factor is considered as a constant value, while abiotic factors can be weather conditions in the years of research, fertiliser doses, and sowing dates.

According to the findings of N. Leshchuk *et al.* (2021), the magnitude of the response of genotypes to changes in abiotic factors is determined by the coefficient of ecological plasticity (b_i), which indicates the deviation of individual sample parameters in relation to the average long-term indicators. The b_i value is proportional to the sensitivity of the variety to changes in environmental conditions. More often than not, the b_i coefficient is positive, and sometimes it is negative. Y. Huang *et al.* (2023) argue that when the regression coefficient b_i is zero or close to it, it indicates that the variety is weakly or not responsive to abiotic environmental factors. P. Arnold *et al.* (2019) report that the plasticity trait is an independent value and is controlled by specific genes. Stability variance – S_i^2 demonstrates how exactly the tested variety fits the plasticity determined by the regression coefficient b_i . Zero and close to zero values of S_i^2 show that there is the smallest difference between the empirical value of the characteristic and the theoretical one.

The simultaneous action of the traits “plasticity” and “stability” of the samples helps the genetic mechanism of plants to maximise the effects of the negative impact of abiotic factors, i.e., to show resistance to them. Thus, the purpose of this study was to identify samples from the cotton collection that are resistant to abiotic factors for further use in breeding.

MATERIALS AND METHODS

The study was conducted at the Institute of Climate-Smart Agriculture of the National Academy of Agrarian Sciences (NAAS) of Ukraine in 2012–2016. The subject of the study was 282 samples of cotton from the collection nursery of different ripeness groups. The agrotechnical conditions of the experiments were generally accepted for the Southern Steppe of Ukraine and were conducted on dark chestnut medium loamy slightly saline soils. Its predecessor is winter wheat. 100 kg/ha of ammonium nitrate was applied for pre-sowing cultivation. Sowing was carried out in the first decade of May, when the soil temperature at a depth of 5 cm reached 18–20°C. The plots of the collection nursery are single-row, 3 m long, with a distance of 15 cm in a row between plants. A standard was placed every 9 samples, which was the zoned cotton variety Dniprovskiy 5 of the Institute of Climate-Smart Agriculture of the NAAS of Ukraine. During the growing season, the crop was irrigated twice at a rate of 400 m³ of water per hectare.

The trait assessment was carried out according to the methodology of Volkodav (2001). The morphological description and classification according to economic properties were made according to the “Broad Unified Classifier – Handbook of the genus *Gossypium hirsutum* L.” (Vozhehova *et al.*, 2015). The coefficient of variation V (%), an indicator of the standard deviation of features, which is given as a percentage of

the mean value, was calculated using the following formula:

$$V = b/m \times 100, \quad (1)$$

where V is the coefficient of variation, %; b is the standard (square) deviation; m is the arithmetic mean. If $V < 10\%$, the variation is considered weak, if $V = 11\text{--}25\%$, it is considered medium, and if $V > 25\%$, it is considered significant.

The weather conditions during the years of the study were typical for the Southern Steppe zone of Ukraine, and therefore an objective assessment of the cotton gene pool was carried out, and valuable samples were identified for their economic characteristics. The year 2012 was the warmest on record, driven by high average monthly temperatures in May (20.8°C), June (23.4°C), and July (23.6°C). Overall, the summer of 2012 was hot, with a shortage of rainfall and soil and air drought throughout the season.

The summer of 2015 was moderately hot, and the summer of 2016 was dry due to lower average monthly air temperatures than in 2012, by 3.8–4.6°C in May and by 3.2–2.2°C in July, respectively. Among the years under study, 2013 saw an extended growing season for cotton plants due to precipitation in June, which amounted to 79.1 mm and 64.4 mm, respectively.

According to the length of the growing season from germination to full ripeness, according to the 9-point scale of the Broad Unified Classifier-Guide to the genus *Gossypium hirsutum* (L.) distinguishes cotton plants: ultra-early ripening, or with a very short vegetation period <110 days (1 point), 111–115 (2 points), early-ripening – short vegetation period 116–120 (3 points), 125–130 (4 points), mid-ripening – medium 131–135 (5 points), 136–140 (6 points), late-ripening – long 140–145 (7 points), 146–150 (8 points), and very late-ripening – very long vegetation period >150 days (9 points). According to the germination–flowering period, cotton plants are divided into 5 groups: very short – <50 days (1 point), short – 50–55 days (2 points), medium – 55–60 days (3 points), long – 65–70 days (4 points), very long – >70 days (5 points) (Vozhehova *et al.*, 2015).

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 Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS AND DISCUSSION

The analysis of 282 collection samples by the duration of the cotton plant vegetation period showed that 29.0% of them were characterised by a very short ripening period, 25.1% – by a short one, 27.9% – by a medium one, and 18.0% – by a long one (Table 1).

Table 1. Distribution of cotton gene pool samples according to the duration of the growing season, days (2012-2016)

Sign and level of its expression	Total weight		Sample name
	pieces	%	
Very short (ultra-early ripening) <110-115 days	81.8	29.0	Dniprovskiy 5 (UF0800009), Pidozerskiy 4 (UF0800003), L 417u (UF0800002), 500u (UF0800000), 510u (UF0800226), Population 1 (UF0800193), Population 2 (UF08000020), Population 3 (UF0800002), Population 3 opr. (UF080018), 170/2 (UF0800039), 2342u (UF0800032), Od 4 (UF0800119), K58 (UF0800114), K67 (UF0800036), K103 (UF0800118), K11 (UF0800107, 1718u (UF0800239) (UKR); 144F (UF0800105), 1135/94 (UF0800204), (UZB); 534 (UF0800535) (RUS); 4521Trakia (UF080006), Interspecific hybrid W64 (UF0800165), Avangard 264 (UF0800020), 32-73 (UF0800262) (BGR), etc.
Short (early-ripening) 116-125 days	70.8	25.1	L 191/13 (UF080028), 501 (UF0800221), 2307u (UF0800227), Population 5 (UF0800035), Line 452u opr. (UF0800190) (UKR); 1135/94 (UF0800204) (UKR); Sizhi 2 (UF0800098), 175F (UF0800063), Andijan 5 (UF0800071), Liniya 446 (UF0800086) (UZB); Beli Izvor 432 (UF0800015), Chirpan 603 (UF0800001), Chirpan 539 (UF0800018), Helius (UF0800277), Ogosta 644 (UF0800017), Ogosta 644 opr. (UF0800181), Balkan 442 (UF0800178), Garant opr. (UF0800180) (BGR); Zeta 2 (UF0800057) (GRC); Maria (UF0800056), Makedonka 21 (055UF0800), Siocra 1-4-C (UF0800046), 115P (UF0800052), Plovdiv 15-81 (UF0800234) (YUG), Zun-Zhong (UF0800003) (CHN), etc.
Medium (mid-ripening) 126-135 days	78.7	27.9	An-Chillyaki (UF0800012), Andijan 4 (UF0800070), C4727 (UF0800067), C7085 (UF0800127), Namangan 77 (UF0800 064), (UZB); A-2 (UF0800054) (YUG); 611B (UF0800033) (RUS); Acra 60 (UF08001490), Acala 90 (UF0800159), Tomcot (UF0800157), Tomcot 1074 (UF0800160), Tomcot 2073 (UF0800158), Tomcot CAMDE (UF0800159), Deltapine ch5 (UF0800167), Delfos 53 (UF0800172), Cocer 100 (UF0800230) (USA); T 073 (UF0800125) (ROU); Varamin (UF0800053) (IRN); Simbaves (UF0800146) (ZWE), etc.
Long (late-ripening) 136-145 days	50.7	18.0	Kyrgyz 6-84 (UF0800082) (KGZ); Tabladilla 16 (UF080014), T 100 (UF080001) (ESP); Nazili 84 (UF0800058) (TUR); Cotton (UF0800279), Joloten 32 (IU14056549) (TKM); Argentina 1 (UF0800147), Argentina 2 (UF0800009), Argentina 3 (UF0800148), Fizinerdo (UF0800007) (ARG); S9 (UF0800024) (SYR), etc.
Total:	282	100	

Source: compiled by the authors of this study

When studying the trait “duration of the growing season” of cotton gene pool samples, not only “germination-full ripeness”, but also “germination-beginning of flowering” were considered – the phase of active growth and development of the plant, formation of fruiting organs, since, according to U. Khan (2003), it is in this phase that the value of the future yield is determined. The shortest germination – flowering period, which lasted from 47 to 50 days, was observed in ultra-early ripening samples in 2012, and slightly

longer – in 2015 (49-52 days) and 2016 (48-51 days). A longer growing season was observed in 2013 (48-52 days) and 2014 (49-53 days). Based on the results of the observations, the environmental plasticity and stability, the index of growing conditions, and the coefficient of variation of the traits of the collection samples by the duration of the period “germination-flowering” of plants in varieties with different growing seasons: ultra-early ripening, early-ripening, mid-ripening, and late-ripening (Table 2).

Table 2. The influence of biotic factors of the year on samples of the cotton gene pool in terms of ecological plasticity (b), stability (S_i^2), the index of growing conditions (I), and the coefficient of variation (V)

Sample name	Duration of the period “germination – beginning of flowering”, days					$\bar{X}$	V, %	Indicators	
	2012	2013	2014	2015	2016			b_i^*	S_i^2
Ultra early-ripening – <110-115 days									
Pidozerskiy 4 (UF080003) (UKR)	46	47	48	48	47	47	5.4	-0.07	1.94
Interspecific hybrid W64 (UF0800165)	49	51	52	49	50	50	9.1	-0.35	5.12
L 417u (UF080002) UKR	46	49	49	48	47	48	10.5	-0.31	5.20
4521Trakia (UF080006) BGR	48	51	50	51	48	50	10.2	-0.38	4.99
1135/94 (UF0800204) UZB	48	51	51	49	49	50	15.4	-0.65	8.75
$\bar{X}$	47	50	50	49	48	49	10.1	-0.36	5.20

Table 2. Continued

Sample name	Duration of the period "germination – beginning of flowering", days					X	V, %	Indicators	
	2012	2013	2014	2015	2016			b_i^*	S_i^2
I_j							–	–	–
Early-ripening – 116-125 days									
Beli Izvor 432 (UF0800015) (BGR)	51	56	55	54	54	54	9.1	-0.43	4.54
Ogosta 644 opr. (UF0800181) (BGR)	51	55	57	55	52	54	10.3	-0.51	0.49
501 (UF0800221) (UKR)	53	53	55	53	53	53	13.7	-0.64	2.13
Makedonka 21 (055UF0800) (YUG)	50	55	58	54	51	54	16.9	-1.09	1.12
175F (UF0800063) (UZB)	52	58	58	55	55	56	14.7	-1.16	1.02
$\bar{X}$	52	56	57	55	53	54	12.9	-0.77	1.86
Mid-ripening – 126-135 days									
C7085 (UF0800127) (UZB)	55	55	58	56	55	56	11.3	-0.52	4.50
T 073 (UF0800125) (ROU)	56	63	62	62	59	60	18.9	-1.17	1.81
An-Chillaki (UF0800012) (UZB)	57	56	63	59	57	58	14.7	-0.70	2.05
$\bar{X}$	56	60	61	59	57	59	15.0	-0.80	2.79
I_j							–	–	–
Late-ripening – 136-145 days									
Tabladilla 16 (UF080014) (ESP)	66	69	71	68	66	68	12.9	-0.68	5.75
Cotton (UF0800279) (TKM)	67	70	71	69	67	69	18.5	-1.59	3.62
Joloten 32 (IU14056549) (TKM)	70	73	76	72	72	73	16.2	-1.28	4.30
Nazili 84 (UF0800058) (TUR)	69	72	73	71	71	72	18.7	-1.53	5.72
$\bar{X}$	68	71	73	70	69	70	16.6	-1.30	4.85
I_j							–	–	–
LSD 05	6.4								

Note: * b_i is the coefficient of regression (plasticity); S_i^2 is the stability variance; I_j is the index of environmental conditions; $\bar{x}$ is the average duration of the period; V is the coefficient of variation

Source: calculated by the authors of this study

The results of the study presented in Table 2 show that according to the obtained data of plasticity (b_i), variances of stability (S_i^2) and coefficient of variation, cotton plants of the ultra-early ripening group in the phase "germination – the beginning of flowering" were less sensitive to abiotic factors. The average regression coefficient (b_i) was -0.36; the variation in the length of the growing season by year was 10.1%, which is significantly less compared to early-ripening varieties – -0.77% and 12.9%, mid-ripening varieties – -0.80% and 15.0%, and late-ripening varieties – -1.30% and 16.6%, respectively.

Among the varieties of the ultra-precocious group, the sample Pidozerskyi 4 (UF080003) (UKR) was more adapted to growing conditions (b_i = -0.07; S_i^2 = 1.94; V = 5.4%), among the early-ripening – Beli Izvor (UF0800015) (BLG) (b_i = -0.43; S_i^2 = 4.54; V = 9.1%),

among the mid-ripening – C7085 (UF0800127) (UZB) (b_i = -0.52; S_i^2 = 4.50; V = 11.3%), late-ripening – Tabladilla 16 (UF080014) (ESP) (b_i = -0.68; S_i^2 = 5.75; V = 12.9%). Sample of 1135/94 (UF0800204) (UZB) (b_i = -0.65; S_i^2 = 8.75; V = 15.4%) was the most sensitive to growing conditions among the ultra-early ripening varieties, the early-ripening – Makedonka 21 (055UF0800) (YUG) (b_i = -1.09; S_i^2 = 1.12; V = 16.9%); mid-ripening – T 073 (UF0800125) (ROU) (b_i = -1.17; S_i^2 = 1.81; V = 18.9%), and late-ripening – Cotton (UF0800279) (TKM) (b_i = -1.59; S_i^2 = 3.62; V = 18.5%).

The maximum average productivity to frosty raw cotton of 49.4 g/plant was formed by Pidozerskyi 4 (UF0800003) (UKR), whose duration of the period "germination-beginning of flowering" was 47 days, and "germination-full ripening" – <103 days. The lowest raw

cotton productivity of 21.2 g/plant was demonstrated by the sample of the late-ripening group Joloten 32 (IU14056549) (TKM), the duration of the growing season of which was 145 days on average over the years of research. Particular attention was paid to samples of ultra-early and early-ripening groups, as the main obstacle to the introduction of cotton in the Southern Steppe of Ukraine is the limit of temperature conditions, as mentioned earlier.

Among the ultra-early ripening group, there were some varieties with shorter or equal vegetation periods

than the standard variety Dniprovskiy 5, which contributed to the formation of high productivity, these are Pidozerskiy 4 (UF0800003) (UKR), 144 F (UF0800062) (UZB), L 417u (UF0800002), 500u (UF0800000), 510u (UF0800226), Population 1 (UF0800193), Population 2 (UF0800020), Population 3 opr. (UF080018), Population 9 (UF0800241), 3737u (UF0800228) (UKR); 4521Trakia (UF0800006), Interspecific hybrid W64 (UF0800165), Avangard 264 (UF0800020) (BGR), etc., that were used in hybridisation as the mother form in most cases in both direct and back crosses (Table 3).

Table 3. Duration of the growing season of parental forms involved in hybridisation for early ripening and productivity (2012-2016)

Sample name, No. in the National Catalogue	Country of origin	Duration of the growing season, days		Productivity	
		days	V, %	g/plant	V, %
Ultra-early ripeness group (<110-115 days)					
Dniprovskiy 5 (UF0800009) St	UKR	107	8.2	42.6	26.9
Pidozerskiy 4 (UF0800003)	UKR	103	5.7	49.4	22.8
144 F (UF0800062)	UZB	102	5.2	39.6	24.8
L 417u (UF0800002)	UKR	104	6.9	46.8	26.0
500u (UF0800000),	UKR	104	6.9	46.9	20.1
510u (UF0800226)	UKR	105	5.4	47.1	26.2
Population 1 (UF0800193)	UKR	105	5.1	46.2	26.8
Population 2 (UF0800020)	UKR	103	6.9	46.9	22.3
Population 3 opr. (UF080018)	UKR	104	4.4	45.1	20.1
Population 9 (UF0800241)	UKR	105	3.0	38.9	22.4
3737u (UF0800228)	UKR	105	2.5	39.8	18.8
4521Trakia (UF0800006)	BGR	103	4.5	39.5	30.5
Interspecific hybrid W64 (UF0800165)	BGR	103	6.9	41.1	37.5
Avangard 264 (UF0800020)	BGR	104	6.8	38.9	36.7
OD 4 (UF0800119)	UKR	114	4.5	39.7	22.4
K58 (UF0800114)	UKR	115	3.5	39.9	35.2
K67 (UF0800036)	UKR	114	4.6	39.1	36.4
K103 (UF0800118)	UKR	113	5.1	39.8	31.0
K11 (UF0800107)	UKR	115	5.8	38.9	24.5
Early-ripening group (116-125 days)					
1135/94 (UF0800204)	UZB	116	5.3	37.9	23.8
Line 452u opr. (UF0800190)	UKR	116	3.1	39.2	37.1
501u (UF0800221)	UKR	117	2.2	47.1	39.9
Beli XXXIzvor 432 (UF0800015)	BGR	117	4.2	36.8	30.6
Chirpan 603 (UF0800001)	BGR	116	3.6	37.1	42.4
Chirpan 539 (UF0800018)	BGR	116	3.4	36.7	32.4
Population 5 (UF0800035)	UKR	116	4.5	36.8	35.6
L 191/13 (UF0800283)	UKR	116	5.1	37.5	23.1
Maria (UF0800056)	YUG	118	4.6	41.1	31.1
Garant opr. (UF0800180)	BGR	118	4.7	35.9	33.6
Ogosta 644 (UF0800017)	BGR	117	3.7	35.8	32.1
Balkan 442 opr. (UF0800178)	BGR	116	3.5	36.7	37.8
Helius (UF08000277)	BGR	117	5.4	37.4	39.1

Table 3. Continued

Sample name, No. in the National Catalogue	Country of origin	Duration of the growing season, days		Productivity	
		days	V, %	g/plant	V, %
Zong Zhong (UF0800003)	CAN	123	6.1	35.8	32.8
2307u (UF0800227)	YUG	125	2.6	36.2	26.6
Line 446 (UF0800086)	UZB	125	3.6	37.8	31.0
175F (UF0800063)	UZB	125	5.1	36.1	27.5
Population 3 (UF0800002)	UKR	125	4.3	47.8	33.8
Siocra 1-4-C (UF0800046)	AUS	125	4.6	35.4	32.1
Plovdiv 15-81 (UF0800234)	BGR	125	3.7	35.7	31.0
Zong Zhong (UF0800003)	CHN	125	4.8	25.6	31.7
Makedonka 21 (055UF0800)	YUG	125	5.7	43.1	38.3
Mid-ripening group (125-135 days)					
Acala 90 (UF0800159)	USA	128	5.4	30.7	31.5
Tomcot 1074 (UF0800160)	USA	135	6.2	29.2	36.8
Cocer 100 (UF0800230)	USA	132	9.1	31.1	22.1
611b (UF0800033)	RUS	132	3.5	31.8	35.0
An-Chillaki (UF0800012)	UZB	131	3.8	31.5	12.0
A-2 (UF0800054)	YUG	134	6.3	25.5	22.2
Andijan 4 (UF0800070)	UZB	134	5.2	25.2	22.1
Delfos 53 (UF0800172)	USA	137	6.2	26.0	21.8
Simbabves (UF0800146)	ZWE	135	5.9	25.1	22.8
T100 (UF0800014)	ESP	132	6.2	25.2	40.1
Late-ripening group (136-145 days)					
Nazili 84 (UF0800058)	TUR	144	4.3	25.0	22.5
Cotton (UF0800279)	TKM	145	4.5	24.1	36.7
Joloten 32 (IU14056549)	TKM	145	4.8	21.2	22.1
Argentina 1 (UF0800147)	ARG	142	10.1	24.8	21.0
Fizinerdo (UF0800007)	ARG	144	5.2	24.8	12.1
Kyrgyz 6-84 (UF0800082)	KGZ	141	4.8	24.7	32.1
LSD ₀₅		0.25		1.8	

Source: compiled by the authors of this study

Samples of the collection with a vegetation period of more than 131 days, which were distinguished by lower plant productivity, but had a large boll, high-quality fibre, and were characterised by a lower coefficient of variation over the years than early-ripening varieties, were used as parents, including Tomcot 1074 (UF0800160), Acala 90 (UF0800159), Cocer 100 (UF0800230) (USA), 611b (UF0800033) (UKR), An-Chillaki (UF0800012) (UZB), A-2 (UF0800054) (YUG), Andijan 4 (UF0800070), Delfos 53 (UF0800172), Simbabves (UF0800146), T100 (UF0800014), Nazili 84 (UF0800058) (TUR), Cotton (UF0800279), Joloten 32 (IU14056549) (TKM), Argentina 1 (UF0800147), Fizinerdo (UF0800007) (ARG), Kyrgyz 6-84 (UF0800082) KGZ, etc. Thus, when creating new cotton varieties, samples with the traits “ultra-early ripeness” and “early ripeness” with high productivity were used as the mother form, and samples of mid- and late-ripening groups, but with a large boll and high-quality fibre, were used as the father form.

Among the local breeding samples, Dniprovskiy 5 and Pidozerskiy 4 are noteworthy – these are varieties developed at the Institute of Climate-Smart Agriculture of the NAAS of Ukraine for the Southern Steppe of Ukraine. They are characterised by a short ripening period and high yield potential of up to 3.2 t/ha. Their short ripening period allows them to be used even as a precursor for sowing winter crops. These two varieties are listed in the Catalogue of Crop Varieties and Hybrids of the Institute of Irrigated Agriculture (now the Institute of Climate-Smart Agriculture) (2019).

The average duration of the period “germination-full ripeness” of the samples of the early ripeness group was 9-8 days longer than that of the ultra-early ripeness group, but the variation during the study was much smaller and the coefficient of variation was 2.2-6.1% versus 2.5-8.2%. As for the years of the study, in 2013 and 2014, the longest “germination-flowering” period was observed in plants, regardless of the ripeness

group, including 47-50 days in ultra-early ripening samples, 56-57 days – in early-ripening samples, 60-61 days – in mid-ripening and 71-73 days in late-ripening samples, respectively. The “germination-flowering” period was the shortest in all samples of the cotton gene pool in 2012 – 47, 52, 56, and 68 days, respectively, in the ripeness groups.

Summarising the results of determining the duration of vegetation periods over the years of research (2012-2016), it can be noted that the “germination-beginning of flowering” period of samples of the late-ripening group was 21 days longer than that of the ultra-early ripening group. In addition, samples of this ripeness group were characterised by a higher coefficient of variation over the years – 16.6% versus 10.1%. The “germination-full ripeness” phase in late-ripening samples occurred 38 days later than in ultra-early ripening samples.

To create new varieties, ultra-early ripening samples from Bulgaria were used for crossing – 4521Traikia (UF0800006), Interspecific hybrid W64 (UF0800165), Avangard 264 (UF0800020), which were 3-4 days ahead of Dniprovskiy 5 in terms of the total growing season with a lower coefficient of variation – $V=4.5-6.9\%$, compared to 8.2% for Dniprovskiy 5 (UF0800009). In terms of productivity, they had the same value as the standard, but differed by a higher coefficient of variation over the years – from 30.5% to 37.5% , which is almost one and a half times lower than the adaptability of the standard variety.

Among the early-ripening group of gene pool samples, the best was the promising line 501u (UF0800221) of Ukrainian selection, which flowered, on average over the years of research, in 53 days with a coefficient of variation of 13.7% , which is 6 days later than the average performance of ultra-early ripening samples. The total vegetation period of the sample was 10 days longer, but it stood out for the highest productivity (47.1 g/plant), compared to the standard Dniprovskiy 5 (UF0800009) – 42.6 g/plant. Therefore, this sample was actively used in backcrosses.

Table 3 shows samples of the gene pool used in crosses to develop more productive ultra-early and early ripening varieties. As a result of the hybridisation, more than 24 promising cotton lines were created, characterised by a short ripening period and high yields of home-grown medium-fibre raw cotton – $2.4-3.2$ t/ha – and meeting the quality indicators of type V fibre. The cotton gene pool is a valuable source for developing varieties adapted to the extreme conditions of the Southern Steppe of Ukraine. Climate change towards warming is helping it to take a leading position in the risky farming zone among conventional crops.

For the introduction of cotton in the Southern Steppe of Ukraine, early-ripening varieties are needed. Cotton production in this zone is closely linked to meteorological conditions. Manufacturers in other countries face similar problems. C. Li *et al.* (2021) argue that

weather conditions are region-specific, and with regard to climate change over the past 50 years and frequent extreme events, they advise that, after assessing their impact on cotton production in different locations, regionally adaptive varieties should be used.

R. Baumhardt *et al.* (2018) emphasise that air temperature is a crucial factor affecting the development of cotton. They have shown through research that low temperatures ($<12^{\circ}\text{C}$) at an early stage lead to stunted growth and development, while hot temperatures ($>35^{\circ}\text{C}$) at the mid-growth stage can negatively affect the fertilisation process and lead to bud drop. Low temperatures during the late growth period prolong the ripening period, which leads to a decrease in yield.

Cotton is a perennial indeterminate crop, and cultivated species are generally not sensitive to photoperiodicity. Therefore, S. Minoli *et al.* (2019) and Y. Jans *et al.* (2021) argue that higher temperatures will increase the rate of plant development, but not necessarily shorten the length of the growing season, if temperature seasonality is the limiting factor and sufficient water and nutrients are available. Although the plant is widely adapted to a variety of environments, the growth and demand for water for cotton irrigation may be challenged by future climate change.

The data obtained from the study showed that in the Southern Steppe of Ukraine, hot temperature conditions not only accelerate plant development, but also affect the reduction of plant maturation. Characteristic of this zone is that early ripening forms of the collection material generate higher productivity than plants with a long growing season. This is caused by a higher number of open pre-frost bolls. Samples of the late-ripening ripeness group form a high total yield of cheese, i.e., home-grown and post-frost, but they are inferior to early-ripening forms in terms of home-grown cheese yield.

Other conclusions based on the study results were made by C.I. Cevheri (2021), who states that higher yields were obtained by introducing varieties with a long growing season. Ensuring crop ripeness is essential for yield maximisation, quality, and economic return. According to L.-M. Chevin and A.A. Hoffmann (2017), plasticity may be non-adaptive to extreme conditions if there are no strong genetic correlations between extreme and non-extreme environmental conditions and the optimal phenotype changes smoothly under its influence. When phenotypic plasticity is adaptive, it can withstand the harmful effects of extreme conditions.

Organismal plasticity can be considered as the response of cotton to environmental conditions or to biological and developmental traits. It helps plants maintain homeostasis in response to changing climatic conditions. According to F.A. Dar *et al.* (2022), the action of biotic factors that cause plastic reactions can to some extent affect the development and timing of the formation of reproductive organs. In the Southern Steppe zone of Ukraine, where the sum of positive temperatures

above 10°C can drop to 1397°C, with a norm of 1492°C, it is necessary to introduce cotton varieties characterised by functional plasticity – the ability of the organism to move functions from one tissue to another as a result of paralogous genes. Y. Huang *et al.* (2023) share a similar opinion. Studies have shown that the samples of the collection of ultra-early ripeness group have greater plasticity and stability compared to early-, mid-, and late-ripening varieties. The same samples were more productive.

However, analysing the findings of this study, it can be stated that the authors do not have a consensus on the influence of environmental factors on the formation of the duration of the cotton growing season for a particular region, and therefore the research conducted on Ukrainian modern varieties in the agroclimatic zone of the South of Ukraine is relevant and deserves further investigation to create new adaptive, plastic varieties that will be characterised by resistance to environmental changes.

CONCLUSIONS

As a result of the study, the ecological plasticity, stability, index of growing conditions, coefficient of variation of samples from the cotton collection on the basis of the duration of the period “germination-beginning of flowering” of plants in varieties with different growing seasons were determined. To increase the genetic potential of the variety trait, samples were selected that were characterised by high values of b_i and low S_i^2 , which will contribute to the creation of new source material for varieties with increased ecological plasticity and stability.

It was found that according to plasticity indicators (b_i), stability variance (S_i^2) and the coefficient of variation of the duration of the “germination-beginning of flowering” period, ultra-early ripening varieties were less sensitive to growing conditions. Pidozerskyi 4 (UF080003) (UKR) was characterised by greater plasticity to growing

conditions among the studied samples of the ultra-early ripeness group, Beli Izvor (UF0800015) (BLG) among the early-ripening group, C7085 (UF0800127) (UZB) among the mid-ripening group, and Tabladilla 16 (UF080014) (ESP) among the late-ripening group.

To extend the process of forming early ripening forms and productivity of raw cotton, parental components with shorter vegetation periods by years were selected from the cotton gene pool, than that of the standard variety Dniprovskiy 5 or on the same level, these include Pidozerskyi 4 (UF080003) (UKR), 144 F (UF0800062) (UZB), L 417u (UF080002), 500u (UF080000), 510u (UF0800226), Population 1 (UF0800193), Population 2 (UF0800020), Population 3 opr. (UF080018), Population 9 (UF0800241), 3737u (UF0800228) (UKR); 4521Traikia (UF080006), Interspecific hybrid W64 (UF0800165), Avangard 264 (UF0800020) (BGR), etc. The average duration of the “germination-full ripeness” vegetation period in the samples of the early ripeness group was 8-9 days longer than in the ultra-early ripeness group, but the coefficient of variation over the years was much lower – 2.2-6.1% versus 2.5-8.2%.

A promising area for further research is the enrichment of the genetic diversity of the cotton gene pool to create valuable source material with high productivity, increased environmental plasticity and stability.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Екологічна пластичність та стійкість бавовнику в умовах Південного Степу України

Раїса Анатоліївна Вожегова

Доктор сільськогосподарських наук, професор, академік
Національної Академії Аграрних Наук України
Інститут кліматично орієнтованого сільського господарства
Національної Академії Аграрних Наук України
67667, вул. Маяцька дорога, 24, смт. Хлібодарське, Україна
<https://orcid.org/0000-0002-3895-5633>

Віра Олександрівна Боровик

Кандидат сільськогосподарських наук, старший науковий співробітник
Інститут кліматично орієнтованого сільського господарства
Національної Академії Аграрних Наук України
67667, вул. Маяцька дорога, 24, смт. Хлібодарське, Україна
<https://orcid.org/0000-0003-0705-2105>

Сергій Олександрович Заєць

Доктор сільськогосподарських наук, старший науковий співробітник
Інститут кліматично орієнтованого сільського господарства
Національної Академії Аграрних Наук України
67667, вул. Маяцька дорога, 24, смт. Хлібодарське, Україна
<https://orcid.org/0000-0002-3895-5633>

Леонід Аркадійович Сергєєв

Кандидат сільськогосподарських наук
Одеська державна сільськогосподарська дослідна станція
Інституту кліматично орієнтованого сільського господарства
Національної Академії Аграрних Наук України
67667, вул. Маяцька дорога, 24, смт. Хлібодарське, Україна
<https://orcid.org/0000-0003-4169-8938>

Інна Миколаївна Когут

Кандидат сільськогосподарських наук, доцент
Одеська державна сільськогосподарська дослідна станція
Інституту кліматично орієнтованого сільського господарства
Національної Академії Аграрних Наук України
67667, вул. Маяцька дорога, 24, смт. Хлібодарське, Україна
<https://orcid.org/0000-0002-4418-5954>

Анотація. Залежно від тривалості періоду вегетації бавовнику, визначається зона впровадження сорту та його найвищі показники потенціалу продуктивності, а своєчасне дозрівання бавовни-сирцю дозволяє провести збір доморозного врожаю, якісно підготувати ґрунт. Мета дослідження – виділити з генофонду бавовнику зразки адаптовані до умов Південного Степу України. При дослідженні використовувалися методики проведення експертизи сортів рослин: фенологічні спостереження, морфологічні дослідження, оцінку селекційного матеріалу за цінними ознаками. За результатами досліджень відібрані батьківські компоненти для розширення процесу формування скоростиглих форм і продуктивності бавовни-сирцю. Проведена оцінка здатності рослин бавовнику до вирощування в екстремальних умовах Південного Степу України та визначена його однорідність або стабільність, індекс умов вирощування, коефіцієнт варіації – різницю у числових значеннях ознаки тривалість періоду «сходи – початок цвітіння» рослин у сортах з різним періодом вегетації та їх коливання навколо середньої величини. Установлено, що за визначеною пластичністю (b_i), варіансою стабільності (S_i^2) та коефіцієнтом варіації тривалості «сходи – початок цвітіння» більшою толерантністю до факторів середовища характеризувались ультраранньостиглі сорти. Середній коефіцієнт регресії (b) складав – 0,36; варіювання тривалості періоду за роками – 10,1 %, що значно менше, у порівнянні зі скоростиглими сортами – 0,77 і 12,9 %, середньостиглими – 0,80 і 15,0 % та пізньостиглими – 1,30 і 16,6 %, відповідно. Максимальну середню продуктивність до морозного бавовно-сирцю 49,4 г/рослину сформував зразок Підозерський 4 (UF0800003) тривалість періоду «сходи – початок цвітіння» якого складав 47 днів, а «сходи – повне досягання» – <103 дня. Найменшу продуктивність бавовни-сирцю 21,2 г/рослину продемонстрував зразок пізньостиглої групи стиглості Joloten 32 (IU14056549) туркменської селекції, тривалість періоду вегетації якого в середньому за роки досліджень дорівнювала 145 днів. Результати досліджень будуть використані у подальшій селекції при створенні високопродуктивних сортів бавовнику з підвищеною екологічною пластичністю і стабільністю та якісним волокном

Ключові слова: зразки бавовнику; тривалість періоду вегетації; групи стиглості; пластичність; стабільність; коефіцієнт варіації; продуктивність



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Adaptive potential of short-stemmed winter bread wheat genotypes in the eastern Forest-Steppe of Ukraine

Andrii Yarosh*

PhD in Agricultural Sciences, Senior Research Fellow

V.Y. Yuriev Institute of Plant Industry of National Academy of Agrarian Sciences of Ukraine

National Centre for Plant Genetic Resources of Ukraine

61060, 142 Heroiv Kharkova Ave., Kharkiv, Ukraine

<https://orcid.org/0000-0002-6009-4139>

Nataliia Kuzmyshyna

PhD in Agriculture, Head of the Laboratory

V.Y. Yuriev Institute of Plant Industry of National Academy of Agrarian Sciences of Ukraine

National Centre for Plant Genetic Resources of Ukraine

61060, 142 Heroiv Kharkova Ave., Kharkiv, Ukraine

<https://orcid.org/0000-0001-8046-1760>

Yegor Kucherenko

PhD in Agriculture Sciences, Head of the Laboratory

V.Y. Yuriev Institute of Plant Industry of the National Academy of Agrarian Sciences of Ukraine

61060, 142 Heroiv Kharkova Ave., Kharkiv, Ukraine

<https://orcid.org/0000-0002-9313-7385>

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Abstract. Among the unresolved issues of breeding short-stemmed winter bread wheat genotypes of different ripeness groups, the problem of obtaining high and stable yields without reducing the adaptive potential in the face of limiting environmental factors that periodically occur in different agroecological conditions of cultivation stays relevant. The preliminary identification of suitable sources with a combination of necessary adaptive properties is a crucial step towards the development of highly promising varieties and improvement of the gene pool of plants in Ukraine. The purpose of this study was to find the level of adaptive potential in short-stemmed winter bread wheat genotypes with different vegetation periods, to investigate the plasticity and stability of yields, and to select highly adaptive source material for the conditions of the eastern part of the Forest-Steppe of Ukraine. To fulfil this purpose, the study employed general scientific, field, and dispersion methods of scientific cognition. It was found that in the eastern part of the Forest-Steppe of Ukraine, among short-stemmed winter bread wheat genotypes, the share of those with the highest genetic potential for adaptability combined with high yields (over 16% of the standard) is highest in the group of mid-ripening varieties and amounts to 53.3%. These include mainly Ukrainian genotypes, namely: Blahodarka Odeska, Zoreslava, Rozdynka Odeska, Kruhozir, Shpalivka (UKR); Ilona (SVK), Urbanus (AUT), and Evklid (FRA). Among

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*Corresponding author

the early-ripening samples, the share of such genotypes was 33.3%, and in the group of late-ripening varieties – 13.3%. High yield stability ($S^2d_i=0.0$) was inherent in 66.7% of the samples of the early-ripening group, 60% of the mid-ripening group, and 26.7% of the late-ripening group. The identified sources are valuable starting material for the development of new and highly promising winter soft wheat varieties adapted to the growing conditions in the eastern part of the Forest-Steppe of Ukraine

Keywords: yield; plasticity; stability; limiting factors; reaction rate; source; variety

INTRODUCTION

Identification of new short-stemmed winter bread wheat genotypes with high adaptability to changing agroecological conditions is an urgent task on the path to successful intensification of agricultural product diversity and food security. For a long time, the development of high-intensity, short-stemmed winter soft wheat varieties has been the main focus of breeding to increase yield potential. As a result, a considerable number of varieties in the State Register of Plant Varieties of Ukraine (n.d.) have a genetic potential of more than 10 t/ha. However, there is a substantial decrease in its stability and, accordingly, a loss of adaptive potential, which is a considerable obstacle to the successful introduction of varieties into production, which requires the early identification of short-stemmed sources of winter soft wheat with the necessary adaptive properties for the selection improvement of the created gene pool.

In many countries of the world, there is an urgent need to address the impact of various stress factors that can limit the yield and adaptability of various food crops, including winter wheat. A. Raza *et al.* (2019) note that abiotic stresses are the main limiting factors that affect soft winter wheat plants in the environment. According to the scientists, genomic selection (GS) with phenotyping of high-yielding samples is a prominent area in identifying drought resistance genes. D. Soto-Gómez and P. Pérez-Rodríguez (2022) emphasise the significance of perennial wheat as a donor of water scarcity resilience, which can help reduce soil erosion and prevent carbon waste in agriculture. Ukrainian scientists M. Ilchuk *et al.* (2019) note that due to its nutritional value and the existing variety of technological uses, soft winter wheat (*Triticum aestivum* L.) plays a major role in ensuring food security of Ukraine, which is the basis for its economic development.

To ensure the effective introduction of new and promising varieties into production, significant breeding work is being carried out to increase yields and adaptability in certain agro-ecological zones. When investigating the adaptability parameters of modern winter wheat varieties in six agro-ecological regions of Poland, M. Studnicki *et al.* (2019) found that yield variability was determined mainly by the influence of the agro-ecological region of cultivation on the studied varieties, and to a lesser extent by the genotype. Researchers have identified the Sailor and Linus varieties, which are highly adaptable to all the agro-ecological

regions under study at different cultivation intensities. Ukrainian scientists N. Zamlila *et al.* (2019) identified breeding lines of soft winter wheat characterised by the necessary parameters of adaptability and a combination of high yield in the central Forest-Steppe of Ukraine – Lutescens 54360 and Lutescens 35232. A lot of research is being done to improve cultivation technologies to increase gross grain yields. Thus, Ukrainian scientists S. Poltoretskyi *et al.* (2020) argue that the choice of sowing dates was accompanied by changes in other elements of winter wheat cultivation technology – selection of varieties according to ripeness groups, determination of seeding rates, and sowing methods. Chinese scientists W-G. Li *et al.* (2022) emphasise the significance of nitrogen application and irrigation as the principal factors in the formation of high yields of soft winter wheat. According to the findings of the study, the highest yields without substantial lodging of crops were obtained with nitrogen application of 240 kg/ha and irrigation of 600 m³/ha. Indian scientists N. Suresh *et al.* (2020) note that to maximise the yield potential of a genotype, it is necessary to sow at the optimum time.

Ukrainian scientists S. Lyfenko *et al.* (2021) note that in the Steppe zone, early-ripening varieties have greater yield advantages over varieties of other ripeness groups, which almost always fall under the influence of fires and summer drought. The findings also suggest that varieties of high-intensity, short-stemmed type considerably outperform varieties of semi-intensive, medium-stemmed type in terms of potential yield, but the stability of medium-stemmed genotypes is often higher than that of short-stemmed ones.

E. Filip *et al.* (2021) notes that the genetic yield potential of a variety is limited by various biotic and abiotic factors, which, accordingly, negatively affects its stability. Polish scientists M. Różewicz *et al.* (2021) note that among biotic factors, fungal diseases cause the most damage, with yields decreasing mainly by 15–20%, and in epiphytotic losses can reach 60%. The main factors that determine the emergence and spread of epiphytotic include the level of genetic protection against pathogens (Kang *et al.*, 2020), weather conditions during the growing season, crop rotation, and soil cultivation system (Iwańska *et al.*, 2019).

The purpose of this study was to determine the adaptive potential of short-stemmed winter wheat genotypes of different ripeness groups in terms of yield

plasticity and stability, to identify sources of high yield potential adapted to the conditions of the eastern part of the Forest-Steppe of Ukraine.

MATERIALS AND METHODS

The research material included 45 short-stemmed samples of soft winter wheat (*Triticum aestivum* L.) of different ripeness groups: early-, mid-, and late-ripening varieties from nine countries, including 11 samples from Ukraine, 10 from Germany, 8 from France, 5 each from Romania and China, 2 each from Austria and the United States of America, and 1 each from Slovakia and Hungary. The early-ripening group included Manera Odeska, Orzhytsia Nova, Soborna (Ukraine); MV Sed (Hungary), Centurion, Retezat, F11208G2, F10222G5-11, F 08034G1-01 (Romania); CA 13014, KN 41, KN 40, T 51 (China); OK 10126, Bentley (United States of America); mid-ripening – Blahodarka Odeska, Svitohliad, Zoloto Ukrainy, Zoreslava, Rodzynka Odeska, Kruhozir, Shpalivka, MIP Feieria (Ukraine); Ilona (Slovakia); Aspekt (Germany); Urbanus, Tacitus (Austria); Evklid, Bardotka (France); Flourish (Canada), and late-ripening – Fagus, Achim, Dromos, Bonanza, Nordkap, Rotax, KWS Malibu, Akrotos, Mattus (Germany); RGT Reform, Matchball, Praktik, Sailor, Boomer, Memory (France). Each ripeness group consisted of 15 samples. The study of samples of different ecological and geographical origin is necessary to determine the adapted genotypes of different ripeness groups to the conditions of the eastern part of the Forest-Steppe of Ukraine.

The study was conducted in 2018–2021 in the laboratory of cereal genetic resources of the National Centre for Plant Genetic Resources of Ukraine (NCPGRU) of the V.Y. Yuriev Plant Production Institute of the National Academy of Agrarian Sciences of Ukraine. The experiments were designed following the requirements of selection field experiments (Dospekhov, 1985). Sowing was carried out in pairs at the optimum time with a seeding rate of 4.5 million grains per 1 ha using a Klen-1.5SP seeder (a selection seeder with a portioned sowing unit, made in Ukraine) on plots of 5 m² in threefold replication. In spring, the crops were fertilised with ammonium nitrate (N₄₀). The standard for the short-stemmed samples was the Bunchuk variety, which was sown through 20 numbers. The samples were studied according to the methodology (Merezhko *et al.*, 1999) and the classifier of the genus *Triticum* L. (CMEA's extended harmonised classifier of the genus *Triticum* L., 1989). The study of plasticity and stability of plant genotypes was carried out according to the method of S.A. Eberhart and

W.A. Russel (1966). The linear regression coefficient (b_i) characterises the average response of the genotype to changes in growing conditions, indicates the level of its plasticity and allows predicting changes in a particular trait within the conditions under study. The fluctuation of the factual yield in relation to the regression line is determined by the standard deviation from the regression line. The variance of the stability of the trait (S^2d_i) indicates how reliably the variety corresponds to the plasticity estimated by the regression coefficient. The smaller the quadratic deviation, at which S^2d_i accordingly approaches zero, the more stable the genotype. Parameters and combinations of these traits indicate the level of adaptability of the genotypes under study to growing conditions. Statistical processing of the results was carried out according to the methodology (Dospekhov, 1985).

To fulfil the purpose of this study, the research methods were employed as follows: general scientific (analysis and synthesis) – to differentiate and generalise the results, field – to determine yield, and dispersion – to determine the levels of plasticity and stability of yield, as well as to assess the reliability of experimental data. 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 Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS

Analysing the weather conditions of the study in 2018–2021 by the growing season, it was found that different values of the hydrothermal coefficient (HTC) contributed to the differentiation of winter bread wheat samples by plasticity and yield stability, as well as by the duration of the growing season. The autumn period was very dry in 2018 (HTC=0.49) and 2020 (HTC=0.46), and quite wet in 2019 (HTC=1.46). The meteorological conditions of the vegetative spring and summer periods of the study differed significantly in terms of moisture availability and temperature: the spring periods of 2019 (HTC=1.26) and 2021 (HTC=1.46) were sufficiently moist, and the spring period of 2020 (HTC=2.05) was excessively moist. In addition, the summer months of 2020 were characterised by sufficient moisture (HTC=1.27), while in 2019 (HTC=0.62) and 2021 (HTC=0.64) they were dry (Fig. 1).

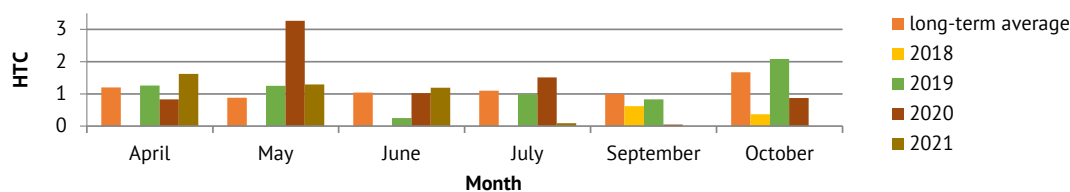


Figure 1. Hydrothermal coefficient of the vegetation periods of the study, 2018–2021

Source: compiled by the authors of this study

The length of the growing season was shortest in 2021 and longest in 2020 was the most favourable year for high yields. In 2019 and 2021, its levels were mostly lower. Notably, moisture deficit and high temperature in July 2021 ($HTC=0.09$) caused grain inflammation, which had a more negative impact on yield formation compared to previous years of the study, which was caused by a decrease in the breeding value of the structural elements of ear productivity. Thus, the contrast of weather conditions in the growing seasons of 2018-2021 contributed to the identification of sources of high yields adapted to the growing conditions in the eastern part of the Forest-Steppe of Ukraine.

During 2018-2020, the duration of the vegetation period (germination-ripening) in short-stemmed winter wheat samples ranged from 275 to 290 days, which made it possible to distinguish sources according to three ripeness groups: early-, mid-, and late-ripening. The group of early-ripening genotypes with a vegetation period ranging from 276 to 279 days included Manera Odeska, Orzhytsia Nova, Soborna (UKR); MV Sed (HUN); Centurion,

Retezat, F 11208G2, F 10222G5-11, F 08034G1-01 (ROU); CA 13014, KN 41, KN 40, T 51 (CHN); OK 10126, Bentley (USA). The vegetation period of the Bunchuk standard was 282 days, and that of the early-ripening standard Aurora Myronivska was 279 days.

In the early maturing group, six sources of high yields (over 16% of the standard) were identified, namely: Manera Odeska (UKR), Centurion (ROU), Retezat (ROU), CA 13014 (CHN), OK 10126 (USA), and Bentley (USA), with a share of 40%; the standard is Bunchuk (UKR) – 5.38 t/ha. As a result of the research, the linear regression coefficient (b_i) determined that the plasticity of the samples under study ranged within 0.50-1.71 in terms of yield in the group of early-ripening samples. High plasticity ($b_i > 1.0$), i.e., a wide ecological norm of reaction, was characterised by six genotypes, which accounted for 40.0% of the total number of samples under study, including Orzhytsia Nova ($b_i=1.71$), Soborna ($b_i=1.69$), Manera Odeska ($b_i=1.32$) (UKR); F11208G2 ($b_i=1.08$) (ROU); CA 13014 ($b_i=1.22$), KN 40 ($b_i=1.07$) (CHN); standard – Bunchuk ($b_i=1.41$) (UKR) (Table 1).

Table 1. Parameters of plasticity and stability of the best early-ripening and short-stemmed samples of soft winter wheat by yield, 2018-2021

Sample name	Country of origin	Vegetation period, days	Yield, t/ha			Regression coefficient, b_i	Stability variance, S^2d_i
			X_{max}	X_{min}	X_{med}		
Bunchuk, standard	UKR	282	7.20	4.30	5.38	1.41	0.73
Manera Odeska	UKR	279	7.80	5.50	6.68	1.32	0.01
Orzhytsia Nova	UKR	278	7.35	4.45	6.18	1.71	0.26
Soborna	UKR	277	6.53	3.65	5.34	1.69	0.16
MV Sed	HUN	278	6.80	5.40	6.22	0.82	0.05
Centurion	ROU	279	6.70	5.82	6.58	0.50	0.00
Retezat	ROU	278	7.15	5.85	6.45	0.73	0.04
F11208G2	ROU	279	6.50	4.62	5.59	1.08	0.00
F10222G5-11	ROU	278	6.24	4.85	5.53	0.79	0.02
F 08034G1-01	ROU	278	5.83	4.55	5.23	0.72	0.00
CA 13014	CHN	276	7.22	5.20	6.59	1.22	0.64
KN 41	CHN	277	6.85	5.65	6.21	0.68	0.03
KN 40	CHN	277	6.85	4.95	5.80	1.07	0.13
T 51	CHN	276	6.45	4.80	5.62	0.94	0.02
OK 10126	USA	278	7.80	6.87	7.37	0.58	0.01
Bentley	USA	278	6.61	5.71	6.26	0.53	0.03
LSD _{0.05}		–	–	–	0.34	–	–
min		276	5.83	3.65	5.23	0.50	0.00
max		279	7.80	6.87	7.37	1.71	0.64
Average in the experiment		278	6.87	5.13	6.06	1.00	0.00

Source: compiled by the authors of this study

Genotypes with best response to changes in growing conditions ($b_i=1.0$) were not observed in the group of early-ripening samples. Low plasticity ($b_i < 1.0$), i.e., a weak response to changes in growing conditions,

was inherent in nine genotypes, which accounted for 60%. The following samples were included: Centurion ($b_i=0.50$), F 08034G1-01 ($b_i=0.72$), Retezat ($b_i=0.73$) (ROU); MV Sed ($b_i=0.82$) (HUN); KN 41 ($b_i=0.68$), T 51

($b_i=0.94$) (CHN); Bentley ($b_i=0.53$) and OK 10126 ($b_i=0.58$).

Ten genotypes (66.7%) were characterised by high productivity stability with the lowest numerical value of the stability variance ($S^2d_i=0.0$). These include the following varieties and lines: Manera Odeska ($S^2d_i=0.01$) (UKR); Centurion ($S^2d_i=0.00$), F11208G2 ($S^2d_i=0.00$), F10222G5-11 ($S^2d_i=0.00$), F 08034G1-01 ($S^2d_i=0.00$), Retezat ($S^2d_i=0.04$) (ROU); T 51 ($S^2d_i=0.02$), KH 41 ($S^2d_i=0.03$) (CHN); OK 10126 ($S^2d_i=0.01$), Bentley ($S^2d_i=0.03$) (USA); the standard is Bunchuk ($S^2d_i=0.73$) (UKR). The most unstable samples were Orzhysia Nova ($S^2d_i=0.26$), Soborna ($S^2d_i=0.16$) (UKR); MV Sed ($S^2d_i=0.05$) (HUN); CA 13014 ($S^2d_i=0.64$), KN 40 ($S^2d_i=0.13$) (CHN).

Thus, in the group of early-ripening and short-stemmed samples, genotypes that combine a low level of plasticity and high yield stability ($b_i<1.0$) and ($S^2d_i=0.0$) prevail, the share of which is 53.3%. These include Centurion, Retezat, F10222G5-11, F 08034G1-01 (ROU); KN 41, T 51 (CHN); OK 10126, Bentley (USA). A prominent level of plasticity and unstable productivity ($b_i>1.0$) and ($S^2d_i>0.0$) were inherent in four samples (26.7%) – Orzhysia Nova, Soborna (UKR); CA 13014, KN 40 (CHN). In addition, two genotypes were selected that combine high plasticity with a stable manifestation of yield ($b_i>1.0$) and ($S^2d_i=0.0$) – Manera Odeska (UKR) and F11208G2 (ROU), the share of which is 13.3%. According to the calculation model by S.A. Eberhart and W.A. Russell (1966), such genotypes are the most valuable because they effectively use favourable factors of growing conditions and are characterised by stable yields. The variety MV Sed (HUN) combined low plasticity and unstable yield ($b_i<1.0$) and ($S^2d_i>0.0$).

As a result of the study of plasticity and stability parameters of the best early-ripening and short-stemmed winter wheat genotypes, it was found that in the eastern

part of the Forest-Steppe of Ukraine, five varieties (33.3%) have the highest adaptive potential in combination with high yield (more than 16% above the standard): Manera Odeska (UKR), Centurion, Retezat (ROU), OK 10126, and Bentley (USA). The group of mid-ripening genotypes with a growing season varying from 283 to 286 days included the following varieties: Blahodarka Odeska, Svitohliad, Zoloto Ukrainy, Zoreslava, Rodzynka Odeska, Kruhozir, Shpalivka, MIP Feieriia (UKR); Ilona (SVK); Aspekt (DEU); Urbanus, Tacitus (AUT); Evklid, Bardotka (FRA); Flourish (CAN). The growing season of the Podolianka mid-ripening standard was 284 days.

In the mid-ripening group, 12 sources of high yields (more than 16% above the standard) were found, including the following samples: Blahodarka Odeska, Svitohliad, Zoloto Ukrainy, Zoreslava, Rodzynka Odeska, Kruhozir, Shpalivka (UKR); Ilona (SVK); Urbanus (AUT); Evklid, Bardotka (FRA); Flourish (CAN), with a share of 80%. It was found that in the mid-ripening group of short-stemmed samples, plasticity (b_i) ranged within 0.27-2.07. The proportion of genotypes with high plasticity ($b_i>1.0$) was 40.0% of the total number of samples. These include the varieties Kruhozir ($b_i=1.52$), Rodzynka Odeska ($b_i=1.18$), Shpalivka ($b_i=1.10$) (UKR); Aspekt ($b_i=1.54$) (DEU); Tacitus ($b_i=2.07$) (AUT); Flourish ($b_i=1.83$) (CAN).

The mid-ripening samples Zoloto Ukrainy ($b_i=0.97$) (UKR) and Evklid ($b_i=0.98$) (FRA) were distinguished by the best response to changes in growing conditions ($b_i=1.0$), with a share of 13.3%. A weak response to changes in growing conditions and, accordingly, a low level of plasticity ($b_i<1.0$) was inherent in seven samples, the share of which was 46.7%. These include the following varieties: Blahodarka Odeska ($b_i=0.36$), Svitohliad ($b_i=0.59$), Zoreslava ($b_i=0.59$), MIP Feieriia ($b_i=0.72$) (UKR); Ilona ($b_i=0.43$) (SVK); Urbanus ($b_i=0.27$) (AUT); Bardotka ($b_i=0.35$) (FRA) (Table 2).

Table 2. Parameters of plasticity and stability of the best mid-ripening and short-stemmed varieties of soft winter wheat by yield, 2018-2021

Sample name	Country of origin	Vegetation period, days	Yield, t/ha			Regression coefficient, b_i	Stability variance, S^2d_i
			X_{max}	X_{min}	X_{med}		
Bunchuk, standard	UKR	282	7.20	4.30	5.38	1.41	0.73
Blahodarka Odeska	UKR	284	7.85	7.15	7.51	0.36	0.01
Svitohliad	UKR	284	8.70	6.00	7.40	0.59	3.06
Zoloto Ukrainy	UKR	285	7.85	6.14	6.78	0.97	0.10
Zoreslava	UKR	285	7.30	6.24	6.68	0.59	0.01
Rodzynka Odeska	UKR	284	7.82	5.62	6.67	1.18	0.02
Kruhozir	UKR	283	8.10	5.32	6.54	1.52	0.01
Shpalivka	UKR	283	7.40	5.40	6.27	1.10	0.01
MIP Feieriia	UKR	285	6.80	5.45	6.08	0.72	0.00
Ilona	SVK	285	7.18	6.35	6.78	0.43	0.02
Aspekt	DEU	285	7.63	4.90	5.94	1.54	0.20
Urbanus	AUT	286	7.00	6.50	6.67	0.27	0.03

Table 2. Continued

Sample name	Country of origin	Vegetation period, days	Yield, t/ha			Regression coefficient, b_i	Stability variance, S^2d_i
			$X_{\max}$	$X_{\min}$	X_{med}		
Tacitus	AUT	285	7.87	4.26	5.54	2.07	0.69
Evklid	FRA	283	7.73	5.88	6.79	0.98	0.03
Bardotka	FRA	286	7.07	6.15	6.58	0.35	0.21
Flourish	CAN	285	7.58	3.95	6.40	1.83	1.22
LSD _{0.05}		–	–	–	0.31	–	–
min		283	6.80	3.95	5.54	0.27	0.00
max		286	8.70	7.15	7.51	2.07	3.06
Average in the experiment		285	7.57	5.60	6.50	1.00	0.00

Source: compiled by the authors of this study

Nine genotypes (60%) were characterized by high stability with the lowest numerical value of the stability variance ($S^2d_i=0.0$). These included the following varieties: MIP Feieriia ($S^2d_i=0.00$), Blahodarka Odeska ($S^2d_i=0.01$), Zoreslava ($S^2d_i=0.01$), Kruhozir ($S^2d_i=0.01$), Shpalivka ($S^2d_i=0.01$), Rodzynka Odeska ($S^2d_i=0.02$) (UKR); Ilona ($S^2d_i=0.02$) (SVK); Urbanus ($S^2d_i=0.03$) (AUT); Evklid ($S^2d_i=0.03$) (FRA). The most unstable samples ($S^2d_i>0$) included Svitoiliad ($S^2d_i=3.06$), Zoloto Ukrainy ($S^2d_i=0.10$) (UKR); Aspect ($S^2d_i=0.20$) (DEU); Tacitus ($S^2d_i=0.69$) (AUT); Bardotka ($S^2d_i=0.21$) (FRA), and Flourish ($S^2d_i=1.21$) (CAN).

Therefore, the group of mid-ripening and short-stemmed samples is dominated by genotypes that combine a low level of plasticity and high yield stability ($b_i<1.0$) and ($S^2d_i=0.0$), the share of which is 33.3%. These include the varieties MIP Feieriia, Blahodarka Odeska, Zoreslava (UKR); Ilona (SVK); Urbanus (AUT). Three genotypes (20%) were identified, which combine high plasticity with a stable manifestation of productivity ($b_i>1.0$) and ($S^2d_i=0.0$), namely: Rodzynka Odeska, Kruhozir, Shpalivka (UKR). Aspekt (DEU), Tacitus (AUT), and Flourish (CAN) varieties were characterised by high sensitivity to growing conditions with unstable productivity ($b_i>1.0$) and ($S^2d_i>0.0$), the share of which is 20%. Less adapted to growing conditions were Svitoiliad (UKR) and Bardotka (FRA), which combined a low level of plasticity with unstable productivity ($b_i<1.0$) and ($S^2d_i>0.0$), the share of which was 13.3%. It is worth noting the variety Evklid (FRA), which combined best response to changes in growing conditions with stable productivity ($b_i=1.0$) and ($S^2d_i=0.0$). The sample of Zoloto Ukrainy (UKR) also combined the best response to changes in growing conditions, but the yield was less stable ($b_i=1.0$) and ($S^2d_i>0.0$).

It was found that among the best mid-ripening and short-stemmed winter bread wheat genotypes in the eastern part of the Forest-Steppe of Ukraine, eight varieties (53.3%) have the highest adaptive potential in combination with high yield (more than 16% above the standard): Blahodarka Odeska, Zoreslava, Rodzynka Odeska, Kruhozir, Shpalivka (UKR); Ilona (SVK); Urbanus (AUT); Evklid (FRA). The group of late-ripening genotypes with a vegetation period ranging from 287 to 290 days included Fagus, Achim, Dromos, Bonanza, Nordkap, Rotax, KWS Malibu, Akratos, Mattus (DEU); RGT Reform, Matchball, Praktik, Sailor, Boomer, Memory (FRA). The growing season of the late-ripening standard Yuivata 60 (UKR) was 288 days. In the late-ripening group of short-stemmed genotypes, five sources of high yields (more than 16% above the standard) were identified, including Fagus, Achim, Dromos, Bonanza (DEU); RGT Reform (FRA), whose share was 33.3%.

It was found that in this group of the varieties under study, plasticity (b_i) ranged from 0.13 to 2.18. The proportion of genotypes with high plasticity ($b_i>1.0$) was 53.3% of the total number of samples. These include Akratos ($b_i=2.18$), Mattus ($b_i=1.38$), KWS Malibu ($b_i=1.21$), Achim ($b_i=1.15$), Dromos ($b_i=1.15$), Nordkap ($b_i=1.11$) (DEU); Sailor ($b_i=1.17$), RGT Reform ($b_i=1.12$) (FRA). Medium plastic genotypes with the best response to changes in growing conditions ($b_i=1.0$) were not observed in the group of late-ripening and short-stemmed samples. A low level of plasticity ($b_i<1.0$) was inherent in seven varieties, which accounted for 46.7% of the total number of samples under study. These included the following varieties: Rotax ($b_i=0.58$), Bonanza ($b_i=0.64$), Fagus ($b_i=0.66$) (DEU); Praktik ($b_i=0.13$), Memory ($b_i=0.73$), Boomer ($b_i=0.78$), Matchball ($b_i=0.91$) (FRA) (Table 3).

Table 3. Parameters of plasticity and stability of the best late-ripening and short-stemmed varieties of soft winter wheat by yield, 2018–2021

Sample name	Country of origin	Vegetation period, days	Yield, t/ha			Regression coefficient, b_i	Stability variance, S^2d_i
			$X_{\max}$	$X_{\min}$	X_{med}		
Bunchuk, standard	UKR	282	7.20	4.30	5.38	1.41	0.73
Fagus	DEU	288	8.33	6.30	7.30	0.66	0.35

Table 3. Continued

Sample name	Country of origin	Vegetation period, days	Yield, t/ha			Regression coefficient, b_i	Stability variance, S^2d_i
			X_{max}	X_{min}	X_{med}		
Achim	DEU	287	8.70	5.80	6.83	1.15	0.00
Dromos	DEU	287	8.33	4.11	6.56	1.15	4.36
Bonanza	DEU	287	7.53	5.00	6.43	0.64	1.73
Nordkap	DEU	288	7.80	5.00	6.00	1.11	0.00
Rotax	DEU	290	6.53	4.63	5.55	0.58	0.47
KWS Malibu	DEU	290	7.47	4.32	5.48	1.21	0.14
Akratos	DEU	287	8.40	4.92	5.84	2.18	3.59
Mattus	DEU	288	6.58	2.13	4.40	1.38	2.29
RGT Reform	FRA	288	8.80	6.00	6.97	1.12	0.04
Matchball	FRA	287	7.73	4.75	6.19	0.91	1.15
Praktik	FRA	288	6.05	5.67	5.84	0.13	0.01
Sailor	FRA	290	7.62	3.45	5.81	1.17	3.67
Boomer	FRA	288	7.05	4.37	5.73	0.78	1.19
Memory	FRA	290	6.50	3.76	5.25	0.73	1.74
LSD _{0.05}		–	–	–	0.40	–	–
min		287	6.05	2.13	4.40	0.13	0.00
max		290	8.80	6.30	7.30	2.18	4.36
Average in the experiment		288	7.54	4.53	5.91	1.00	0.00

Source: compiled by the authors of this study

A low level of plasticity ($b_i < 1.0$) was inherent in seven varieties, which accounted for 46.7% of the total number of samples under study. These include the following varieties: Rotax ($b_i = 0.58$), Bonanza ($b_i = 0.64$), Fagus ($b_i = 0.66$) (DEU); Praktik ($b_i = 0.13$), Memory ($b_i = 0.73$), Boomer ($b_i = 0.78$), Matchball ($b_i = 0.91$) (FRA). Four genotypes (26.7%) were characterised by high productivity stability ($S^2d_i = 0.0$). These included the following varieties: Achim ($S^2d_i = 0.00$), Nordkap ($S^2d_i = 0.00$) (DEU); Praktik ($S^2d_i = 0.01$), RGT Reform ($S^2d_i = 0.04$) (FRA). In the sample of late-ripening samples, the share of the most unstable samples ($S^2d_i > 0.0$) was 73.3%, which included Dromos ($S^2d_i = 4.36$), Akratos ($S^2d_i = 3.59$), Mattus ($S^2d_i = 2.29$), Bonanza ($S^2d_i = 1.73$), Rotax ($S^2d_i = 0.47$), Fagus ($S^2d_i = 0.35$), KWS Malibu ($S^2d_i = 0.14$) (DEU); Sailor ($S^2d_i = 3.67$), Memory ($S^2d_i = 1.74$), Boomer ($S^2d_i = 1.19$), Matchball ($S^2d_i = 1.15$) (FRA).

Thus, the group of late-ripening and short-stemmed samples is dominated by genotypes that combine a low level of plasticity with yield instability ($b_i < 1.0$) and ($S^2d_i > 0.0$), the share of which is 40%. These include the Fagus, Bonanza, Rotax (DEU); Matchball, Boomer, Memory (FRA) varieties. Varieties Achim, Nordkap (DEU) and RGT Reform (FRA) were distinguished by a combination of high plasticity and yield stability ($b_i > 1.0$) and ($S^2d_i = 0.0$), their share is 20% of the total number of the late-ripening varieties under study. Varieties Dromos, KWS Malibu, Akratos, Mattus (DEU); Sailor (FRA) were characterised by high sensitivity to growing conditions with unstable productivity ($b_i > 1.0$) and ($S^2d_i > 0.0$), the share of which is also 33.3%. Variety Praktik (FRA) was characterised by low sensitivity to growing conditions

with stable productivity ($b_i > 1.0$) and ($S^2d_i = 0.0$). Based on the conducted study, it was found that among the best late-ripening and short-stemmed winter bread wheat genotypes, the highest adaptive potential in combination with high yields (more than 16% above the standard) was achieved by Achim (DEU) and RGT Reform (FRA), with a share of 13.3%.

Proceeding from the above findings, it can be argued that among short-stemmed winter bread wheat genotypes there are sources characterised by a combination of high levels of adaptive potential and yield in the eastern part of the Forest-Steppe of Ukraine, which are mostly represented in the group of mid-ripening samples. The in-depth search for such genotypes allows thoroughly differentiating the genetic diversity of winter soft wheat by adaptive properties and to identify the best varieties and breeding lines of the Ukrainian plant gene pool to meet the needs of humanity.

DISCUSSION

Due to the abnormally high air temperatures and droughts observed in Ukraine in recent decades, as noted by Yu. Kolupaev *et al.* (2023), there are prerequisites for an in-depth investigation of the adaptive properties of plants and the selection of the best gene pool for breeding. Varieties of different ecological and geographical origins are being introduced into production, which differ in their physiological and morphological-biological characteristics, specifically: winter hardiness, length of the growing season, height, structural elements of yield, etc., and therefore it is quite significant and relevant, especially with climate change, to differentiate the

source material by yield and adaptive properties in relation to the region of their cultivation (Bondarenko & Nazarenko, 2020). Generally, the level of genotype-environment interaction (Studnicki *et al.*, 2019), weather conditions (Harkness *et al.*, 2020), and sowing dates (Petrychenko *et al.*, 2021) have the greatest influence on the formation of a variety's yield.

According to many scientists, namely J. Laugerotte *et al.* (2022), the stress resistance of winter wheat varieties to biotic and abiotic factors is crucial for the stabilisation and development of agriculture. Ukrainian researchers M. Sydorenko and S. Chebotar (2020) note that among abiotic factors, as environmental stressors affecting plant homeostasis, the problems of water deficit caused by drought, usually accompanied by hot temperatures, which is widely predicted with climate change, are becoming increasingly important. Chinese scientists W-G. Li *et al.* (2022) note that under conditions of dehydration, there are considerable disruptions in metabolic processes, protein breakdown, changes in the chemical state of the cytoplasm, and a decrease in the amount of organic matter accumulated by plants, which often leads to a significant decrease in gross grain yields and yield instability.

The problem of high and stable yields by a genotype, due to the intensification of cultivation technologies and the creation of high-intensity short-stemmed varieties, does not cause consensus among scientists on the possibility of solving this problem. Ukrainian scientists S. Lyfenko *et al.* (2021) note that semi-intensive, medium-sized varieties mostly have horizontal leaves, which better protects the soil surface from drying out and overheating, and yield formation becomes more stable compared to high-intensive varieties. During the formation of a productive stem stand of 600-800 pcs./m², high-intensity, short-stem varieties are quite significant for them to have an erectoid leaf position, which contributes to better illumination of the leaves of different tiers. A. Zviahin *et al.* (2008) argue that there are no soft winter wheat varieties that can combine high resistance to stress factors, including drought, and high yield stability. However, T. Yurchenko *et al.* (2020), researchers at the the V.M. Remeslo Myronivka Institute of Wheat under the National Academy of Agrarian Sciences of Ukraine, note that the negative correlation between stress resistance and yield is not absolute, and therefore there are prospects for the best combination of these traits in one genotype. It was found that the variety Oberih Myronivskyi combines a high level of yield and is characterised by high drought tolerance according to a range of drought tolerance indices (DSI – drought susceptibility index; TOL – drought tolerance index; MP – mean productivity; YSI – yield stability index; YI – yield index; STI – stress tolerance index; GMP – geometric mean productivity).

A range of scientists, including K. Dhiwar *et al.* (2020), N. Suresh *et al.* (2020) note that highly plastic plant genotypes, characterised by high yields under

improved growing conditions, but with their deterioration or reduction of the agrophysical background, are most often characterised by a considerable decrease in yield levels. Samples with low plasticity show less variability in yield under different growing conditions and are characterised by the formation of low, but mostly stable yields even under stressful growing conditions. UK scientists J. Pennacchi *et al.* (2019) identified four modern British soft winter wheat varieties – Gladiator, Humber, Mercato, and Zebedee – which combine relatively high yield potential with stable yields over three years of contrasting growing conditions in the UK.

According to Yu. Lavrynenko *et al.* (2020), a necessary step towards the development of highly promising and adaptive winter wheat varieties is to assess not only the potential yield, but also the parameters of its plasticity and stability, which reflect the adaptive properties of genotypes. In a breeding process aimed only at increasing yields, the adaptive potential of varieties decreases over time. Thus, the findings of the study on the adaptability of high-yielding varieties and breeding lines of winter bread wheat in the eastern part of the Forest-Steppe of Ukraine are unique, and the identified sources require further research, including biochemical studies. Summarising the above, it is believed that with a targeted search for the necessary highly adaptive and productive winter bread wheat genotypes among different ripeness groups, according to the parameters of plasticity and yield stability, it is quite possible to identify varieties that are the most adapted to certain growing conditions.

CONCLUSIONS

The highest genetic potential for adaptability among short-stemmed soft winter wheat genotypes in the conditions of the eastern part of the Forest-Steppe of Ukraine in combination with high yield are mid-ripening varieties, namely: Blahodarka Odeska ($b_i=0.36$; $S^2d_i=0.01$), Zoreslava ($b_i=0.59$; $S^2d_i=0.01$), Rodzynka Odeska ($b_i=1.18$; $S^2d_i=0.02$), Kruhozir ($b_i=1.52$; $S^2d_i=0.01$), Shpalivka ($b_i=1.10$; $S^2d_i=0.01$) (UKR); Ilona ($b_i=0.43$; $S^2d_i=0.02$) (SVK), Urbanus ($b_i=0.27$; $S^2d_i=0.03$) (AUT), and Evklid ($b_i=0.98$; $S^2d_i=0.03$) (FRA), the share of which is 53.3%. Early-ripening varieties were slightly inferior to them in quantitative composition – Manera Odeska ($b_i=1.32$; $S^2d_i=0.01$) (UKR), Centurion ($b_i=0.50$; $S^2d_i=0.00$), Retezat ($b_i=0.73$; $S^2d_i=0.04$) (ROU), OK 10126 ($b_i=0.58$; $S^2d_i=0.01$), and Bentley ($b_i=0.53$; $S^2d_i=0.03$) (USA), which made up 33.3% of the total sample of the group of samples under study. The smallest share of well-adapted and high-yielding varieties (13.3%) was in the group of late-ripening varieties, which included Achim ($b_i=1.15$; $S^2d_i=0.00$) (DEU) and RGT Reform ($b_i=1.12$; $S^2d_i=0.04$) (FRA).

It was found that plasticity (b_i) with increasing duration of the vegetation period increased from 1.71 – the maximum level of its manifestation in the early-ripening group to 2.18 in the late-ripening group.

Therewith, the stability parameters had the opposite nature, which according to the variance of stability (S^2d_i) in the early-ripening group within 0.00-0.64, in the mid-ripening group – within 0.00-3.06, and in the late-ripening group – within 0.00-4.36. The proportion of samples with high plasticity ($b_i > 1.0$) in the early- and mid-ripening groups was 40.0% each, and 53.3% in the late-season group. Genotypes with the best response to changes in growing conditions ($b_i = 1.0$) were observed only in the group of mid-ripening samples, which included Zoloto Ukrainy ($b_i = 0.97$) (UKR) and Evklid ($b_i = 0.98$) (FRA), with a share of 13.3%. High stability ($S^2d_i = 0.0$) was observed in 66.7% of the samples in the early-ripening group, 60% in the medium-ripening group, and 26.7% in the late-ripening group.

The identified high-yielding and short-stemmed sources of different ripeness groups with high adaptive

potential are valuable source material for the development of new and highly promising winter bread wheat varieties adapted to stressful growing conditions in the eastern part of the Forest-Steppe of Ukraine. The prospect of further research is to investigate the morphological, physiological, and anatomical features of the architectonics of plants with different adaptive properties of the material. The study of the anatomical structure of the stem and ear, the structure of the leaf apparatus will open more opportunities for systematising the research findings.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Адаптивний потенціал короткостеблих генотипів пшениці м'якої озимої в умовах східного Лісостепу України

Андрій Васильович Ярош

Кандидат сільськогосподарських наук, старший науковий співробітник
Інститут рослинництва імені В.Я. Юр'єва Національної академії аграрних наук України
Національний центр генетичних ресурсів рослин України
61060, просп. Героїв Харкова, 142, м. Харків, Україна
<https://orcid.org/0000-0002-6009-4139>

Наталія Василівна Кузьмишина

Кандидат сільськогосподарських наук, завідувач
Інститут рослинництва імені В.Я. Юр'єва Національної академії аграрних наук України
Національний центр генетичних ресурсів рослин України
61060, просп. Героїв Харкова, 142, м. Харків, Україна
<https://orcid.org/0000-0001-8046-1760>

Єгор Юрійович Кучеренко

Кандидат сільськогосподарських наук, завідувач
Інститут рослинництва імені В.Я. Юр'єва Національної академії аграрних наук України
61060, просп. Героїв Харкова, 142, м. Харків, Україна
<https://orcid.org/0000-0002-9313-7385>

Анотація. Серед невирішених питань селекції короткостеблих генотипів пшениці м'якої озимої різних груп стиглості залишається актуальною проблема отримання високих та стабільних врожаїв, без зниження адаптивного потенціалу в умовах прояву лімітуючих факторів зовнішнього середовища, які періодично виникають у різних агроекологічних умовах вирощування. Попереднє виділення відповідних джерел з поєднанням необхідних адаптивних властивостей є досить важливим етапом на шляху створення високоперспективних сортів та покращення генофонду рослин України. Мета досліджень – визначення рівня адаптивного потенціалу в короткостеблих генотипів пшениці м'якої озимої різних за тривалістю вегетаційного періоду, вивчення пластичності та стабільності врожайності, а також виділення високоадаптивного вихідного матеріалу для умов східної частини Лісостепу України. Для досягнення поставленої мети використовували загальнонауковий, польовий та дисперсійний методи досліджень. Визначено, що в умовах східної частини Лісостепу України, серед короткостеблих генотипів пшениці м'якої озимої, частка з найвищим генетичним потенціалом адаптивності у поєднанні з високою врожайністю (понад 16 % до стандарту) найбільшою є у групі середньостиглих сортів і складає 53,3 %. До них відносяться переважно вітчизняні генотипи, а саме: Благодарка одеська, Зореслава, Родзинка одеська, Кругозір, Шпалівка (UKR); Ilona (SVK) Urbanus (AUT) та Evklid (FRA). Серед ранньостиглих зразків частка таких генотипів складала 33,3 %, а у групі пізньостиглих сортів – 13,3 %. Висока стабільність урожайності ($S^2d_i=0,0$) була характерною для 66,7 % зразків ранньостиглої групи, 60 % – середньостиглої та 26,7 % – пізньостиглої. Виділені джерела є цінним вихідним матеріалом для створення нових та високоперспективних сортів пшениці м'якої озимої, адаптованих до умов вирощування у східній частині Лісостепу України

Ключові слова: урожайність; пластичність; стабільність; лімітуючі чинники; норма реакції; джерело; сорт



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Soil physical health under different farming systems of rice fields and its effect on rice (*Oryza sativa* L.) productivity

Ganjar Herdiansyah*

Lecturer

Sebelas Maret University

57126, 36 Ir. Sutami, Surakarta, Indonesia

<https://orcid.org/0000-0001-5841-4642>

Mujiyo Mujiyo

Professor of Pedology and Soil Survey

Sebelas Maret University

57126, 36 Ir. Sutami, Surakarta, Indonesia

<https://orcid.org/0000-0002-6161-7771>

Aktavia Herawati

Lecturer

Sebelas Maret Universitas

57126, 36 Ir. Sutami, Surakarta, Indonesia

<https://orcid.org/0000-0001-5278-8811>

Hanindyo Bramastomo

Undergraduate Student

Sebelas Maret University

57126, 36 Ir. Sutami, Surakarta, Indonesia

<https://orcid.org/0009-0009-3828-0531>

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Abstract. Soil physical health was affected by several factors including farming systems, and the plant growth and soil productivity were directly affected. The study of soil physical health in different farming systems will help manage soil and water used processes. The purpose of this study was to determine the effect of the paddy field management system on soil physical health status, to identify determinants of soil physical health status, and to formulate appropriate management solutions to improve soil physical health. This study employed descriptive, explorative, and survey approaches with purposive sampling methods in soil sampling. The findings showed that the physical health status of soil on paddy fields in the Tirtomoyo District was classified into moderately healthy and healthy categories. Differences in the management system of paddy fields affect soil physical health. Organically managed rice fields have the highest soil physical health value of 76.69. Semi-organic and inorganically managed paddy fields have lower health values of 71.48 and 69.11,

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*Corresponding author

respectively. Soil penetration resistance, soil porosity, and soil texture are determining factors because they can improve indicator conditions and soil physical health values. Efforts can be made to improve soil physical health status by applying organic fertilisers and biochar to paddy fields. Having established the relationship between soil physical health values and rice yield productivity, it is anticipated that farmers and stakeholders will be able to increase crop productivity through improving soil physical condition

Keywords: determinant factors; farming system; organic carbon; rice yield; soil penetration; soil porosity

INTRODUCTION

Soil health is essential in maintaining biodiversity and sustainable agricultural production. Soil health can be defined as the ability of soil to function sustainably in maintaining soil productivity, air, and water quality in the environment, and improving plant, animal, and human health. Soil with good physical conditions such as crumb structure, optimal porosity, and efficient drainage will help maximise the absorption of nutrients and water by plant roots, as well as making soil and plants integrated with each other in adapting to micro and macro climate changes in rice fields, thereby minimising land degradation and the risk of farmer losses (Pahalvi *et al.*, 2021). To achieve agricultural productivity, food security, and environmental sustainability, the physical health of the soil is a crucial aspect that needs to be studied because of its fundamental impact.

The assessment of soil health is investigated in various aspects, including soil physical properties (Simarmata *et al.*, 2020). Poor physical soil health has resulted in various problems in Indonesia. Low soil physical health leads to poor soil structure conditions, such as low percentage of pore space, limited drainage, and water infiltration. This affects plant health and productivity by restricting plant root growth and nutrient uptake. The inundation of rice fields makes the soil aggregate characteristics of rice fields different from those of dry land. The findings of H. Tang *et al.* (2020) show that soil and land processing factors greatly influence soil aggregates in rice fields. Soil health research has developed various methods such as assessment using VSA (Visual Soil Assessment) and vertical soil physical health assessment methods using VNIR, ECa data, and penetration resistance sensors. Research conducted by M. That *et al.* (2020) produced data that soil health on organic land can reduce NO_3^- , P, K, Mg^{2+} , and increase Ca^{2+} compared to conventional cultivation systems, while simultaneously increasing soil respiration significantly by 20 times. H. Williams *et al.* (2020) stated that soil health on agricultural land with intensive management is lower than on land without tillage or with minimal tillage. Conservation farming systems and the addition of organic matter can improve the physical health of the soil.

The continuous use of chemical fertilisers will increase the accumulation of chemical residues in the soil, polluting the soil ecosystem (Rabot *et al.*, 2018). Inorganic farming practices are still quite common in Indonesia, one of which is in the Tirtomoyo District,

Wonogiri Regency, Central Java Province. In 2018, Tirtomoyo District became the 4th highest rice-producing area in the Wonogiri Regency and supports the value of rice production in Central Java province (Central Bureau of Statistics, 2022). However, in the last 2 years, in 2021 and 2022, the harvested area and rice production have decreased, with the harvested area decreasing from 1.70 million hectares (in 2021) to 1.69 million hectares (in 2022), and rice production decreasing from 9.62 million tonnes (in 2021) to 9.36 million tonnes (in 2022). This condition necessitates the assessment of the physical health of the soil on rice fields in Tirtomoyo District. Evaluation of the physical health of the soil is expected to maintain and increase rice production in Tirtomoyo District. The study suggests that maintaining soil health is a crucial factor in achieving sustainability goals and should be considered as an ongoing process.

Research conducted by J. Lehmann *et al.* (2020) on soil health highlights the significance of soil health to strike an environmentally and economically sustainable balance. The role of technology and data analysis in advancing soil health is also important. M. That *et al.* (2020) found that soil health in the organic field has an improvement compared to inorganic fields and can economically address global food demands. These researchers assume that the soil farming system will influence the level or status of the soil physical health and impact the productivity of rice crops in rice fields (case study in Tirtomoyo District, Wonogiri, Indonesia). Generally, the physical health of the soil, such as water retention and the structure of rice fields, will be different from other land uses. The other objectives of the study are to find the relationship between the farming system, the physical health of the soil, and the productivity of rice yields, and innovative approaches to establishing the determining factors of the soil physical health. This purpose of this study was to examine how paddy field management affects soil health, identify its determinants, and develop strategies to enhance soil physical well-being.

MATERIALS AND METHODS

Study area description. This study was conducted in Tirtomoyo District, Wonogiri Regency, Central Java Province rice fields. Geographically, Tirtomoyo District is located at 111°0'14.32" – 111°8'57.39" E and 7°54'31.45" – 8°0'54.03"S with an altitude of 150 to 1,100 m above sea level. Hilly areas surround the

Tirtomoyo District, which is divided into two by the Wiroko River (Mujiyo *et al.*, 2018).

Pre-survey. The unit of analysis is based on the Land Map Unit (LMU) overlay results from maps of soil type, slope, rainfall, and rice field farming systems in the Tirtomoyo District using ArcGIS 10.3 software. The

soil type found in the area der study is Inceptisols. The slope is categorised into flat (0-8%), sloping (8-15%), slightly steep (15-25%), steep (25-45%), and very steep (>45%) (Fig. 2). Rice field farming systems in Tirtomoyo District consists of organic, semi-organic, and inorganic rice fields (Fig. 1).

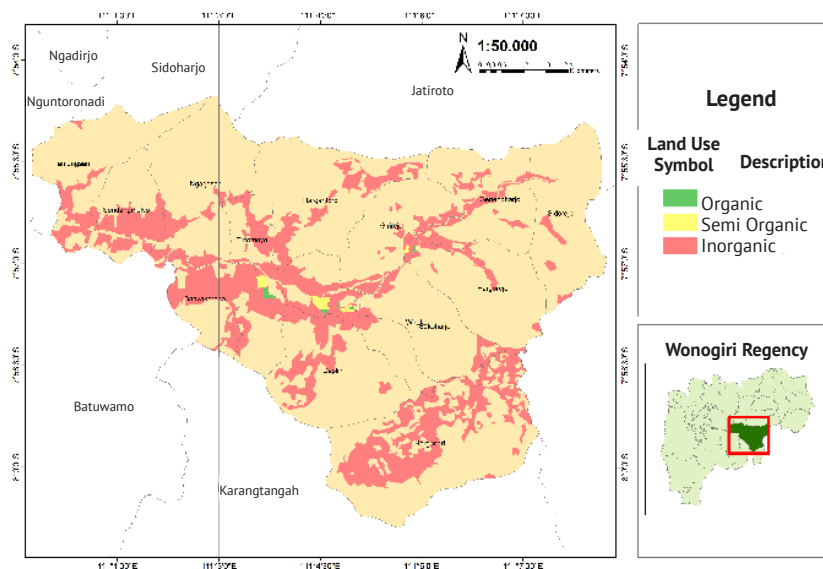


Figure 1. Map of rice fields farming system

Source: Indonesian Seamless Digital Elevation Model (DEM)

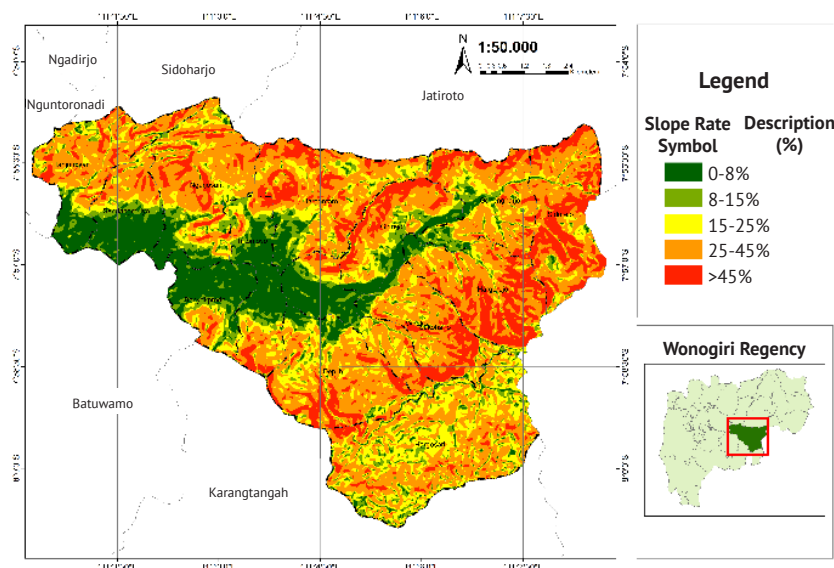


Figure 2. Map of slope

Source: Indonesian Seamless Digital Elevation Model (DEM)

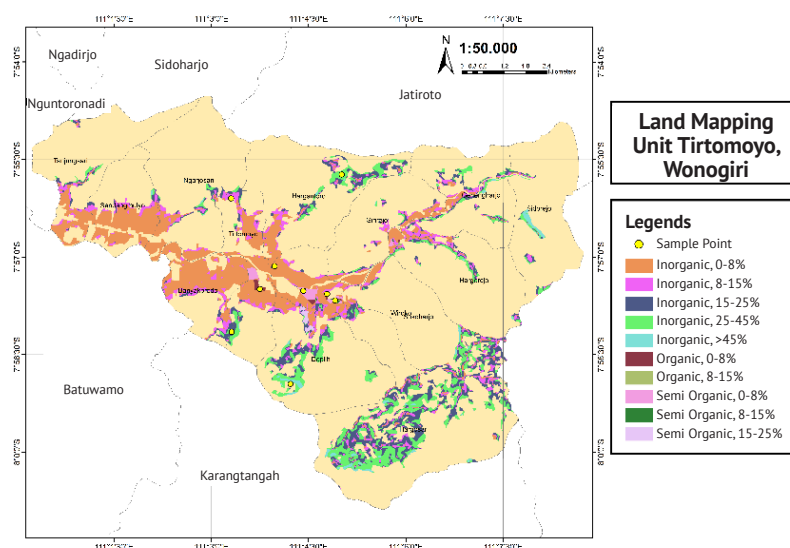
Purposive sampling methods were arranged to determine the sample points for each LMU. It is proportional and in a representative area. This study investigated 30 sample points from the results of 10 LMU with 3 replicates (Fig. 3). Land characteristics for each LMU are presented in Table 1. Soil samples were taken at rooting depth (1-20 cm) for each point, which were

then used for laboratory analysis. Parameters of soil effective depth, soil penetration resistance, and crop productivity were carried out directly in the field. Soil effective depth was measured using the soil drill method, penetration resistance – using the penetrometer method, and crop productivity – using the 1×1 m *ubinan* method (Arinta & Lubis, 2018).

Table 1. Characteristics of LMU in the area under study

Soil Type	Farming Systems	Rainfall (mm year ⁻¹)	The degree of Slope (%)
Inceptisols	Inorganic	2,119.9	0-8%
Inceptisols	Inorganic	2,119.9	8-15%
Inceptisols	Inorganic	2,119.9	15-25%
Inceptisols	Inorganic	2,119.9	25-45%
Inceptisols	Inorganic	2,119.9	>45%
Inceptisols	Organic	2,119.9	0-8%
Inceptisols	Organic	2,119.9	8-15%
Inceptisols	Semi organic	2,119.9	0-8%
Inceptisols	Semi organic	2,119.9	8-15%
Inceptisols	Semi organic	2,119.9	15-25%

Source: secondary data (Digital map and field survey)

**Figure 3.** Field observation and sampling point of the study

Source: secondary data

Laboratory analysis. Soil samples taken from the field were then dried for laboratory analysis. Analysis of research parameters refers to the technical guidelines of the Soil Research Institute (2006). The parameters observed were texture (by pipette method), moisture content (by gravimetric method), porosity (by pycnometer method), permeability (by permeameter method), and organic carbon (according to the method suggested by A. Walkley and C.A. Black (1934).

Soil physical health status determination. Soil physical health analysis using the method suggested by B.N. Moebius-Clune *et al.* (2017) was modified according to concrete research conditions. This study used 6 indicators of soil physical health: soil texture, permeability, penetration resistance, effective depth, moisture content, and porosity. Indicators in each replicate at each LMU were entered into a scoring table (Table 2), calculated, and categorised according to soil health values (Table 3).

Table 2. Scores of the soil physical health indicator

No.	Indicators	Very Low	Low	Medium	High	Very High
		Score 1	Score 2	Score 3	Score 4	Score 5
1.	Soil texture ⁽¹⁾	S, C, Si	SiC	SiL, SC	LS, SiCL, SCL	L, SL, CL
2.	Permeability (cm/h) ⁽²⁾	<0.025-0.125	0.125-0.5	0.5-2.0 and >25	2.0-6.25 and 12.5-25	6.25-12.5
3.	Soil Penetration Resistance (kg/cm ²) ⁽³⁾	>1.5	1.4-1.5	1.2-1.3	1-1.1	<1
4.	Soil Effective Depth (cm) ⁽⁴⁾	<10	10-25	25-50	50-90	>90
5.	Moisture Content (%) ⁽⁵⁾	31-41	1-3	4-10	11-18	19-30
6.	Total Porosity (%) ⁽⁵⁾	<5	5-10	10-25	25-40	>40

Note: S=Sand, C=Clay, Si=Silt, SiC=Silty Clay, SC=Sandy Clay, LS=Loamy Sand, SiCL=Silty Clay Loam, SCL=Sandy Clay Loam, L=Loam, SL=Sandy Loam, CL=Clay Loam

Sources: L. Puspitasari (2018); M. Aliero *et al.* (2018); L. Rachman (2019)

Table 3. Classification of soil physical health status

Soil Physical Health Value	Status
0-20	Very Low
20-40	Low
40-60	Medium
60-80	High
80-100	Very High

Source: B.N. Moebius-Clune et al. (2017)

The score results were then included in the calculation of soil health status using the formula 1 according to B.N. Moebius-Clune et al. (2017) as follows:

$$\text{Soil Physical Health Value} = \frac{\text{Score Summary}}{\text{Maximum Score}} \times 100. \quad (1)$$

Rice yield productivity. Observations of rice plant productivity were carried out at harvest time using the tile technique and calculating the yield of harvested dry grain (GKP) at the research location. The tile method is a technique that aims to determine the productivity or crop yield per hectare of rice field (Agoes et al., 2018). The tiling method is carried out by making sampling plots of 1×1 m area on the planted land and the resulting data is converted into t/ha units (Arinta & Lubis, 2018). Ubinan calculation was performed according to formula 2.

$$\text{Rice productivity} = \frac{10,000 \text{ m}^2}{\text{Ubinan total area}} \times \text{weight of dry grain}. \quad (2)$$

Statistical analysis. In this study, researchers used a determining factor approach. Determining factors are those considered to accurately determine the physical health of the soil based on the results of statistical tests. The statistical tests conducted in this study were one-way ANOVA, DMRT, and Pearson's correlation test. One-way ANOVA was used to determine the effect of rice field farming system (organic, semi-organic, and inorganic) on the soil physical health indexes and properties. Furthermore, the relationship between the physical health of the soil and the productivity of rice plants, as well as the determinants of soil physical

health (relationships between indicators and the physical health status of the soil) were analysed using Pearson's correlation. Crop productivity sampling was conducted using the method suggested by the Indonesian Central Bureau of Statistics in 2020. Data from the ANOVA analysis and Pearson's correlation were then used as the basis for determining suitable and efficient recommendations for land management in an effort to maintain the health of rice fields' soil. The materials and methods used in this study have followed national guidelines in Indonesia. In addition, researchers referred to the standards requirements and provisions of Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1979).

RESULTS AND DISCUSSION

Data and information of soil health status is essential in supporting the growth of plant. Soil physical health contributes to the decomposition of organic matter and the recycling of nutrients. A healthy soil with good physical properties is essential for maintaining a balanced and functioning ecosystem (Guo, 2021). The higher the value of the soil health, the healthier the soil physical condition. Soil physical properties such as texture, permeability, penetration resistance, effective depth, soil moisture content, and porosity influence soil physical health condition. The study results showed that rice fields in the area under study are classified into 2 categories – medium and high. Rice fields with medium category are found in LMU 1 and LMU 2. Other LMUs of the rice fields were classified into high category (Table 4).

Table 4. Data results of soil physical health in each LMU

LMU	Replication No.	Indicators Score						Accumulation Score	Status	Rice Productivity (ton/ha)
		T	PE	PR	ED	MC	PO			
		(cm/h)	(kg/cm)	(cm)	(%)	(%)	(%)			
1	1	1	2	3	3	4	5	60	Medium	6.75
	2	1	2	4	3	4	5	63.33	High	6.86
	3	5	2	4	3	4	5	76.67	High	7.59
2	1	1	2	3	3	4	5	60	Medium	6.80
	2	1	2	3	3	5	5	63.33	High	6.86
	3	5	2	3	3	5	5	76.67	High	7.44
3	1	5	2	3	3	4	5	73.33	High	7.28

Table 4. Continued

LMU	Replication No.		Indicators Score					Accumulation Score	Status	Rice Productivity (ton/ha)
	T	PE	PR	ED	MC	PO				
							(cm/h)			
4	2	4	2	3	3	4	5	70	High	7.03
	3	5	2	3	3	5	4	73.33	High	7.34
	1	5	2	2	3	4	4	66.67	High	6.88
	2	5	2	3	3	5	5	76.67	High	7.45
	3	5	2	2	3	4	5	70	High	7.10
5	1	4	2	2	3	5	4	66.67	High	6.89
	2	5	2	3	3	4	5	73.33	High	7.32
	3	4	2	2	3	4	5	66.67	High	6.98
6	1	4	2	4	4	4	5	76.67	High	7.56
	2	3	2	5	3	5	5	76.67	High	7.58
	3	3	2	4	4	5	5	76.67	High	7.65
7	1	1	2	5	4	4	5	70	High	7.14
	2	4	2	5	4	5	5	83.33	High	7.59
	3	4	2	4	4	4	5	76.67	High	7.61
8	1	1	2	5	3	4	5	66.67	High	6.98
	2	3	2	5	3	5	5	76.67	High	8.02
	3	1	2	4	3	4	5	63.33	High	6.95
9	1	4	2	4	3	4	5	73.33	High	7.96
	2	4	2	3	3	4	5	70	High	7.17
	3	4	2	3	3	5	5	73.33	High	7.88
10	1	4	2	4	3	4	5	73.33	High	7.38
	2	4	2	3	3	4	5	70	High	7.23
	3	4	2	5	3	4	5	76.67	High	8.23

Notes: T – Texture; PE – Permeability; PR – Penetration Resistance; ED – Effective Depth; MC – Moisture Content; PO – Porosity

Source: compiled by the authors of this study

The analysis results (Fig. 4) suggest that soil physical health significantly correlates with rice productivity ($r=0.465$; $P\text{-value}=0.010$; $n=30$). The higher the soil physical health value, the higher the yield productivity of the rice crop. Good soil health will increase crop productivity.

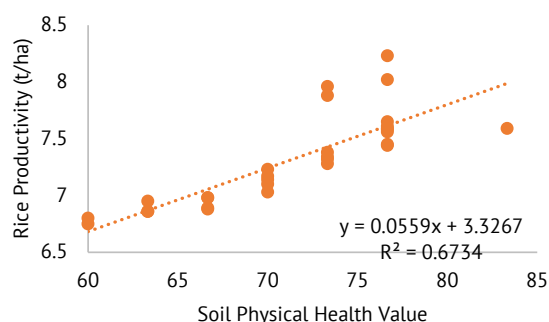


Figure 4. Correlation of soil physical health index with rice yield productivity

Source: compiled by the authors of this study

The analysis of variance showed that the rice field farming system significantly affects soil physical health status ($F=4.569$; $P\text{-value}=0.02$; $n=30$). Organic rice fields have the highest average soil health with 76.69,

while inorganic and semi-organic rice fields have an average of 69.11 and 71.48 (Fig. 5).

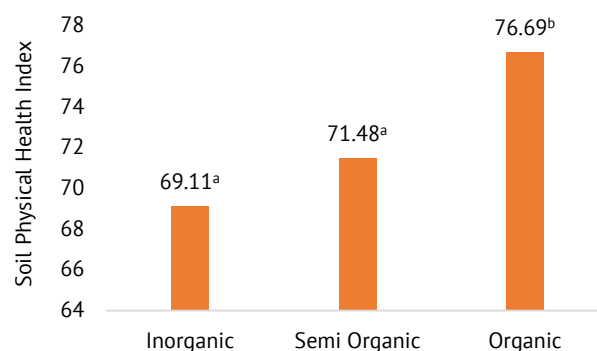


Figure 5. Effect of rice field farming system on soil physical health

Note: different letters on the chart are significantly different values in the 5% level of DMRT

The differences in average values of soil physical indicators are presented in Table 5. Another factor that can directly or indirectly affect soil and physical health indicators is the organic carbon content in the soil. Organic carbon is important in supporting sustainable agriculture since it can improve the physical

properties of the soil. Based on the analysis of variance, the farming system significantly influenced organic carbon in the soil and several physical characteristics such

as penetration resistance ($F=12.765$, $P\text{-value}=0.000$, $n=30$), effective depth ($F=42.528$, $P\text{-value}=0.000$, $n=30$), and porosity ($F=18.071$, $P\text{-value}=0.000$, $n=30$).

Table 5. Effect of rice field farming system on soil physical health properties

Parameter	Rice field System		
	Organic	Semi-organic	Inorganic
Organic carbon	1.53a	1.32b	1.19c
Soil penetration resistance	0.88b	0.97b	1.28a
Soil effective depth	56.67a	39.44b	38.67b
Soil porosity	46.39a	44.36b	42.43c
Soil permeability	0.34a	0.34a	0.37a
Soil moisture content	19.59a	17.39a	18.79a

Note: numbers followed by the same letter are not significantly different at the 5% of Duncan's test

Source: compiled by the authors of this study

Correlation tests between soil physical health indicators and soil physical health values were conducted to establish the determinants of soil physical health (Table 6). The correlation test results showed that indicators of penetration resistance, porosity, and texture

determine soil physical health. Soil physical health was significantly correlated with penetration resistance ($r=-0.424$; $P\text{-value}=0.019$; $n=30$), porosity ($r=0.473$; $P\text{-value}=0.046$; $n=30$), and soil texture ($r=0.627$; $P\text{-value}=0.000$; $n=30$).

Table 6. Correlation between soil physical health indicators and soil physical health (as determinant factors)

	Soil Texture	Permeability	Soil Penetration Resistance	Soil Effective Depth	Moisture Content	Porosity	Organic Carbon	Soil Physical Health
Soil Texture	1							
Permeability	0.261	1						
Soil Penetration Resistance	0.330	0.205	1					
Soil Effective Depth	-0.055	-0.330	-0.330	1				
Moisture Content	-0.078	-0.083	-0.083	0.044	1			
Porosity	0.032	-0.147	-0.471**	0.589**	-0.266	1		
Organic Carbon	0.387*	-0.052	-0.380*	0.507**	0.240	0.046	1	
Soil Physical Health	0.627**	0.047	-0.424*	0.336	0.181	0.473**		1

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

Efforts to improve soil physical health are focused on improving the conditions of determinant factors, including penetration resistance, porosity, and texture. Based on the study results presented in Table 4, soil physical properties are distributed in various values. Soil texture in each replication at each LMU provides a variety of values. Soil texture with the lowest value was found at LMUs 1, 2, 7, and 8, namely clay with a value of 1. Soil texture with the highest value was found at LMUs 1, 2, 3, 4, and 5, namely sandy loam and loamy sand with a value of 5. The effects of farming systems on soil texture in fields are complex and depend on factors such as the presence of plants, soil texture, and the addition of organic matter (Bacq-Labreuil et al., 2018). These factors interact to influence the diversity of pore

sizes, porosity, and pore connectivity, ultimately affecting water availability, nutrient uptake, and overall soil health.

Soil permeability in all LMUs under study had the same values, categorised as 2. Permeability in paddy fields in Tirtomoyo District ranges within 0.26-0.44 cm/h (Table 4). Soil permeability with a value range within 0.0125-0.5 cm/h is included in the slow category. Permeability is related to soil texture. Soil permeability is classified as very slow, and in some LMUs, the soil is classified as clay. This is in line with the findings of F. Louati et al. (2018), which explain that soil permeability is at a very slow level with a high proportion of clay. Soil penetration resistance values tend to vary from 0.5 to 1.5 kg/cm. Soil penetration resistance with

a score of 2 was found at LMUs 4 and 5. Penetration resistance with the highest score of 5 was found at LMUs 6, 7, 8, and 10 (Table 4). Soil penetration resistance indicates whether or not the soil is easy for plant roots to penetrate. The easier it is to penetrate the soil, the smaller the penetration resistance value.

The highest effective soil depth was found in LMUs 6 and 7 at 60 cm with a score of 4, while effective soil depth with a score of 3 was found in all LMUs except LMU 7 (Table 4). Soil moisture content in the Tirtomoyo District ranges within 12.04-26.01%. Soil moisture content with a score of 4 was found in all LMUs, while soil moisture content with a score of 5 was found in LMUs 2, 3, 4, 5, 6, 7, 8, and 9 (Table 4). Soil moisture content is better in soil with high organic matter than soil with low organic matter. Organic matter in the soil enables better infiltration and retention. It acts as a sponge, holding water and making it available to plants. This results in higher soil moisture content (Tokova *et al.*, 2020). Soil porosity in Tirtomoyo District ranges within 40.97-47.27%. Soil porosity with a score of 4 was found at LMUs 3, 4, and 5, while the rest of LMUs porosity had the highest score of 5 (Table 4).

Rice productivity in the area under study had various values, ranging within 6.75-8.23 t. The highest rice productivity was at LMU 10 with 8.23 t, while the lowest was at LMU 1 with 6.75 t (Table 4). Rice field system affects rice productivity. Using organic fertilisers positively affects rice productivity since it improves soil properties. According to M. Salam *et al.* (2021), using organic fertilisers can increase rice plant production by 16.67%. The study results showed that organic farming and semi-organic farming of rice fields have higher productivity than inorganic fields. Organic rice fields have a productivity of 7.52 t/ha and semi-organic – 7.53 t/ha, higher than inorganic rice fields with 7.1 t/ha. According to P. Gao *et al.* (2023), combining organic and inorganic fertilisers is better for maintaining and increasing crop yields. Semi-organic rice fields have high productivity because it gets inputs from organic and inorganic fertilisers, and therefore rice plants' macro and micronutrient needs can be satisfied.

An analogous study was conducted by I. Chahal *et al.* (2021), where soil health indicators were positively correlated with crop productivity. Proper soil structure and aeration are essential for root development and nutrient uptake, ultimately contributing to higher yields. Additionally, well-structured soil helps in water infiltration and retention, reducing the risk of waterlogging or drought stress (Goswami *et al.*, 2020). On the other hand, compacted or poorly drained soil can hinder root growth, nutrient availability, and water movement, leading to lower rice yields. Therefore, maintaining favourable soil physical conditions through residue incorporation and reduced tillage can enhance rice productivity. Index values in organic farming are the highest. Soil physics indicators in organic

farming have better conditions than in semi-organic and inorganic farming. Soil physical health properties are essential for improving soil health to achieve maximum productivity. Increased values of soil properties in organic rice fields are due to the provision of organic matter in higher fertilisers than in semi-organic and inorganic rice fields.

Farming systems in the area under study significantly differ in soil physical health values and properties such as organic carbon content, penetration resistance, effective depth, and porosity (Table 5). Organic rice fields had the highest organic carbon content, with an average of 1.53%, while inorganic rice fields had the lowest average organic carbon content with 1.19%. Semi-organic rice fields have a moderate organic carbon content of 1.32% (Table 5). Organic farmers promote the growth of diverse plant species and encourage the accumulation of organic matter in the soil. This organic matter, such as plant residues and animal manure, the decomposition process is influenced by the presence of soil fauna. The activity of soil fauna in organic farming systems can enhance the decomposition process, releasing nutrients essential for plant growth (Frouz, 2018). This is in line with the earlier study conducted by Supriyadi *et al.* (2020), which found that different treatments on organic and inorganic rice fields affect the organic carbon content. According to J. Gerke (2022), providing organic matter in the soil positively affects the organic carbon content. Organic rice fields have the highest organic carbon content compared to semi-organic and inorganic rice fields due to the application of higher organic fertilisers during management.

The rice field farming system has a significant effect on soil penetration resistance. Inorganic farming had the highest average penetration resistance of 1.28 kg/cm², while semi-organic fields had a lower average of 0.97 kg/cm². Organic fields had the lowest average of penetration resistance at about 0.88 kg/cm² (Table 5). According to J. Zemke *et al.* (2020), soil penetration resistance is a critical property that supports plant root growth and is influenced by the soil's organic matter content. In line with research conducted by J. Filho *et al.* (2022), soil organic matter content affects soil penetration resistance, especially when the soil is in a dry condition.

Organic rice fields have the highest average effective depth of 56.67 cm, significantly different from inorganic and semi-organic rice fields with an average effective depth of 38.67 cm and 39.44 cm, respectively (Table 5). This is because rice plants' roots in organic fields are stronger than in semi-; and inorganic fields. The root system of rice crops on organic farming can reach deeper areas due to longer and stronger roots. This condition has a positive impact on plant growth and productivity, as it allows for the absorption of nutrients. High availability of nutrients in organic-rich allowing for better root penetration and exploration of

the soil profile. According to A. Jayasekara *et al.* (2022), organic matter input in rice fields can increase the depth of plant roots. That proves organic rice fields have a greater effective soil depth than inorganic and semi-organic rice fields. Deeper effective soil depth is explained by higher organic matter in organic rice fields. Providing organic materials in solid and liquid organic fertilisers can increase the effective depth of the soil. According to I. Wedhana *et al.* (2018), soil with a shallow effective depth will inhibit the development of plant roots. The deeper effective depth of the soil will positively affect the movement of rice plant roots. Soil that is easily penetrated will provide best growing space for plants. The combination of fertiliser application increased soil organic carbon (SOC), leading to improved soil aggregate and decreased soil bulk density.

Organic rice fields have the highest soil porosity value, averaging 46.39%. Inorganic rice fields have the lowest soil porosity value, with an average of 42.43%, while semi-organic rice fields have an average of 44.36% (Table 5). The three rice field farming systems have significantly different soil porosity values when compared to each other. The difference in porosity value is caused by applying organic fertiliser on organic and semi-organic paddy fields. According to D. Holthusen *et al.* (2018), soil porosity is influenced by differences in farming system. The study results proved that the rice field farming systems have significantly different soil porosity values when compared to each other. Organic rice fields have higher total porosity than semi-organic and inorganic rice fields. According to H. Fang *et al.* (2021), the provision of organic matter can affect soil porosity through several aspects, such as increasing soil aggregate stability, reducing soil compaction, and improving soil structural conditions. The difference in porosity value is caused by the provision of organic materials in solid and liquid fertilisers, which are higher in organic rice fields. Using organic fertilisers can increase the total porosity in the soil because decomposed organic matter in the soil will produce aggregates that are beneficial to soil porosity.

Determinant factors of soil physical health include soil texture, penetration, and porosity (Table 7). The higher the penetration resistance value, the lower the soil's physical health status. Low penetration resistance indicates that it is easier for roots to penetrate the soil to obtain nutrients, and therefore it can improve the physical health status of the soil. High values of soil penetration resistance are commonly found in soil with compact condition. A low compaction indicates better water holding capacity and lesser soil erosion potential. However, many factors determine soil physical health, such as particle size distribution, pore volume, hydrophobicity, and pore connectivity (He *et al.*, 2021). Therefore, it is essential to consider other indicators and factors when evaluating soil health, rather than relying solely on soil bulk density. Soil compaction

relates to soil porosity. Low value of soil compaction indicates a high soil porosity. The higher the porosity value, the higher the soil physical health. This condition causes good soil porosity conditions to correlate with improved soil physical health status. Soil texture was significantly correlated with soil physical health status (Table 7). According to B.N. Moebius-Clune *et al.* (2017), texture is essential for various processes in the soil. Soil texture affects other soil health indicators. Soils with higher clay content generally have a higher ability to retain nutrients and accumulate soil organic matter. Good soil texture conditions will affect other physical indicators and improve soil physical health status.

The factors are enhanced by increasing the organic carbon content in the soil. The efforts to improve soil physical health will be focused on increasing the organic carbon content in the soil. That is because organic carbon content is significantly correlated with the determinants of soil physical health, namely soil penetration resistance and soil porosity. According to K. Kakar *et al.* (2020), applying organic fertilisers to the soil can improve soil physical properties. That will increase the ability of the soil to hold water and nutrients needed for crop production. According to B. Lei *et al.* (2022), providing organic matter as manure will improve soil health and organic carbon content. Improved soil physical health will indirectly increase the productivity of rice plants. The organic carbon content in rice fields can be increased by applying organic fertiliser from livestock manure or crop residues. An increase in organic carbon content in the soil causes an increase in total soil porosity. The organic carbon content in paddy fields can be increased by applying organic fertilisers from animal manure and crop residues. Organic matter in the form of composted straw and animal manure applied to the soil can increase the organic carbon content in the soil (Yuniarti *et al.*, 2019). Using compost and manure can boost the organic carbon content in the soil. The more organic fertiliser is added to the soil, the more organic carbon content is released.

Another effort to increase organic carbon content in the soil can be made by applying biochar. According to A. Mohammadi *et al.* (2020), biochar application can be used to improve the condition of soil properties. Biochar is organic material burned at temperatures between 300°C and 700°C with low oxygen concentration. Biochar can improve soil physical health through several mechanisms. Biochar has a high porosity and large surface area, which can improve soil water retention and reduce water evaporation. This enhances soil moisture availability for plant growth. Additionally, biochar can improve soil structure and aggregation, leading to better soil aeration and drainage. This can enhance plant root penetration and nutrient uptake. Introducing biochar such as rice husks can increase the organic carbon content in the soil. Increasing the dose of biochar positively correlates with the organic carbon content

(Ebido *et al.*, 2021). Therefore, it is best to use biochar at the beginning and post-harvest of tillage. Previous research has shown that biochar can significantly improve the physical health of the soil by increasing organic carbon content, soil water retention, soil structure and aggregates, and plant root penetration and nutrient uptake.

CONCLUSIONS

Rice fields managed by flooding affect the physical characteristics of rice fields and have an impact on the soil's ability to support plant growth. Low soil physical health conditions will inhibit plants' absorption of water and soil nutrients. Based on the findings of this study, the physical health status of organic, semi-organic, and inorganic rice fields is classified as medium and high (index range within 60.00-83.33). Organic rice fields have the highest physical soil health compared to semi-organic and inorganic rice fields, with respective values for organic rice fields 76.69b, semi-organic rice fields 71.48a, and inorganic rice fields 69.11a. The findings of the study on rice fields suggest that the indicators of soil penetration resistance ($r=0.424$), soil porosity ($r=0.473$), and soil texture ($r=0.627$) greatly determine the soil physical health. Soil health is positively correlated with soil porosity and soil texture that has a high clay content. However, conversely, the lower the soil penetration, the higher the soil physical health. The physical health of the soil is significantly related to

determining rice production results $r=0.465$). This means that the physical health of the soil determines rice yield production in the area under study. The higher the soil physical health index, the greater the yield. Recommended farming system efforts to overcome the determinants of physical soil health found include expanding the coverage of rice fields with an organic system, or by providing organic fertiliser and biochar during tillage in preparation for planting. Future research in soil physical health, with a focus on sustainable agriculture and environmental protection, has the potential to enhance soil resilience to climate change impacts, such as extreme temperatures, prolonged droughts, and increased flooding. Developing practical tools to assess soil physical health can aid in understanding the complex interactions between physical properties, soil health levels, and optimising crop production through water availability for plant growth.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Фізичний стан ґрунту за різних систем землеробства на рисових полях та його вплив на продуктивність рису (*Oryza sativa* L.)

Ганджар Гердіансях

Викладач

Університет Себелас Марет

57126, вул. Ір. Сутамі, 36, м. Суракарта, Індонезія

<https://orcid.org/0000-0001-5841-4642>

Муджійо Муджійо

Професор педології та обстеження ґрунтів

Університет Себелас Марет

57126, вул. Ір. Сутамі, 36, м. Суракарта, Індонезія

<https://orcid.org/0000-0002-6161-7771>

Актавія Гераваті

Викладач

Університет Себелас Марет

57126, вул. Ір. Сутамі, 36, м. Суракарта, Індонезія

<https://orcid.org/0000-0001-5278-8811>

Ханіндьо Брамастомо

Бакалавр

Університет Себелас Марет

57126, вул. Ір. Сутамі, 36, м. Суракарта, Індонезія

<https://orcid.org/0009-0009-3828-0531>

Анотація. На фізичний стан ґрунту впливають декілька факторів, у тому числі системи землеробства, що безпосередньо впливає на ріст рослин і продуктивність ґрунту. Вивчення фізичного стану ґрунту в різних системах землеробства допоможе управляти процесами використання ґрунту та води. Метою цього дослідження є визначення впливу системи управління рисовими полями на фізичний стан ґрунту, виявлення детермінант фізичного стану ґрунту та розробка відповідних заходів для покращення фізичного стану ґрунту. У цьому дослідженні були використані описовий, дослідницький та опитувальний підходи із застосуванням методів цілеспрямованого відбору зразків ґрунту. Результати показали, що фізичний стан ґрунту на рисових полях у підрайоні Тіртомойо був класифікований як помірно здоровий і здоровий. Відмінності в системі управління рисовими полями впливають на фізичний стан ґрунту. Органічно керовані рисові поля мають найвищий показник фізичного здоров'я ґрунту – 76,69. Напіворганічні та неорганічні рисові поля мають нижчі показники здоров'я – 71,48 та 69,11. Стійкість ґрунту до проникнення, пористість і текстура ґрунту є визначальними факторами, оскільки вони можуть покращити індикаторні умови і фізичний стан ґрунту. Можна докласти зусиль для покращення фізичного стану ґрунту шляхом внесення органічних добрив та біомаси на рисові поля. Знаючи взаємозв'язок між показниками фізичного стану ґрунту та врожайністю рису, можна сподіватися, що фермери та інші зацікавлені сторони зможуть підвищити врожайність культури за рахунок покращення фізичного стану ґрунту

Ключові слова: визначальні фактори; система землеробства; органічний вуглець; врожайність рису; проникнення в ґрунт; пористість ґрунту



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Evaluation of aflatoxin M1 and nutritional content in Kosovo's market cheese samples

Arieta Camaj Ibrahim

PhD in Food Science and Technology, Assistant Professor

University Haxhi Zeka

30000, "UÇK" Str., Pejë, Republic of Kosovo

<https://orcid.org/0000-0002-3560-3704>

Bajram Berisha

PhD in Agricultural Sciences, Full Professor, Doctor Habilitatus

University of Prishtina

10000, 31 George Bush Str., Prishtine, Republic of Kosovo

<https://orcid.org/0000-0001-9112-054X>

Hata Dibrani Sopjani

Master of Science, Chemist

Food and Veterinary Agency

10000, "Nëna Terezë" Sq., Prishtinë, Republic of Kosovo

<https://orcid.org/0009-0008-6245-9024>

Aferdita Camaj Isa

PhD in Chemistry, Quality Control Manager

Kosova Medicines Agency

10000, Lagjia e Spitalit, Prishtinë, Republic of Kosovo

<https://orcid.org/0000-0001-7044-8810>

Shyhrete Muriqi*

PhD in Agroecconomics, Assistant Professor

University Haxhi Zeka

30000, "UÇK" Str., Pejë, Republic of Kosovo

<https://orcid.org/0000-0002-3389-0594>

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Abstract. The ubiquitous presence and potent carcinogenic effects of aflatoxins, a group of mycotoxins, pose a significant threat to global food safety, especially within the dairy sector. This research aimed to evaluate the prevalence and distribution of Aflatoxin M1 in diverse cheese varieties within Kosovo's markets in 2022 and discern potential correlations with cheese types, processing conditions, and nutritional parameters to enhance understanding and ensure the safety of dairy products. In the latter half of 2022, a total of 93 different types of manufactured cheese samples from 15 different

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*Corresponding author

countries were analysed for Aflatoxin M1 toxin content using the enzyme-linked immunosorbent assay technique. The results of the research indicate that several countries have exceeded the concentration of 0.05 µg/kg for Aflatoxin M1, set as the maximum tolerable limit for milk and milk products in many countries. Notable, cheese samples from Albania exhibited substantially ($p=0.000$) higher levels of Aflatoxin M1 compared to other nations. The results showed that the Aflatoxin M1 concentration did not exhibit statistically significant variations based on the regulated total fat content ($p=0.902$), carbohydrate content ($p=0.741$), protein concentration ($p=0.298$), or salt content ($p=0.122$) within the cheese. The results demonstrated that Aflatoxin M1 levels were not significantly ($p=0.926$) affected by cheese consistency. While different starter cultures were associated with varying Aflatoxin M1 concentrations, these differences were not statistically significant ($p=0.472$). Similarly, ageing time did not exert a significant ($p=0.691$) impact on AFM1 levels, as concentrations remained relatively consistent across different ageing periods. These findings provide valuable insights into the variations in Aflatoxin M1 levels in cheese samples among different countries. They highlight the importance of regulatory measures and continuous monitoring to ensure food safety and adherence to maximum allowable limits

Keywords: cheese types; ELISA; Aflatoxin M1 contamination; dairy product safety

INTRODUCTION

Aflatoxins, toxic by-products created predominantly by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, are a type of mycotoxin. These toxins frequently contaminate food and are especially prevalent in the basic foods of numerous developing nations. Aflatoxins are generated through fungal activity at various stages, including production, harvesting, storage, and processing of food. The US Food and Drug Administration (FDA) (2022) regards these toxins as unavoidable food contaminants. Of all the various aflatoxins, Aflatoxin B1 (AFB1), poses a distinct risk to the dairy industry. When ruminants consume feed contaminated with AFB1, their bodies metabolize it, leading to the secretion of aflatoxin M1 (AFM1) in their milk. Aflatoxins are known to cause liver toxicity, cancerous effects, and suppression of the immune system (Ferrari *et al.*, 2023). As noted by the World Health Organization (2018) also, AFM1 is associated with liver and kidney cancers and may potentially compromise the immune system, weakening its disease-fighting capacity.

These substances can be present in numerous crucial agricultural and food items, predominantly influenced by factors such as product moisture content, water activity, relative air humidity, temperature, pH value, composition of the food matrix, the extent of physical damage, and the existence of mould spores (Pleadin *et al.*, 2019). The resilience of AFM1 across various temperatures and pH levels, which enables it to remain stable through different phases of dairy processing, is very concerning. Consequently, conventional heat methods like pasteurization and sterilization are unable to eliminate it effectively. Existing and developing physical techniques, including pulsed electric fields and various nonthermal processes, along with the use of chemical substances like acids, enzymes, gases, and absorbents in livestock management, have proven to be successful in diminishing the levels of mycotoxins in both feed and food products (Sipos *et al.*, 2021). Although AFM1 is less toxic than its precursor AFB1, it is still considered a potential human liver carcinogen and is categorized as

Group 2B by the International Agency for Research on Cancer (IARC, 2002).

The European Commission (2006), enforces strict regulations on AFM1 levels, given its ubiquitous presence and serious health consequences. Continuous monitoring and effective control measures are crucial for managing AFM1 contamination in dairy products, ensuring food safety and public health, as AFM1 could be passed from milk into milk products. N. Salari *et al.* (2020) conducted a systematic review and meta-analysis to assess the global occurrence of AFM1 in milk. They examined 122 articles, encompassing a total of 18,921 samples from January 1988 to February 2020, and found that 79.1% of the milk samples contained AFM1, indicating a widespread presence of this toxin in milk around the world. According to a study done by J. Kos *et al.* (2023), as the climate conditions in Europe continue to provide increasingly favourable conditions for the proliferation of *Aspergillus flavus*, there is an escalating likelihood of cereals being exposed to elevated risks of contamination by highly hazardous aflatoxins in the future.

The transfer of AFM1 from milk to cheese is unavoidable, considering that this toxin is not removed through conventional treatment methods. Nevertheless, the concentration of AFM1 in various types of cheese and other dairy products has been found to vary. Kosovo, a Balkan region with substantial dairy production and consumption, is no exception to this challenge. Yet, comprehensive, and systematic data on AFM1 contamination in the region is scant. This issue becomes even more critical considering the local population's dietary habits, with milk and dairy products playing a key role (FAO, 2013). As per a study done by A. Camaj *et al.* (2018), high percentage of milk samples which were non-compliant with AFM1 maximum levels. However, a subsequent study by A. Camaj *et al.* (2023) concluded that the prevalence of AFM1 contamination of raw milk in Peja, Kosovo, was lower than previously reported. All samples complied with European Union regulations for

AFM1, indicating increased farmer awareness regarding feed storage and regular AFM1 monitoring. However, the study emphasized the need for regular institutional monitoring of imported UHT milk samples. Yet, published data on AFM1 levels in cheese consumed in Kosovo, particularly those imported, is lacking. This study is crucial as a variety of foreign cheese types are available in Kosovo's markets.

Accordingly, this study aimed to assess the presence and distribution of AFM1 in various cheese types available in Kosovo's markets during 2022, including both locally produced and imported cheeses, and to assess if there is a correlation between cheese types, processing conditions, and nutritional parameters, to the aflatoxin M1 level in cheese. This study is the first article of this context done in Kosovo.

MATERIALS AND METHODS

Sample Collection. In the latter half of 2022, a total of 93 different types of manufactured cheese samples were procured from the largest food suppliers across Kosovo for the analysis of the AFM1 toxin content. The chemical analysis of the toxin AFM1 was conducted in the laboratories of the Food and Veterinary Agency of Kosovo. Additionally, other parameters like, the cheese's nutritional parameters including fat total (g/100g), carbohydrates total (g/100g), protein (g/100g), and salt (g/100g); parameters such as, consistency (hard, semi-hard, semi-soft, semi-soft creamy, soft, and soft-creamy); starter cultures (bacterial cultures, bacterial-rennet, bacterial-rennet-fungal, rennet, bacterial-rennet-processed, rennet-processed); and ageing time (none, 1-3 months, 4-6 months, 7-12 months, <13 months) are considered for the comparative analysis. These data were gathered from the producers' declarations on labels as well as internet sources. These samples originated from fifteen distinct countries, including: Kosovo (6 samples), North Macedonia (6), Albania (3), Slovenia (3), Greece (5), Italy (27), Germany (7), France (5), Croatia (5), Netherlands (3), Turkey (3), Hungary (5), Lithuania (3), Austria (9), and Denmark (3). To ensure optimal preservation and accurate analysis, these samples were stored in deep freeze conditions at -18°C. They were then brought up to room temperature prior to the execution of the analyses.

Laboratory analyses. The procedure for quantitatively analysing AFM1 in the cheese samples was carried out using the enzyme-linked immunosorbent assay (ELISA) technique. The assessment of AFM1 was conducted utilizing the MEIZHENG Bio-Tech Co. Aflatoxin M1 ELISA Test Kit, strictly adhering to the protocol provided by the manufacturer, briefly outlined below. Each testing reagent

Kit offered a certificate with the validation results. All the samples were brought to room temperature before analysis. The dilution factor was 9. For testing, 1 g of each sample and 4 mL of methanol were mixed into a centrifuge tube and put to vortex to mix well. Then the mixture was put into the centrifuge at 4000 rpm for 5 minutes. From the mixture, 1 mL of the supernatant was transferred into a centrifuge tube and blow dry under 50-60°C Termovap Sample Concentrator. In each tube was added 1 mL of Sample Diluting Buffer II and vortexed to mix well. For the assay was taken 50 µL of the sample dilution.

The Aflatoxin M1 ELISA Test Kit operates on the principle of an indirect competitive enzyme-labelled immunoassay. The Aflatoxin M1 antigen is pre-embedded in the wells. Within the sample, the aflatoxin M1 vies for binding with the aflatoxin M1 antibody against the aflatoxin M1 antigen already present on the well. Meanwhile, the M1 antibody associates with the enzyme conjugate. Subsequently, the substrate solution is added to the wells, which prompts a colour change. The colour intensity of the unknown samples is compared to that of the standards, enabling the determination of Aflatoxin M1 concentration. Using a plate reader set to 450 nm, absorbance values were recorded. The AFM1 level was then calculated from a logarithmic standard curve, and the mean value of the duplicates was recorded as results.

Based on the information provided in the manufacturer's certificate, the sensitivity was 0.015 µg/kg, equivalent to the Kit's Limit of Detection (LOD) for raw milk determination. This implies that the Kit might detect aflatoxin M1 at concentrations even lower than the LOD. Typically, sensitivity aligns with the concentration of the second standard (Table 1). The percent recovery was within the range of 100%±30%, and the intra-lab assay precision was denoted with a Coefficient of Variation (CV%) less than 10%. In this study, it's crucial to note that the kit's LOD for cheese determination was 0.06 µg/kg, which is slightly above the maximum permissible limit of aflatoxin M1 in cheese as adapted by regulatory standards in Kosovo. (0.05 µg/kg). The observations included results that fell below the Kit's stated LOD. This suggests that some cheese samples contained extremely low, or potentially even undetectable, levels of aflatoxin M1.

Aflatoxin M1 ELISA Test Kit standard summary. The ELISA test kit validation certificate of analysis by the manufacturer provides reference points for comparing the results obtained from samples tested with the Aflatoxin M1 ELISA Test Kit. Table 1 shows the absorbance values A450, B/B0 ratios, and CV% values for different concentrations of Aflatoxin M1 standards used in the validation.

Table 1. Aflatoxin M1 ELISA test Kit validation results

Aflatoxin M ₁ (ng/kg)	A ₄₅₀	B/B ₀	CV%
Negative control			
0	1.836	100.0	2.1

Table 1. Continued

15	1.410	76.2	4.2
45	0.901	49.1	3.7
150	0.356	19.4	0.4
500	0.090	4.9	3.1

Notes: A_{450} = Average absorbance using 450 nm primary filter and 630 nm differential filter

Source: developed by the authors

CV% (Coefficient of Variation) is calculated as:

$$CV = (SD/\mu) \times 100\%, \quad (1)$$

where SD is the standard deviation of the replicates (two replicates for each concentration of the standard), and μ is the average of replicates.

Statistical analysis. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS). The data were categorized into fifteen groups based on the origin of the cheese. The AFM1 content was reported as mean values with standard deviation (SD), median, minimum, and maximum concentration. To determine if there were significant differences between groups, statistical tests were employed. To explore variations in AFM1 levels between different groups, the dataset was categorized based on the concentration of AFM1. These classifications were established according to their adherence to a designated threshold value falling within the range of >0.050 and <0.051 $\mu\text{g/kg}$. For comparisons between two groups, an

Independent Sample t test was employed to assess differences in AFM1 levels within this defined range. One-way Analysis of Variance (ANOVA) was used to assess the influence of AFM1 levels on the consistency, starter cultures, and ageing time of different types of cheeses. The grouping of cheese samples into three or more categories was based on relevant factors such as cheese variety, production methods, and geographical origin. A significance level of $p < 0.05$ was used for the test.

RESULTS AND DISCUSSION

Comparative analysis of cheese production among countries. In relation to the presence of Aflatoxin M1 in a total of 93 cheese samples, the outcomes (Table 2) revealed noteworthy findings. The mean concentration of Aflatoxin M1 detected in Italian cheese samples was recorded as 0.061 ± 0.072 $\mu\text{g/kg}$, with the range spanning from 0.01 to 0.34 $\mu\text{g/kg}$. Out of these samples, 4 (14.81%) exceeded the LOD (Limit of Detection) and 12 (44.4%) exceeded the maximum level of 0.05 $\mu\text{g/kg}$.

Table 2. Overview of cheese sample characteristics: a general analysis

Origin of Cheese	N	% of total samples	$\bar{x} \pm \sigma$	Median $\mu\text{g/kg}$	Min $\mu\text{g/kg}$	Max $\mu\text{g/kg}$	No. (%) of samples exceeding LOD**	No. (%) of samples exceeding the maximum level of 0.05 $\mu\text{g/kg}$ *
Italy	27	29.0%	0.061 ± 0.072	0.048	0.01	0.34	4(14.81%)	12(44.4%)
France	5	5.4%	0.191 ± 0.334	0.334	0.02	0.79	2(40%)	2(40%)
Croatia	5	5.4%	0.036 ± 0.016	0.016	0.02	0.06	0(0%)	1(20%)
Netherlands	3	3.2%	0.066 ± 0.011	0.011	0.06	0.08	1(33.33%)	3(100%)
Germany	7	7.5%	0.063 ± 0.022	0.022	0.03	0.09	4(57.14%)	4(57.14%)
Slovenia	3	3.2%	0.023 ± 0.020	0.020	0.00	0.04	0(0%)	0(0%)
Turkey	3	3.2%	0.056 ± 0.005	0.005	0.05	0.06	0(0%)	2(66.66%)
Greece	5	5.4%	0.152 ± 0.195	0.195	0.04	0.50	2(40%)	3(60%)
Albania	3	3.2%	0.597 ± 0.342	0.342	0.20	0.83	3(100%)	3(100%)
North Macedonia	6	6.5%	0.072 ± 0.036	0.036	0.04	0.12	3(50%)	5(83.33%)
Hungary	5	5.4%	0.048 ± 0.024	0.024	0.01	0.08	1(20%)	2(40%)
Lithuania	3	3.2%	0.046 ± 0.005	0.005	0.04	0.05	0(0%)	0(0%)
Austria	9	9.7%	0.056 ± 0.045	0.045	0.02	0.16	2(22.22%)	4(44.44%)
Denmark	3	3.2%	0.030 ± 0.016	0.016	0.01	0.04	0(0%)	0(0%)
Kosovo	6	6.5%	0.062 ± 0.007	0.007	0.06	0.07	2(33.33%)	6(100%)
Pulled	93	100%	0.086 ± 0.145	0.145	0.00	0.83	24	47(50.53%)

Notes: *Kosovo regulation; **LOD – Limit of detection (0.06 $\mu\text{g/kg}$)

Source: developed by the authors

Conversely, in the case of French cheese samples, the mean Aflatoxin M1 concentration was determined to be $0.191 \pm 0.334 \mu\text{g/kg}$, and the range of detected values varied from minimum 0.02 to $0.79 \mu\text{g/kg}$. According to the study, 2 (40%) of the French cheese samples tested exceeded the LOD for AFM1 and 2 (4%) exceeded the maximum level of $0.05 \mu\text{g/kg}$. Croatian cheese samples exhibited a mean Aflatoxin M1 concentration of $0.036 \pm 0.016 \mu\text{g/kg}$, with the range observed between 0.02 and 0.06 for the entire sample set. Out of the samples tested, none of the samples exceeded the LOD while, 1 (20%) surpassed the maximum level of $0.05 \mu\text{g/kg}$. In the case of Netherlands cheese samples, the mean concentration of AFM1 was found to be $0.066 \pm 0.011 \mu\text{g/kg}$, with a range of 0.06 to $0.08 \mu\text{g/kg}$ for the entire sample set. Conversely, for Netherlands cheese samples, 1 (33.33%) exceeded the LOD and 3 (100%) exceeded the maximum level of $0.05 \mu\text{g/kg}$. Additionally, Dutch cheese samples exhibited a mean Aflatoxin M1 concentration of $0.063 \pm 0.022 \mu\text{g/kg}$, with the range falling between 0.03 and $0.09 \mu\text{g/kg}$. Out of all the samples, 4 (57.14%) exceeded the LOD of AFM1 and 4 (57.14%) exceeded the maximum level of $0.05 \mu\text{g/kg}$. In the context of additional countries, Slovenia exhibited Aflatoxin M1 concentrations with a mean value of $0.023 \pm 0.020 \mu\text{g/kg}$. The range of observed values spanned from 0.00 to $0.04 \mu\text{g/kg}$. Notably, none of the samples tested exceeded the LOD and the maximum level of $0.05 \mu\text{g/kg}$. These findings suggest that the levels of AFM1 in Slovenian cheese samples are within acceptable limits.

Turkish cheese samples had a mean Aflatoxin M1 concentration of $0.056 \pm 0.005 \mu\text{g/kg}$, with the lowest recorded content being 0.05 and the highest $0.06 \mu\text{g/kg}$, based on the results (Table 2) none of the samples tested exceeded the LOD, while 2 (66.66%) surpassed the maximum level of $0.05 \mu\text{g/kg}$. On the other hand, Greek cheese samples displayed a mean Aflatoxin M1 content of $0.152 \pm 0.195 \mu\text{g/kg}$, with the range varying from 0.04 to $0.50 \mu\text{g/kg}$. Out of all the samples, 2 (40%) exceeded the LOD and 3 (60%) exceeded the maximum level of $0.05 \mu\text{g/kg}$. For Albanian cheese samples, the mean Aflatoxin M1 concentration was determined as $0.597 \pm 0.342 \mu\text{g/kg}$, and the recorded minimum and maximum values spanned from 0.20 to $0.83 \mu\text{g/kg}$. Notably, all the samples tested 3 (100%) exceeded the Limit of Detection and the maximum level of $0.05 \mu\text{g/kg}$. These findings reveal that the levels of AFM1 in Albanian, Greece, and France cheese samples analysed notably exceed acceptable limits.

In relation to cheese samples from North Macedonia, the analysis revealed a mean Aflatoxin M1 content of $0.072 \pm 0.036 \mu\text{g/kg}$, with a range spanning from 0.04 to $0.12 \mu\text{g/kg}$, from the total sample tested 3 (50%) exceeded the LOD, whereas 5 (83.33%) exceeded the maximum level of $0.05 \mu\text{g/kg}$. Hungarian cheese samples exhibited a mean Aflatoxin M1 content of $0.048 \pm 0.024 \mu\text{g/kg}$,

characterized by a minimum content of 0.01 and a maximum content of $0.08 \mu\text{g/kg}$, while 1 (20%) of the sample tested exceeded the Limit of Detection, while 2 (40%) surpassed the maximum level of $0.05 \mu\text{g/kg}$ for AFM1. In contrast, Lithuanian cheese samples displayed a mean Aflatoxin M1 content of $0.046 \pm 0.005 \mu\text{g/kg}$, with the detected values ranging from 0.04 to $0.05 \mu\text{g/kg}$. The results of the study indicated that none of the samples tested exceeded the Limit of Detection (LOD) and exceeded the maximum level of $0.05 \mu\text{g/kg}$. However, Austrian cheese samples indicated a mean Aflatoxin M1 content of $0.056 \pm 0.045 \mu\text{g/kg}$, and the minimum and maximum values observed were 0.02 and $0.16 \mu\text{g/kg}$, respectively. Results showed that 2 (22.22%) exceeded the LOD, whereas 4 (44.44%) exceeded the maximum level of $0.05 \mu\text{g/kg}$. In terms of Danish cheese samples, the mean Aflatoxin M1 content was determined to be $0.030 \pm 0.016 \mu\text{g/kg}$, while the range of detected values varied from 0.01 to $0.04 \mu\text{g/kg}$. The results showed that none of the samples tested exceeded the Limit of Detection (LOD) and exceeded the maximum level of $0.05 \mu\text{g/kg}$. Lastly, the analysis of Kosovo cheese samples demonstrated a mean Aflatoxin M1 content of $0.062 \pm 0.007 \mu\text{g/kg}$, with the range of observed values falling between 0.06 and $0.07 \mu\text{g/kg}$. Resulting that 2 (33.33%) of the Kosovar cheese samples tested exceeded the Limit of Detection (LOD) for AFM1.

The homogeneity of variances was tested using Levene's test, which revealed a statistically significant result (Levene Statistic=6.677, $df_1=14$, $df_2=78$, $p=0.000$). This indicates that the variances of the dependent variable differed significantly across the groups. The current study aimed to investigate the variations in average levels of Aflatoxin M1 (AFM1) in cheese across fifteen countries included in the study. There is a significant difference between groups as determined by one-way ANOVA ($F(14,78)=5.26$, $p=0.000$). A Tukey post hoc test, One Way Anova, revealed that the cheese with Albania origin content is statistically significantly higher level of AFM1 (0.59 ± 0.34) compared to other countries as Italy (0.061 ± 0.072), France (0.191 ± 0.334), Croatia (0.036 ± 0.016), Netherlands (0.066 ± 0.011), Germany (0.063 ± 0.022), Slovenia (0.023 ± 0.020), Turkey (0.056 ± 0.005), Greece (0.152 ± 0.195), North Macedonia (0.072 ± 0.036), Hungary (0.048 ± 0.024), Lithuania (0.046 ± 0.005), Austria (0.056 ± 0.045), Denmark (0.030 ± 0.016), and Kosovo (0.062 ± 0.007).

However, no significant differences ($p>0.05$) were observed among the remaining countries, indicating comparable levels of AFM1 in cheese samples from Italy, France, Croatia, Netherlands, Germany, Slovenia, Turkey, Greece, North Macedonia, Hungary, Lithuania, Austria, Denmark, and Kosovo. The mean levels of Aflatoxin M1 (AFM1) in cheese samples collected from countries compliant with the maximum allowable limit set by the European Commission (2006) (0.05) were as follows: Slovenia (0.023 ± 0.020),

Denmark (0.030 ± 0.016), Croatia (0.036 ± 0.016), Lithuania (0.046 ± 0.005), and Hungary (0.048 ± 0.024) as illustrated in Figure 1. The results of the research indicate that most of the countries have exceeded the

permitted limit ($0.05 \mu\text{g/kg}$) of AFM1 in cheese products. These countries include Albania, France, Greece, North Macedonia, The Netherlands, Germany, Kosovo, Italy, Austria, and Turkey.

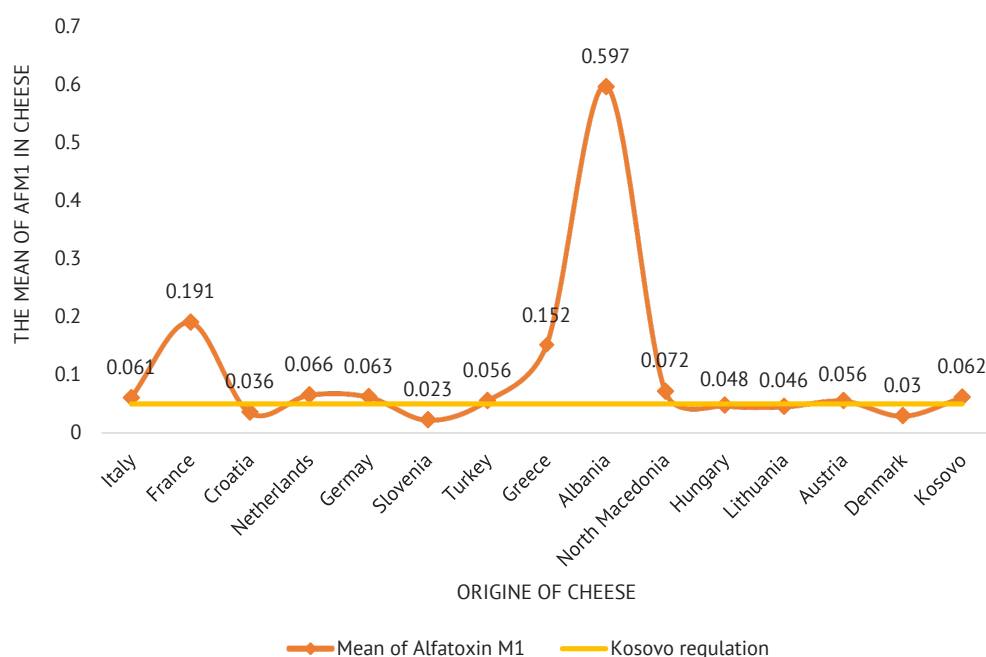


Figure 1. Mean AFM1 concentration in cheese samples from different countries

Source: developed by the authors

In relation to the Balkans, there are numerous studies that document the presence of AFM1 in dairy products. A research project, conducted by G. Ilievska *et al.* (2022), studied 974 dairy samples from North Macedonia's markets. The samples included 404 ultra-high temperature (UHT) milk, 291 ice cream, 178 yogurts, and 101 cheese, all of which were scrutinized for Aflatoxin M1 contamination. The study found that AFM1 concentrations averaged 49.1 ± 68.4 , 30.9 ± 30.0 , 35.1 ± 40.4 , and 40.1 ± 90.1 ng/kg for UHT milk, ice cream, yogurt, and cheese samples, respectively. On a similar note, a separate study in Albania by D. Topi *et al.* (2022), examined 1,200 milk samples from various Albanian regions over a span of two years. Their findings indicated that 82.3% of the samples contained AFM1, with an average concentration of $0.051 \mu\text{g/kg}$. Moreover, they found that 10.8% of the milk samples exceeded the European Union's maximum permissible limit of $0.05 \mu\text{g/kg}$ for AFM1. These findings underscore the widespread nature of AFM1 contamination in the region and the need for rigorous monitoring and control measures. A global systematic review and meta-analysis by T. Mahmudiono *et al.* (2023) which included thirty-one scientific papers with 34 data reports (Sample size=2,277) in their study, concluded that the lowest and highest prevalence of AFM1 in cheese was related to El Salvador (12.18%) and Serbia (100.00%). The pooled prevalence of AFM1

was 49.85%, 95% CI (37.93-61.78%). In our study, there were not analysed the cheese samples originated from Serbia. Cheese produced in Serbia, because of the political issues, it is not common in Kosovo's markets even though it is a neighbouring country.

Comparative Analysis: Examining AFM1 Levels in Cheese Alongside Nutritional Parameter. The following Table 3 presents a comprehensive overview of various nutritional parameters intrinsic to cheese. These parameters include total fat content, complete carbohydrate composition, protein constitution, and sodium chloride content. The empirical findings evince that, with regard to the aggregate fat content, the mean attains a value of 24.10 g per 100 g. Notably, the lower and upper bounds of this continuum manifest at 1.50 g per 100 g and 35 g per 100 g, respectively. Conversely, in relation to the composite carbohydrate content encompassing the entire cheese sample set, the mean is ascertained at 1.89 g per 100 g. The minimum and maximum values are reported 1.00 g per 100 g and 12.50 g per 100 g. Pertaining to the protein profile of cheese, the total mean registers at 19.87 g per 100 g, accompanied by an amplitude stretching from 1.50 g per 100 g to 37 g per 100 g. The ultimate nutritional parameter, namely sodium chloride content, is reported at 2.11 g per 100 g, characterized by a range from 0.50 g per 100 g to 5.60 g per 100 g in terms of minimum and maximum values, respectively.

Table 3. The characteristics of various nutritional parameters of cheese

Nutritional parameters	Mean±SD	Min	Max
Fat-total (g/100g)	24.10±6.17	1.50	35
Carbohydrates-total(g/100g)	1.89±2.45	1.00	12.50
Protein (g/100g)	19.87±7.46	1.50	37
Salt(g/100g)	2.11±.93	0.50	5.60
Other parameters	Subcategory	N (Percentage)	
Consistency	Hard	9(9.9%)	
	Semi hard	24(26.4%)	
	Semi soft; semi soft creamy	4(4.4%)	
	soft; soft-creamy	54(59.3%)	
Starter cultures	Bacterial cultures	11(12.5%)	
	Bacterial-rennet	38(43.2%)	
	Bacterial-rennet-fungal	6(6.8%)	
	Rennet	17(19.3%)	
	Bacterial-rennet-processed	4(4.5%)	
Ageing time	Rennet-processed	12(13.6%)	
	None	35(40.2%)	
	1-3 months	34(39.1%)	
	4-6 months	4(4.6%)	
	6-12 months	11(12.6%)	
	>13 months	3(3.4%)	

Source: developed by the authors

Table 3 presents a range of descriptive data regarding the complete collection of cheese samples, concerning diverse factors like texture, starter cultures, and cheese maturation duration. Drawing from the outcomes of the entire cheese sample pool, it was identified 9 samples as hard (constituting 9.9%), 24 as semi-hard (accounting for 26.4%), 4 as semi-soft/semi-soft creamy (equivalent to 4.4%), and 54 as soft/soft-creamy (comprising 59.3%) cheeses. Among the starter cultures analysed in the total sample size, the distribution was as follows: 11 samples (12.5%) were identified as bacterial cultures, 38 samples (43.2%) were categorized as bacterial-rennet cultures, 6 samples (6.8%) were classified as bacterial-rennet-fungal cultures, 17 samples (19.3%) were attributed to rennet cultures,

4 samples (4.5%) were characterized as bacterial-rennet-processed cultures, and 12 samples (13.6%) were associated with rennet-processed cultures. Regarding the duration of ageing, out of the total sample of 93, 40.2% (n=35) were categorized as "none." In the 1-3 months range, there were 34 samples, accounting for 39.1% of the total. The 4-6 months bracket had 4 samples, representing 4.6% of the group. For the 6-12 months category, there were 11 samples, making up 12.6%. Additionally, 3 samples (3.4%) were recorded as having an ageing duration of more than 13 months. To assess the potential differences of the examined nutritional parameters on the levels of Aflatoxin M1 (AFM1) in cheese, an Independent Sample T-test was employed (Table 4).

Table 4. Comparison of nutritional parameters in different concentrations of Aflatoxin M1 in cheese samples

Nutritional parameters	AFM1 concentration >0.050 µg/kg	AFM1 concentration <0.051 µg/kg	Mean Difference	t-value	Sig.
	Mean	Mean			
Fat total	24.90	24.75	0.151	0.124	0.902
Carbohydrates	2.14	1.95	0.19	0.331	0.741
Protein	21.65	19.92	1.72	1.04	0.298
Salt	1.90	2.26	-0.35	-1.56	0.122

Source: developed by the authors

The ensuing Table 3 presents the outcomes of a thorough comparative analysis pertaining to the concentrations of AFM1 in discrete classifications of cheese samples. These classifications are established according to their adherence to a designated threshold value falling within the range of >0.050 and <0.051 µg/kg. In relation to the concentration of AFM1 concerning the

total fat content, the average concentration observed in cheese samples surpassing the specified threshold of 0.050 µg/kg is measured at 24.90 µg/kg. In contrast, samples that fall below the threshold exhibit a slightly lower average concentration of 24.75 µg/kg. Consequently, the results collectively suggest an absence of statistically significant (p=0.902) differentiation in

AFM1 concentration based on the regulated total fat content within the cheese. In the case of AFM1 concentration of carbohydrate content, the mean concentration varied from 2.14 µg/kg in cheese samples to the predefined threshold, while a slightly diminished mean of 1.95 µg/kg characterizes samples situated below the threshold. The results show no significant differences ($p=0.175$) between the two groups in the concentration of carbohydrate content. In terms of protein, the mean concentration is 21.65 µg/kg in samples exceeding the predefined threshold, while a slightly diminished mean of 19.92 µg/kg characterizes samples situated below the threshold. The findings revealed no statistically significant ($p=0.298$) differences across AFM1 level for protein concentration. Turning attention to AFM1 concentration with respect to salt content, samples surpassing the established

threshold exhibit a mean concentration of 1.90 µg/kg, whereas their sub-threshold counterparts display a marginally higher mean concentration of 2.26 µg/kg. The results indicate an absence of statistically significant ($p=0.122$) divergence in AFM1 concentration, predicated upon the regulated salt content within the cheese.

The current investigation utilized a One-way ANOVA to aspects compare the consistency, starter cultures and ageing time of cheese in the levels of AFM1 in different types of cheeses. The results represented in Table 5 indicate that there was no statistically significant ($p=0.926$) difference in the average levels of AFM1 across consistency for hard (0.077 ± 0.10 µg/kg), semi-hard (0.078 ± 0.14 µg/kg), semi-soft, semi-soft cream (0.061 ± 0.019 µg/kg), soft, and soft-creamy (0.095 ± 0.159 µg/kg) cheese.

Table 5. Comparison of consistency, starter culture and ageing time parameters in Aflatoxin M1 concentration in Cheese Samples

Variables	Level of AFM1	
Consistency	Mean±SD	
Hard	0.077±0.11 ^a	0.02-0.34
Semi hard	0.078±0.15 ^b	0.01-0.76
Semi soft; semi soft creamy	0.061±0.019 ^c	0.04-0.08
Soft; soft-creamy	0.095±0.163 ^d	0.00-0.83
Starter cultures		
Bacterial cultures	0.15±0.24 ^a	0.04-0.83
Bacterial-rennet	0.10±0.16 ^b	0.01-0.79
Bacterial-rennet-fungal	0.16±0.17 ^c	0.06-0.50
Rennet	0.04±0.02 ^d	0.01-0.08
Bacterial-rennet-processed	0.06±0.01 ^e	0.06-0.08
Rennet-processed	0.05±0.03 ^f	0.02-0.16
Ageing time		
None	0.09±0.15 ^a	0.00-0.83
1-3 months	0.10±0.17 ^b	0.01-0.79
4-6 months	0.01±0.00 ^c	0.01-0.02
7-12 months	0.04±0.01 ^d	0.02-0.08
<13 months	0.06±0.00 ^e	0.06-0.07

Notes: means with same superscript letters differ significantly at $p<0.05$. *Minimum and maximum levels of AFM1 in cheeses. SD, standard deviation

Source: developed by the authors

According to the results represented in Table 5, the mean AFM1 concentration was highest in cheeses made with bacterial-rennet-fungal cultures (0.16 µg/kg), bacterial cultures (0.15 µg/kg), followed by those made with bacterial-rennet cultures (0.10 µg/kg), bacterial-rennet-processed cultures (0.06 µg/kg), rennet cultures (0.05 µg/kg), and rennet (0.04 µg/kg). The results also indicate that there were no statistically significant ($p=0.472$) differences in mean AFM1 concentration between the starter culture types. According to

the results, the mean AFM1 concentration was highest in cheeses aged for 1-3 months (0.10 µg/kg), followed by those aged for less than 1 month (0.09 µg/kg), those aged for more than 13 months (0.06 µg/kg), those aged for 7-12 months (0.04 µg/kg), and those aged for 4-6 months (0.01 µg/kg). The results also indicate that there were no statistically significant ($p=0.691$) differences in mean AFM1 concentration between in the ageing time categories of cheese. These findings suggest the consistency, starter cultures, and ageing time of cheese do

not have a significant impact on the levels of AFM1 present within it. These parameters are all important in determining the final characteristics of cheese and can be carefully controlled to produce cheeses with specific qualities.

Compared to other articles published in the last five years, some studies have identified a relationship between aflatoxin M1, nutritional parameters, and the manufacturing process of cheese. A study by D. Costamagna *et al.* (2019), concluded that the concentration levels of AFM1 increased both in curd and in long maturing cheese, suggesting that AFM1 concentration in milk could be a good predictor of its fate in milk products. A. Zinedine *et al.* (2021) and A. Vaz *et al.* (2022) studied the distribution of AFM1 during the production of two different cheeses, where the amount of the toxin was monitored on a wet and a dry basis. On the wet basis, there was a continuous increase in concentration. The same study on a dry basis concluded that under the tested conditions, there is no degradation of the toxin, and the concentration increase observed previously was in fact due to the loss of humidity. Current study results imply that the levels of AFM1 in cheese are not notably affected by factors such as consistency, starter cultures, and ageing time.

A study by G. Chavarría *et al.* (2017), aimed to investigate the distribution and fate of AFM1 in fresh cheese and whey throughout cheese manufacturing and storage. The research aimed to understand the interaction of this toxin with various proteins in milk, cheese, and whey. The findings revealed that during the production of fresh cheese, up to 70% of AFM1 levels present in milk spiked with 0.5 and 1.5 µg/L were released into whey. Additionally, a significant reduction in AFM1 concentration was observed during ripening, showing an inverse correlation with lactic acid bacteria plate counts and a direct correlation with the whey-draining process that occurs during the maturation or storage of fresh cheese. A study done by E. Sarmast *et al.* (2021), found that cheese production increased aflatoxin M1 (AFM1) levels by 77.2% ($P < 0.001$) compared to milk. The order of increased AFM1 levels in cheese was hard (168%) > semi-hard (68.7%) > soft (60.6%). Cheese from milk without a starter culture had a higher increase than that with a starter culture. Overall, there was a significant reduction in AFM1 levels after ripening/storage (-11%, $P = 0.007$), particularly in cheese from milk with a starter culture (-21.1%, $P = 0.009$). In our study, in contrast, the findings suggest that the ageing time of cheese does not have a significant impact on the levels of AFM1 present within it. Also, the findings of this study revealed no statistically significant ($p = 0.298$) differences across AFM1 level for protein concentration. The results of this study also indicate that there were no statistically significant ($p = 0.472$) differences in mean AFM1 concentration between the starter culture types.

In a recent study done by S. Sabatelli *et al.* (2023) in Italy, which aimed to define the enrichment factor (EFs) of cheese with different moisture content on a fat-free basis (MFFB). The milk used for cheesemaking was naturally contaminated with different AFM1 concentrations. This research found that the Enrichment Factor (EF) values for soft and semisoft cheeses were half of the suggested EFs established by the Italian Ministry of Health in 2019. They propose that a comprehensive examination of EF should also consider technological aspects and quality parameters that may influence EF variability. This approach could assist regulatory bodies in establishing an appropriate legal limit, whether based on EFs or concentration values, to protect public health and the diverse range of Italian cheese productions. European Commission (2006) emphasizes the significance of determining the enrichment factor (EF), a crucial parameter that needs to be specified for assessing the permissible level of the toxin in cheese. This is essential to guarantee that cheese is derived from milk that adheres to the regulatory standards. Similarly, I. Pecorelli *et al.* (2020) suggest that the grouping of cheeses into two wide categories (hard and soft cheeses), based on MFFB, and the consequent attribution of the suggested EF, may not be accurate because the experimental evidence shows that soft and semi-soft, as well as semi-hard and very hard cheese categories, may present different results addressing for different categorization. In Kosovo, there is a lack of institutional similar data. The data found in this study could be a significant aid in determining the Enrichment Factors (EF) for cheese, taking into consideration the factors suggested by the above-cited work.

CONCLUSIONS

The findings revealed considerable variations in AFM1 contamination levels among different countries. Notably, current research found that there were cheese samples from some countries that exceeded the concentration of 0.05 µg/kg for AFM1, like, Albania, France, Greece, North Macedonia, The Netherlands, Germany, Kosovo, Italy, Austria, and Turkey. This highlights the importance of continuous monitoring and stringent quality control measures to ensure the safety of cheese products for consumers. The countries with the mean levels of Aflatoxin M1 (AFM1) in cheese samples compliant with the level of 0.05 µg/kg were as follows: Slovenia, Denmark, Croatia, Lithuania, and Hungary. The occurrence of AFM1 in cheese samples was notable, with 47 out of the total 93 different cheese samples (50.53%) exhibiting concentrations higher than 0.05 µg/kg for AFM1.

To evaluate potential variations in Aflatoxin M1 (AFM1) levels in cheese associated with examined nutritional parameters, an Independent Sample T-test was applied. The collective results indicate the absence of statistically significant differentiation ($p = 0.902$) in AFM1 concentration based on the regulated total fat

content in the cheese. Similarly, no significant differences ($p=0.175$) were observed in AFM1 concentration between two groups regarding carbohydrate content. The findings further reveal no statistically significant differences ($p=0.298$) in AFM1 levels concerning protein concentration. Moreover, there is no significant divergence ($p=0.122$) in AFM1 concentration based on the regulated salt content in the cheese. In assessing the consistency, starter cultures, and ageing time of various cheese types in relation to AFM1 levels, a One-way ANOVA was employed. The results indicate no statistically significant difference ($p=0.926$) in average AFM1 levels across different consistencies, including hard (0.077 ± 0.10 µg/kg), semi-hard (0.078 ± 0.14 µg/kg), semi-soft, semi-soft cream (0.061 ± 0.019 µg/kg), soft, and soft-creamy (0.095 ± 0.159 µg/kg) cheese. Examining the mean AFM1 concentration in cheeses produced with bacterial-rennet-fungal cultures, the results show the highest level at 0.16 µg/kg. However, no statistically significant differences ($p=0.472$) were observed between different starter culture types. Similarly, no significant differences ($p=0.691$) were found in mean AFM1 concentration across various ageing time categories of cheese, with the highest mean concentration

observed in cheeses aged for 1-3 months at 0.10 µg/kg. Overall, these findings collectively suggest that the consistency, starter cultures, and ageing time of cheese do not exert a significant impact on the levels of AFM1 present within it.

Overall, this study underscores the need for ongoing efforts to address and mitigate AFM1 contamination in cheese production. Stringent monitoring, adherence to quality standards, and continuous research into effective control measures are crucial to ensure the safety and quality of cheese products consumed worldwide. As the findings have shown, a collaborative approach between regulatory bodies, producers, and researchers is essential to safeguarding public health and maintaining consumer confidence in cheese products.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Оцінка вмісту альфатоксину М1 та поживних речовин у зразках ринкового сиру в Косові

Арієта Камай Ібрагімі

Кандидат з харчових наук та технологій, доцент
Університет Хакші Зека
30000, вул. «УЏК», м. Печ, Республіка Косово
<https://orcid.org/0000-0002-3560-3704>

Байрам Беріша

Кандидат сільськогосподарських наук, професор, доктор габілітований
Приштинський університет
10000, вул. Джорджа Буша, 31, м. Приштина, Республіка Косово
<https://orcid.org/0000-0001-9112-054X>

Хата Дібрані Сопджані

Магістр наук, хімік
Агентство з питань продовольства та ветеринарії
10000, площа «Нана Тереза», м. Приштина, Республіка Косово
<https://orcid.org/0009-0008-6245-9024>

Афердіта Камай Іса

Кандидат хімічних наук, менеджер з контролю якості
Косовське агентство з лікарських засобів
10000, Ладжія та Спіталіт, м. Приштина, Республіка Косово
<https://orcid.org/0000-0001-7044-8810>

Шихрет Мурікі

Кандидат наук з агроелектроніки, доцент
Університет Хаджі Зека
30000, вул. «УЏК», м. Печ, Республіка Косово
<https://orcid.org/0000-0002-3389-0594>

Анотація. Присутність та потужна канцерогенна дія афлатоксинів, групи мікотоксинів, становить значну загрозу глобальній безпеці харчових продуктів, особливо в молочному секторі. Це дослідження мало на меті оцінити поширеність і розподіл афлатоксину М1 у різних сортах сиру на ринках Косова у 2022 році та виявити потенційні кореляції з типами сиру, умовами переробки та поживними параметрами, щоб покращити розуміння та забезпечити безпеку молочних продуктів. У другій половині 2022 року загалом 93 зразки різних видів сирів з 15 різних країн були проаналізовані на вміст токсину афлатоксину М1 за допомогою методу імуноферментного аналізу. Результати дослідження вказують на те, що в кількох країнах було виявлено перевищення концентрації афлатоксину М1 у 0,05 мкг/кг, що є максимально допустимою межею для молока та молочних продуктів у багатьох країнах світу. Примітно, що зразки сиру з Албанії показали значно ($p=0,000$) вищі рівні афлатоксину М1 порівняно з іншими країнами. Результати показали, що концентрація афлатоксину М1 не мала статистично значущих відмінностей залежно від загального вмісту жиру ($p=0,902$), вуглеводів ($p=0,741$), білків ($p=0,298$) або солі ($p=0,122$) в сирі. Результати продемонстрували, що рівень афлатоксину М1 суттєво ($p=0,926$) не залежав від консистенції сиру. Хоча різні закваски були пов'язані з різними концентраціями афлатоксину М1, ці відмінності не були статистично значущими ($p=0,472$). Аналогічно, час визрівання не мав значного ($p=0,691$) впливу на рівні АФМ1, оскільки концентрації залишалися відносно постійними протягом різних періодів визрівання. Ці результати дають цінне уявлення про варіації рівнів афлатоксину М1 у зразках сиру в різних країнах. Вони підкреслюють важливість регуляторних заходів і постійного моніторингу для забезпечення безпеки харчових продуктів і дотримання максимально допустимих меж

Ключові слова: типи сирів; ІФА; забруднення афлатоксином М1; безпечність молочних продуктів



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Risk analysis of *Apis mellifera* colony losses and health assessment in Albania from 2021 to 2023

Kastriot Korro*

Associated Professor, Lecturer
Agricultural University of Tirana
1025, Paisi Vodica Str., Tirana, Albania
<https://orcid.org/0000-0002-9304-1041>

Vitor Malutaj

President of the Federation
Federation of Beekeepers of Albania
1001, Muhamet Deliu Str., Tirana, Albania

Lecturer

Agricultural University of Tirana
1025, Paisi Vodica Str., Tirana, Albania
<https://orcid.org/0009-0004-7462-4577>

Gani Moka

Professor, Lecturer
Agricultural University of Tirana
1025, Paisi Vodica Str., Tirana, Albania
<https://orcid.org/0009-0005-5991-838X>

Merije Elezi

Associated Professor, Lecturer
University of Tetova
1200, Ilinden Str., Tetova, Republic of North Macedonia
<https://orcid.org/0000-0002-5073-2483>

Besnik Elezi

Associated Professor, Lecturer
University of Tetova
1200, Ilinden Str., Tetova, Republic of North Macedonia
<https://orcid.org/0009-0008-7867-9187>

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Abstract. The research relevance is determined by the decline of bee populations in Albania, as the need to understand the dynamics of colony loss and the factors contributing to it is of paramount importance. The study aimed to comprehensively investigate the prevalence and main causes of colony losses, with special attention to

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*Corresponding author

Varroa mite infestation, *Nosema* disease, viral pathogens, pesticides, and bacterial infections. Using the stratified sampling method, 15,493 beekeepers of different ages and experiences participated in the study. Both electronic and face-to-face surveys were used to collect data on bee family losses, management practices and environmental factors affecting bee health. In addition, monitoring programmes allowed a detailed assessment of bee family health and environmental conditions in the apiary, providing valuable information on temporal trends and patterns. The findings indicate alarming rates of *Varroa* mite infestation, prevalence of *Nosema* and a complex interplay of factors contributing to colony loss, particularly during the summer and winter months. For example, *Varroa* mite infestation was found in 61% of the 29,474 bee samples collected during summer sampling, with rates ranging from 0.5% to 70.2%. Similarly, during autumn sampling, 65% of 43,037 bee samples contained *Varroa* mites, with an average infestation rate of 5.3%. Moreover, *Nosema* disease is also a complex problem, with clinical prevalence ranging from 0.1% in autumn to 1.3% in summer and spring. These key figures highlight the urgent need to develop effective strategies to reduce *Varroa* mite infestation and *Nosema* disease, thereby maintaining bee populations and ecosystem health. The results of the study make a valuable contribution to bee management and policy development, emphasising the importance of holistic approaches to maintaining bee health and resilience in Albania

Keywords: varroaosis; winter losses; *Nosema*; queen; seasonal diseases

INTRODUCTION

Bees are crucial to pollination, ecosystem health and food production. Understanding the factors contributing to bee colony loss is critical to protecting bee populations and ensuring global food security. The research problem revolves around the increasing rates of colony loss observed in *Apis mellifera* populations, particularly in Albania, and the complex interplay of factors such as *Varroa* mite infestation, *Nosema*, viral pathogens, pesticides and bacterial infections contributing to these losses. Understanding the specific dynamics of bee family health and the cause of deterioration is paramount to implementing effective mitigation strategies and conserving bee populations.

D. Pavlova *et al.* (2022) concluded that honeybees pollinate about 35% of the world's crops. According to authors plant pollination by honeybees provides an efficient mechanism for seed formation and hence for the maintenance of biodiversity. This utility provided by bees has a cascading effect on the food chain, ensuring the yield and quality of many crops. In addition to their role in pollination, S. Lippi and M. Sanfilippo (2023), identify the production of valuable products by honey bees. In her opinion, honey, as a result of beekeeping, not only serves as a source of nutrients but also has applications in medical practice and other human activities.

Beekeeping in Albania is a long-standing and traditional industry. According to the Food and Agriculture Organisation, as of 2022, there were about 100,000 beekeepers in Albania, managing approximately 1.5 million bee families (FAO partners with Albania..., 2023). These figures reflect the significant spread and importance of beekeeping in rural and peri-urban areas of Albania. Beekeeping is not only a source of income for many families but also plays a key role in maintaining the ecosystem, pollinating plants, and producing valuable bee products such as honey and other products (Tyliszczak *et al.*, 2017).

M. Kuliçi (2021b) notes the significant contribution of *Apis mellifera* bees to agricultural production and biodiversity conservation in Albania. The studies emphasise that the activities of these bees contribute to the pollination process of different crops, which leads to increased yields of fruits, as well as important crops such as wheat, maize, and vegetables. The role of bees in maintaining biodiversity by pollinating wildflowers has also received special attention. This process not only favours the formation of new generations of plants but also ensures the preservation of Albania's natural ecosystem.

The statement of B. Bekić Šarić *et al.* (2023) on the effect of bee population reduction on crop yields is confirmed by numerous scientific studies. The researchers suggest that this problem may lead to higher food prices and lower economic activity in the agricultural sector. Moreover, studies show that insufficient pollination can also affect the quality of the crop, affecting the size, shape and maturity of fruits and seeds. This can negatively affect the profitability and competitiveness of agricultural production. M. Kuliçi (2021a) notes the additional problem of possible increased production costs for farmers who depend on bees to pollinate their crops. This problem becomes relevant when bee populations are reduced or unavailable for pollination. If bees are not available for pollination, farmers may be forced to seek alternative pollination methods such as hand pollination or the use of other insect pollinators (Kuzbakova *et al.*, 2022). These methods can be more labour-intensive and costly than natural pollination by bees.

Despite the rather extensive views of researchers, their studies lack more specific and relevant information on the situation of colony loss risk analysis, statistical information on this topic, as well as a more detailed assessment of the health and diseases of honey bee families. Based on the aforementioned, the study aims to assess the current status of the honey bee population in Albania, to analyse the factors affecting the

decline in their numbers, and to develop recommendations to reduce the risks of loss of *Apis mellifera* colonies in this country.

MATERIALS AND METHODS

In conducting the survey and monitoring of apiaries across Albania between 2021 and 2023, a stratified sampling approach was used to ensure representation of the different beekeeping communities. Working with the Association of Beekeepers, public institutions and local communities, efforts were made to recruit 15,493 beekeepers of different ages (22-75 years) and experience levels (3-58 years). Survey questionnaires were distributed through both electronic and in-person distribution channels to gain insight into bee family losses, management practices, and environmental factors affecting bee health. Face-to-face interviews conducted by field teams facilitated deeper engagement with beekeepers, allowing qualitative data and first-hand observations to be collected on beekeeping practices and challenges faced in different regions.

In addition, monitoring programmes were established in collaboration with local beekeepers' associations and extension services to assess bee family health and environmental conditions at apiaries. Trained staff made field visits to apiaries four times a year, where they systematically assessed bee family health, recorded bee family counts, and identified potential stressors affecting bee populations. Data collected during surveys and monitoring activities were systematically analysed to identify trends, patterns and correlations related to family loss and beekeeping practices. Ethical considerations, including informed consent and data confidentiality, were prioritised throughout the research process to ensure the integrity and privacy of participants. Through this integrated approach, the study aimed to provide science-based insights and recommendations to promote sustainable beekeeping practices and improve bee family health throughout Albania.

Univariate analyses were performed to assess the relationship between modifying factors and colony losses in summer/winter. Statistical methods were used to quantify the effects of *Varroa* mites, parasites of the genus *Nosema*, colony characteristics and queen health on colony losses in different periods. seasons. These analyses provided detailed information on the relative importance of different factors affecting colony health dynamics.

Various methods of data collection and analysis were used, including official reports, surveys, statistical software, and review of research literature. Baseline data were obtained from reports of the Albanian Ministry of Agriculture, European and International beekeeping organisations, and through questionnaires distributed to beekeepers to collect information on management practices, bee diseases and pesticide use. Statistical software R was used to analyse the collected data. These methods were selected due to their suitability for the study objectives and to reflect the multifaceted nature of bee family health and losses. A longitudinal observational study allowed the examination of temporal trends and patterns, while univariate analyses allowed the identification of specific factors contributing to colony collapse disorder in different seasons. Survey-based data collection ensured that the diverse perspectives of beekeepers and stakeholders were considered, thus enhancing the comprehensiveness and relevance of the research findings. The research was conducted in accordance with the rules of The Declaration of Helsinki (2013).

RESULTS

A study conducted with the University of Tirana shows a marked increase in the number of bee families in Albania between 2021 and 2023. In 2021, 298,000 bee families were registered, which in itself is a significant indicator for the country's agricultural sector, especially given the importance of bees in pollination and honey production (Table 1).

Table 1. Number of bee families in Albania from 2021 to 2023

No.	Year	Bee families
1	2021	298,000
2	2022	320,000
3	2023	360,000

Source: compiled by the authors

By 2022, a growth of 7.38% is observed, reaching the 320,000 bee families mark. This increase can be attributed to improved conditions for beekeeping, the introduction of more advanced technologies and methods of bee care, and the growing interest in beekeeping as a sustainable and profitable agricultural activity. A notable jump occurred in 2023, when the number of bee families increased to 360,000, up 12.5% from the previous year. This growth can be attributed not only to

internal factors, such as improved beekeeping practices and expansion of apiary areas but also to external factors, such as increased demand for honey and other bee products in both domestic and foreign markets. Analyses of colony health show that approximately 60% of colonies tested were of normal size and viability. Conversely, only 12% of colonies were categorised as particularly weak, while 18% were found to be exceptionally resilient. Interestingly, among this 12% subset

of weakened colonies, signs of potential collapse were evident during the autumn season. The main causes of this dangerous condition are various factors, particularly cases of uterine mortality, the introduction of a new uterus, the establishment of new families, and clinical signs of varroosis and nosemosis. Varroosis has emerged as the predominant parasitic disease, with 42,494 clinical observations recorded during the field visits. Consequently, the clinical prevalence of varroosis in the sampled apiaries was an alarming 66%.

Analyses of families experiencing summer losses show clear patterns: lack of mothers and *Varroa* mite infestation become important risk factors. When closely examined in July, typical families struggling with summer losses tended to be either motherless or affected by varying degrees of *Varroa* mite infestation, as shown in Table 2. Despite the infrequency of summer losses, only 3,600 cases were observed among the 3,600,000 families, the model Consideration of these cases sheds light on critical risk factors affecting colony health.

Table 2. Bee family losses in Albania by year and season

Period	Spring*	%	Summer*	%	Autumn*	%	Winter*	%	Overall	% ¹
2021	13,708	20	3,427	5	10,281	15	41,124	60	68,540	23
2022	12,642	18	3,529	5	10,560	15	43,648	62	70,379	22
2023	15,840	22	3,600	5	8,640	12	43,920	61	72,000	20
Overall	42,190		10,556		29,481		128,692		210,919	

Note: * – number of dead families at a particular time of year; ¹ – percentage of all bee families in Albania in a particular year

Source: compiled by the authors

The relatively low probability of summer losses kept the modelled probability around 5% for all identified risk factors. However, the absence of a queen significantly increased the probability of summer loss, increasing the probability by a factor of about 54 compared to families with intact mates. For families equipped with mates, the probability of summer loss mortality hovered around 0.2% under average conditions. Conversely, families deprived of mates experienced a significantly higher probability of summer mortality, as high as 3.2%. Moreover, colony strength emerged as a key determinant of summer survival: weaker colonies experienced a staggering 25-fold increase in the probability of summer mortality compared to their stronger counterparts. Notably, the size of colonies, both normal and resistant, showed no significant differences in the probability of summer mortality.

The effect of *Varroa* mite infestation on summer losses requires close attention, as the model predicts a gradual increase in summer mortality as mite levels increase. Specifically, for every 2% increase in *Varroa* mite infestation, the probability of summer mortality increased by a factor of 2.37. Notably, the model predictions identify a summer loss probability of 0.93% when *Varroa* mite infestation levels reach 30%, with all other covariates remaining at average levels. These results emphasise the complex interplay of factors affecting colony health during the summer months. By elucidating the subtle relationships between queen status, bee family vigour, *Varroa* mite infestation and summer losses, beekeepers can proactively implement targeted management strategies to improve bee family resilience and prevent the detrimental effects of these risk factors. Through vigilant monitoring and adaptive intervention, beekeepers can strive to maintain healthy

and thriving families, ensuring the long-term sustainability of bee populations in the face of changing environmental challenges.

The number of colony losses increased markedly during the winter months, especially when colonies were managed by queens that had reached the yearling mark. This observation highlights the disturbing trend that older queen mothers over a year old develop a range of physiological disorders that have a detrimental effect on colony development and overall health. Research suggests that as queens age, they may be prone to certain physiological abnormalities that interfere with optimal colony function and sustainability. Indeed, the presence of older mothers correlates with a cascade of adverse effects, including reduced productivity and a propensity for smaller colony sizes, both of which are often cited as precursors to colony mortality. The complex relationship between queen age and family health emphasises the key role that queen viability plays in maintaining family dynamics and longevity during the challenging winter season (Bruckner *et al.*, 2023).

The mechanisms underlying the detrimental effects of older queen mothers on family health are multifaceted. Physiological changes associated with ageing can compromise a queen's ability to maintain reproductive vigour and effectively regulate colony dynamics. As a result, colonies headed by older queen mothers may experience reduced brood production, reduced feeding efficiency and increased susceptibility to environmental stressors. Moreover, the link between queen senescence and declining productivity highlights the complex social dynamics within bee families. Worker bees rely heavily on signals and pheromonal cues emitted by the queen to coordinate their activities and maintain family cohesion.

In the presence of an older queen, these regulatory mechanisms may be disrupted, leading to suboptimal

colony functioning and increased vulnerability to unfavourable environmental conditions (Table 3).

Table 3. Review of the results of univariate analyses of factors associated with summer and winter losses

Parameter	Modifying factor	Summer losses	Winter losses
Summer diseases	Nosemite parasite	-	-
	<i>Varroa</i> mite	↑	↑
Winter diseases	Nosemite parasite	-	↑
	<i>Varroa</i> mite ↑		↑
Bee colony characteristics	Spring nest colony	-	↓
	Migrating colony	-	-
	Summer-weakened colony	↑	-
	Winter-weakened colony	-	↑
	Non-summer-active queen	↑	-
	Non-winter-active queen	-	↑
	Old-at-summer queen	↑	↑
	Old-at-winter queen	↑	↑

Note: “↑” – defines a marked increase in the probability of colony loss when the factor is applied; “↓” – defines a significant decrease in the probability of colony loss; “-” – indicates a negligible effect on the probability of colony loss

Source: compiled by the authors

The bee families surveyed in Albania remained healthy throughout the study period. Visible clinical symptoms of only two diseases were observed in about 12.8% of apiaries. Varroosis became the predominant disease, consistently occurring in families and correlating with losses in both summer and winter. The causes of colony losses in late summer and winter appeared to be similar, often related to the general weakness of each colony. Varroosis was one and a half times more frequent in late summer and autumn than in spring. Nosemosis was recorded in 15,120 colonies from different apiaries and confirmed by laboratory analyses at the Laboratory of Wild Animals and Bees at the Agricultural University of Tirana. Its clinical prevalence varied from 3% in autumn to 5% in summer and spring. During visits, dead bees were found in front of the colony, as well as signs of diarrhoea. The queen is one of the most important factors in assessing the probability of loss in both summer

and winter. A colony has a higher risk of summer loss if it is left without a queen in July. This indicates that the timing of queen replacement is a very sensitive phase in colony development. Loss of a queen during mating flight or hive manipulation, failed mating, introduction of a weak or sick queen and rejection of the new queen by worker bees are some of the problems faced by beekeepers.

When analysing the table of family losses by cause, it is evident that Varroatoxis appears to be the main culprit, highlighting its widespread impact on bee families (Table 4). The ubiquitous presence of *Varroa* mites, clinically observed by vigilant beekeepers or veterinary specialists during routine inspections, emphasises the severity of the infestation. In addition, confirmation of *Varroa* mite infestation through laboratory analyses performed in local, regional, or national laboratories confirms its role as a significant factor in bee mortality in affected families.

Table 4. Percentage of bee family losses by cause of loss

Cause	Number of dead bee families in 2021	Number of bee families lost per 2022	Number of bee families lost per 2023	Overall values
Varroatoxis	65%	69%	64%	66%
Nosematosis	20%	15%	21%	19%
Pesticides	-	-	-	-
Viruses (Wing deformation Virus (WDV))	10%	10%	12%	10%
Bacterial diseases	-	-	-	-
Fungal diseases (Ascospherosis and Aspergillosis)	2%	2%	2%	2%
Other causes (damage from bears, traffic, landslides, flooding, rainstorms)	3%	4%	51%	3%
Overall	100%	100%	100%	100%

Source: compiled by the authors

In the study, researchers collected bee samples during both the summer and autumn seasons to assess the prevalence of *Varroa* mite infestation in families. In summer sampling, *Varroa* mites were detected in 61% of the 29,474 bee samples collected, indicating a high rate of infestation. These rates varied considerably, from 0.5% to an astounding 70.2%. As the season moved into autumn, the presence of *Varroa* mites remained at similar levels, with 65% of 43,037 bee samples containing these parasitic mites. The average infestation rate for this period was 5.3%, showing a wider range of infestation levels, from 0.9% to approximately 92.8%. Notably, the highest recorded infestation level of 92.8% was recorded in an apiary heavily affected by *Varroa* infestation, where the average autumn infestation level reached 22.7%.

The discovery that half of the colonies had already died of *Varroa* infestation at the time of sampling and most of the remaining colonies suffered the same fate in the following months was of great concern. This grim reality emphasises the detrimental impact of *Varroa* mites on honey bee families. It is important to note that the absence of *Varroa* mites in a sample does not necessarily guarantee the absence of infestation within a family. Nevertheless, detailed analysis revealed a clear correlation between the presence of *Varroa* mites and colonies diagnosed with varroosis. Colonies affected by *Varroa* mites had six times higher rates of *Varroa* infestation than colonies without a diagnosis. For instance, during summer sampling, families without varroosis had an average infection rate of 18.4%, while families diagnosed with varroosis had a much higher rate of 22.5%. Similarly, during autumn sampling, families without varroosis had an average infection rate of 20.1%, in stark contrast to the 34.6% rate observed in families diagnosed with varroosis. These results emphasise the urgent need for effective strategies to control *Varroa* mite infestations and mitigate their devastating effects on honey bee populations.

The presence of *Nosema* disease is a complex problem in beekeeping. Clinical prevalence ranged from 0.1% in autumn to 1.3% in both summer and spring, showing no significant differences between the four visits. Bee inspectors documented various symptoms including bee deaths in front of the family (469 cases), signs of diarrhoea (6,983 cases), bee deaths combined with diarrhoea (996 cases) and non-flying bees near the family (1,352 cases). Analysis was carried out on 2,947 cases using dead bees as matrix and 7,251 samples included faecal samples. Among these samples, 5,728 were infested with *Nosematosis*. However, the presence of *Varroa* mites in almost all cases makes it difficult to determine the primary cause of bee mortality. This coincidence blurs the distinction between *Varroa* and *Nosema*, emphasising the interrelated nature of bee health problems.

The lack of diagnostic tools for viral diseases in Albania is a particular problem. Diagnosis, especially of wing deformity virus-WDV, is based solely on visible

clinical signs. Unfortunately, the lack of diagnostic facilities limits a comprehensive understanding of viral diseases affecting bee families, resulting in many viral pathogens remaining undiagnosed and their impact underestimated. Although pesticides and bacterial diseases have deleterious effects on bee populations, their effects are different in nature. Unlike Varroaosis and *Nosema*, which can wipe out entire families, pesticide exposure and bacterial infections usually result in reduced bee numbers and productivity rather than total mortality. Consequently, they are not listed in the loss table, although their impact on colony health and productivity should not be overlooked. The complex nature of bee losses emphasises the complexities of beekeeping and the challenges associated with maintaining healthy and thriving families. Effective mitigation strategies require a holistic approach that considers the interactions of *Varroa* mites, *Nosema*, viral pathogens, pesticides, and bacterial infections, protecting bee populations and preserving their vital role in both ecosystems and agricultural systems.

DISCUSSION

The results of the present study emphasise the importance of winter as a critical season for colony mortality in Albania. In the study, 73% of all family losses occurred during winter. This trend mirrors observations in other European countries. A study conducted by V.R. Olave-Olave *et al.* (2021), in Norway, found a comparable ratio of losses between summer and winter, with 75.3% of losses occurring in winter 2020/2023 and 22.7% in summer 2020. An extensive study by E. Alonso-Prados *et al.* (2020), which examined summer and winter losses in 17 European countries, confirmed these findings, emphasising the highest loss rates in the winter season. However, in southern European countries, as well as in studies conducted by L. Insolia *et al.* (2022), in the USA, reported similar rates of loss for both winter and summer seasons. These differences suggest that the relative importance of winter and summer mortality varies by country and climatic region. Potential factors contributing to these differences include, among others, differences in land use practices, pesticide application rates, bee family dynamics and beekeeping methodologies.

Extensive beekeeping experience significantly reduces the probability of winter losses of bee families, as evidenced by many years of experience (Dvykaliuk *et al.*, 2023). A study by S. More *et al.* (2021) found a remarkable positive correlation between beekeeper age and winter losses. Surprisingly, their data set showed that the highest winter losses occurred among apiaries managed by older beekeepers with limited families and minimal beekeeping experience. Conversely, the study by the researchers showed that beekeepers with more experience, i.e. older beekeepers, tended to control more bee families compared to novice beekeepers. Possession of a significant number of colonies often reflects an increased level of

professionalism. Consequently, the results of both studies emphasise that European beekeepers with a high level of professionalism, demonstrated by managing numerous bee families and having extensive experience, suffered fewer losses than their colleagues with a lower level of professionalism. In the present study, additional factors such as apiary and enterprise size and a higher level of beekeeping education were considered when assessing professionalism. However, these factors either showed no correlation with losses or failed to increase the explanatory power of the model, hence excluding them from the multivariate analysis. The number of bee families managed by beekeepers often matches their goals and significantly determines their management approach. As stated by K.P. Hester *et al.* (2023), these differences may explain seasonal differences in family losses among different types of beekeepers. Similar trends have been observed in both domestic and international studies. Homestead beekeepers consistently had higher winter losses than their commercial counterparts, except for the 2021-2022 period.

A. Leska *et al.* (2021) add that many commercial beekeepers rely on their bee families as a source of income and therefore prioritise strong families at the beginning of the brood-rearing season to ensure efficient pollination services in the spring. These beekeepers are more inclined to use chemical control agents such as acaricides to control the critical biotic risk factor *Varroa*. This proactive approach potentially reduces the risk of winter mortality associated with parasite pressure; an idea echoed in recent studies. Interestingly, beekeepers unanimously identified *Varroa* as the main cause of winter losses across all three years of the study, regardless of the type of operation. This suggests that homestead beekeepers are also aware of the risks of *Varroa*, but their hesitancy to use synthetic acaricides and the timing of treatment may affect the effectiveness of their management strategies. In contrast, commercial beekeepers suffered significantly higher family losses in summer compared to other types of beekeeping operations. While they diligently care for their families in the autumn to ensure strong families in the spring, the use of in-hive acaricides, which can promote the emergence of resistant *Varroa* mites, along with exposure to agricultural chemicals, may explain the increased summer losses. Losses during the brood-rearing season are a concern and may be related to what beekeepers believe to be the main cause of summer losses – “partner problems” (Kirimbayeva *et al.*, 2023).

Studies by A. De Carolis *et al.* (2023) showed that residues of chemicals used for *Varroa* control can adversely affect the reproductive health and behaviour of partners and potential partners. Large-scale beekeeping operations, such as long-distance transport for pollination services, may expose honeybees to significant stress. However, previous results do not indicate a significant negative relationship between these activities and winter colony mortality rates. These activities likely

that either such activities are not detrimental to the family as a whole, or any negative effects are offset by subsequent hive management practices. Future research should examine the relationship between beekeepers' management activities and bee family losses to improve recommendations for best management practices. Furthermore, following L. Barascou *et al.* (2023), Polish beekeepers actively monitored and applied *Varroa* control practices and reported markedly lower rates of bee family mortality compared to their colleagues who did not engage in such practices. This observation echoes data from the United States, where the use of barricades, especially amitraz, consistently showed a positive correlation with reduced winter losses.

Similarly, a study conducted by N. Steinhauer *et al.* (2021) in Luxembourg emphasised the importance of preventive treatment of *Varroa*, highlighting the efficacy of timely treatment given during both summer and winter seasons. This strategic approach was associated with a marked reduction in colony loss. Similarly, study by K. Naharki and S. Regmi (2020), in Canada highlighted the significant impact of *Varroa* infestation on bee health: approximately 79% of winter family mortality was associated with the presence of *Varroa* mites. These collective results confirm the crucial role of *Varroa* control measures in mitigating bee family losses in different geographical regions. By actively controlling and applying effective *Varroa* control strategies, Albanian beekeepers can increase the resilience of their families and contribute to the overall health and sustainability of bee populations.

To mitigate losses and increase the resilience of bee families, M. Stanković *et al.* (2023) recommend several preventive measures. Firstly, improving the genetic quality of mothers is of paramount importance. Selecting and breeding mothers with desirable characteristics can enhance colony health and productivity over subsequent generations. In addition, efforts to reduce the occurrence of laying workers or laying mothers are critical. The presence of such individuals can disrupt colony dynamics and jeopardise overall hive productivity. Controlling Africanisation by replacing European queens is another strategy to mitigate losses. By introducing European queens into hives prone to Africanisation, beekeepers can help maintain the desired genetic and temperament traits in their families (Salyuk, 2023).

As noted by N. Capela *et al.* (2023), minimising pesticide exposure is critical to protecting bee health. This can be achieved by working with farmers to coordinate pesticide application schedules and selecting strategic locations for apiaries that reduce the risks of pesticide exposure. Moreover, it is vital to improve the diagnosis and control of varroosis and other diseases and pests. Regular monitoring, timely intervention and the use of effective treatment protocols can help mitigate the detrimental effects of disease outbreaks and pest infestations (Kończak *et al.*, 2023).

At the national level, it is critical to promote educational initiatives and training resources for beekeepers. R. Bava *et al.* (2023) add that providing accessible and comprehensive educational materials can provide beekeepers with the knowledge and skills necessary to identify potential threats to bee family health, take preventive measures and respond effectively to problems as they arise. By fostering a culture of continuous learning and equipping beekeepers with the tools and knowledge necessary to address the multifaceted challenges faced by bee populations, a sustainable and productive beekeeping industry can be worked towards.

CONCLUSIONS

A study conducted between 2021 and 2023 in Albania has revealed serious problems related to the loss of bee families caused by various factors. Through careful data collection involving 15,493 beekeepers, the main factors causing bee family deaths at different times of the year were observed.

One of the main causes of bee family death is Varroaosis, caused by parasitic *Varroa* mites. This disease causes bees to become emaciated and reduce their ability to reproduce, eventually leading to colony collapse. In addition, Nosematosis caused by microscopic parasitic protozoa is also a significant cause of bee family loss. Symptoms of *Nosema* include diarrhoea and weight loss of the bees, leading to exhaustion and death. The third cause of bee family loss is weakness,

caused by various factors such as lack of a queen, low numbers or an ageing queen. Weak families are more susceptible to disease and more likely to die. Interestingly, bee family losses are higher in the winter months than in summer, which is explained by the fact that bees are dormant in winter and less able to resist diseases and stresses. On average, winter losses were 20%, while summer losses were only 5%. Thus, the study showed that the loss of bee families in Albania is a serious problem and measures are needed to control Varroaosis, *Nosema* and other factors threatening beekeeping in the region.

Further research on bee family loss in Albania should focus on several key aspects, including genetic resistance of bees, development of new parasite control methods, study of environmental factors, development of sustainable beekeeping practices, education and public awareness, and establishment of a monitoring and information exchange system among stakeholders. These efforts will contribute significantly to the conservation of beekeeping in the region, ensuring the sustainability of ecosystems and agricultural production in Albania and beyond.

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CONFLICT OF INTEREST

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Аналіз ризиків втрати колоній *Apis mellifera* та оцінка стану здоров'я в Албанії з 2021 по 2023 рік

Кастріот Корро

Доцент, викладач

Сільськогосподарський університет Тирани

1025, вул. Паїсі Водіца, м. Тирана, Албанія

<https://orcid.org/0000-0002-9304-1041>

Вітор Малутай

Президент федерації

Федерація бджолярів Албанії

1001, вул. Мухамет Деліу, м. Тирана, Албанія

Викладач

Сільськогосподарський університет Тирани

1025, вул. Паїсі Водіца, м. Тирана, Албанія

<https://orcid.org/0009-0004-7462-4577>

Гані Мока

Професор, викладач

Сільськогосподарський університет Тирани

1025, вул. Паїсі Водіца, м. Тирана, Албанія

<https://orcid.org/0009-0005-5991-838X>

Меріє Елезі

Доцент, викладач

Університет Тетова

1200, вул. Ілінден, м. Тетова, Республіка Північна Македонія

<https://orcid.org/0000-0002-5073-2483>

Беснік Елезі

Доцент, викладач

Університет Тетова

1200, вул. Ілінден, м. Тетова, Республіка Північна Македонія

<https://orcid.org/0009-0008-7867-9187>

Анотація. Актуальність дослідження зумовлена скороченням чисельності бджолиних сімей в Албанії, оскільки необхідність розуміння динаміки втрати бджолосімей та факторів, що її спричиняють, має першочергове значення. Метою дослідження було комплексне вивчення поширеності та основних причин загибелі бджолосімей, приділяючи особливу увагу зараженню кліщем *Varroa*, нозематозу, вірусним патогенам, пестицидам та бактеріальним інфекціям. Використовуючи метод стратифікованої вибірки, у дослідженні взяли участь 15 493 бджолярів різного віку та досвіду. Для збору даних про втрати бджолиних сімей, практики управління та фактори навколишнього середовища, що впливають на здоров'я бджіл, використовувалися як електронні, так і особисті опитування. Крім того, програми моніторингу дозволили детально оцінити стан здоров'я бджолиних сімей та умови навколишнього середовища на пасіці, надавши цінну інформацію про часові тенденції та закономірності. Отримані дані свідчать про тривожні темпи зараження кліщем *Varroa*, поширеність нозематозу та складну взаємодію факторів, що призводять до втрати бджолосімей, особливо в літні та зимові місяці. Наприклад, кліщ *Varroa* був виявлений у 61 % з 29,474 зразків бджіл, відібраних під час літнього відбору проб, з показниками від 0,5 % до 70,2 %. Аналогічно, під час осіннього відбору 65 % з 43,037 зразків бджіл містили кліщів *Varroa*, а середній рівень зараження становив 5,3 %. Крім того, нозематоз також є складною проблемою, оскільки його клінічна поширеність коливається від 0,1 % восени до 1,3 % влітку та навесні. Ці ключові цифри підкреслюють нагальну потребу в розробці ефективних стратегій для зменшення зараження кліщем *Varroa* та нозематозу, тим самим підтримуючи популяції бджіл та здоров'я екосистеми. Результати дослідження є цінним внеском в управління бджільництвом та розробку політики, підкреслюючи важливість цілісних підходів до підтримки здоров'я та стійкості бджіл в Албанії

Ключові слова: вароатоз; зимові втрати; нозематоз; матка; сезонні хвороби



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Analysis of the factors influencing the quality of farmed mud crabs in the Mekong Delta, Vietnam

Danh Le Ngoc*

Doctor of Agricultural Economics

Kien Giang University

320 A, 61 Highway, Minh Lương Town, Kien Giang Province, Vietnam

<https://orcid.org/0009-0002-8976-9037>

Tuan Ngo Anh

Doctor of Agricultural Economics

Can Tho University of Technology

256 Nguyen Van Cu, Can Tho City, Vietnam

<https://orcid.org/0009-0009-1537-3823>

Tac Nguyen Van

Doctor of Agricultural Economics

Nam Can Tho University

168 Nguyen Van Cu Ext Str., Can Tho City, Vietnam

<https://orcid.org/0009-0002-2522-4628>

Hien Nguyen Thi Bich

Kien Giang University

320 A, 61 Highway, Minh Lương Town, Kien Giang Province, Vietnam

<https://orcid.org/0009-0304-0045-3491>

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Abstract. Enhancing the quality of cultivated mud crabs is a crucial element for elevating productivity, quality, and economic effectiveness within the mud crab farming sector. This study investigates factors influencing the quality of farmed crabs in the Mekong Delta, Vietnam. The study employs descriptive statistical methods and synthesis approaches to analyse the current situation of crab farming in the Mekong Delta, Vietnam. Additionally, multivariate regression analysis was employed to identify factors affecting the quality of farmed crabs in the Mekong Delta, Vietnam. The survey results from 304 crab farmers in the Mekong Delta, Vietnam indicate that the total area of the Mekong Delta, Vietnam is 465 thousand hectares, with a production of 68 thousand tons. Among the provinces in the Mekong Delta, Vietnam, Kien Giang, Ca Mau, and Bac Lieu are the top three provinces with the highest area and production. Furthermore, factors influencing the quality of crab products during the farming process were analysed, and three main factors were identified: the decision to catch crabs at the fourth moult stage (Y4), the

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*Corresponding author

number of crab traps used, and the duration of the farming period. This research represents the first study on the quality of farmed crab in the Mekong Delta, Vietnam. Therefore, these findings provide guidelines for improving the quality of farmed crab and serve as a foundation for further research in this field

Keywords: decision to catch crabs; Mekong Delta; mud crab quality; farming

INTRODUCTION

The total output of farmed mud crabs in the Mekong Delta in 2020 was 67 thousand tons, an increase of 19% compared to 2015 (Directorate of Fisheries, 2020). Converting the specialized shrimp farming model into extensive crab farming initially brings high profits. On average, each farmer earns 30 million VND/hectare/crab crop (Long, 2019). In addition to the rapid growth in output and profits for mud crab farmers, the mud crab industry faces many difficulties in consumption as well as stable development. The rapid and unstable development has caused current difficulties for the mud crab industry such as a lack of specific farming area planning for mud crabs, unquarantined breeds, and lack of cooperation between entities, the undeveloped mud crab brand, lack of a set of standards in the production stage, inconsistent mud crab quality, slow transportation and long waiting time, and lack of product diversity for consumers. These reasons caused unstable development of mud crab products in recent years and the products have not been exported to international markets.

T. Trang and N. Toan (2020) and A. Coronado Mondragon *et al.* (2021) believe that product quality contributes to the expansion of competitive advantage while products are designed and manufactured to achieve customer requirements related to high product performance. D. Love *et al.* (2021) believe that there are many product features used to evaluate the quality of that product. Among them, the most common attributes are technical, practical, aesthetic, economic, ergonomic, ecological, and logistical factors. Fishery quality refers to the objective or subjective value attributed to one or all of the four identified qualitative characteristics. The quality of aquatic products directly influences the revenues of crab farmers and affects the economic chain. Quality can include four characteristics: nutrition, safety, function, and sensory attributes, depending on the type of seafood. Assessing the quality of aquatic products will involve product research, experience, beliefs, and other attributes. Li *et al.* (2018) suggest that, in particular, product research is done before purchasing (price, colour, size), experience is evaluated after the process of purchasing and consuming the product (smell, taste), beliefs are related to food safety (being free from chemical or microbiological contamination) and other attributes cannot be observed both before and after consumption such as production method or place of production (Stadtler, *et al.*, 2015; Booncharoen & Anal, 2021).

N. Mangun *et al.* (2021) define that quality standards include all activities and measures to ensure that

products are delivered to consumers with the best quality and that the products have the maximum satisfaction of consumer needs at the lowest cost. Analysis of product quality standards must also be performed throughout the product life cycle from production and distribution to product consumption. However, T. Phong *et al.* (2021) determined that product quality for mud crab products depends largely on the production stage. In addition, analysing mud crab quality is to manage the following quality attributes: (1) nutritional quality; (2) sensory quality (colour, flexibility, size) and eating quality (fatness, sweetness); (3) product quality (tying quality, shipping quality, aesthetic quality); (4) food hygiene and safety quality (soil environment, water, air; aquatic chemicals; storage and sale); (5) preservation quality; and (6) seed quality. Furthermore, mud crab is a commodity whose meat needs to be fresh, and the quality of mud crab is greatly affected by the farming process of farmers. This is also the biggest barrier that makes the mud crab industry develop slowly (Samah & Kamaruddin, 2015).

Given the current status of mud crab production by farmers in the Mekong Delta and the context of international economic integration, the Mekong Delta region requires practical and effective solutions to enhance quality and satisfy consumer requirements related to competitive prices and help develop the mud crab industry in the Mekong Delta sustainably. The study aims to establish theoretical foundations and demonstrate the logical methodology of determining factors influencing the quality of farmed mud crabs among crab farmers in the Mekong Delta, Vietnam. The hypotheses of this study posit the existence of relationships between factors such as experience, training, number of harvests, decision to catch crabs Y4; the number of crab traps; the farming time of crabs; pond renovation and breeds.

MATERIALS AND METHODS

Following Binh Dien Wholesale Market (2020), the mud crab market in Vietnam is regulated by a set of criteria, such as type, size, and quality of mud crabs. Crab parts are divided into 3 main types per crab: meat crabs (male or female crabs without crab roe over 180g), gravid crabs (female crabs with roe over 200g) and mixed crabs (crabs with missing claws, sticks or soft shells). However, each type of crab is different based on size and gender and thus the market divides mud crabs into 4 types. The 3 types of which meet quality standards (Y1 crab, Y4 crab, gravid crab) and 1 type does not meet standards about quality (mixed crab).

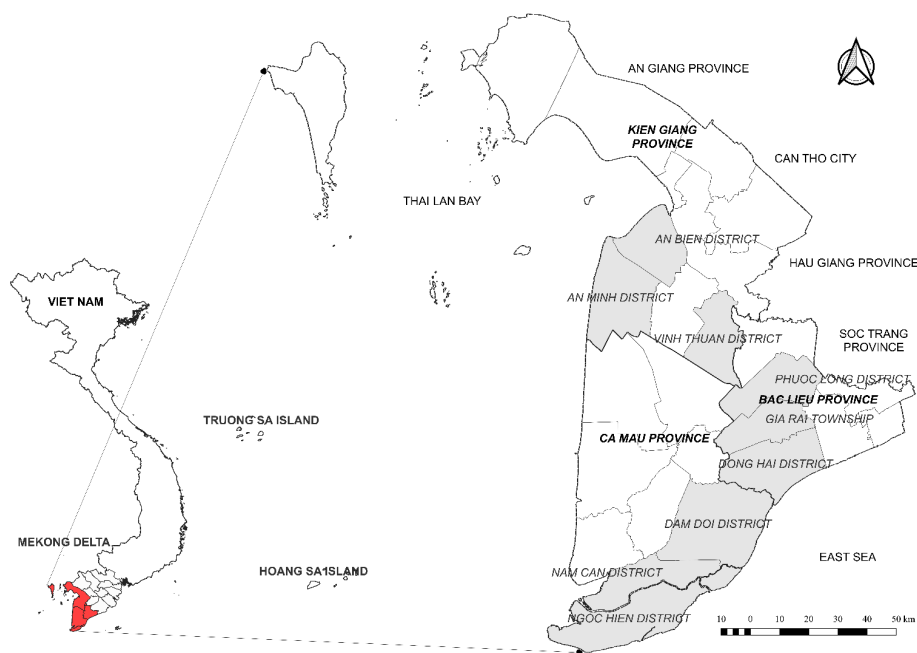
Table 1. Criteria for classifying mud crabs in the Mekong Delta

No.	Crab type	Description	Size	Quality
1	Y1 crab (meat crab)	Y crab is a male crab or meaty crab, with big claws and a long Y-shaped apron	>270g/crab	All intact parts intact, no dirt or parasites clinging to the crab, good reflexes, and more than 70% flesh in the body
2	Y4 crab (meat crab)	Y crab is a male crab or meaty crab, with big claws and a long Y-shaped apron	180-265 g/crab	All intact crab parts, no dirt or parasites clinging to the crab, good reflexes, and more than 70% flesh in the body
3	Gravid crab	Female crab carries crab roe	>200g/crab	All intact crab parts, no dirt or parasites clinging to crab, good reflexes, and dark yellow roe
4	mixed crab	The type of meaty crab only has one claw or has a broken stick, the shell is soft, and the meat in the crab body is low		No dirt or parasites clinging to crab, and good reflexes

Source: compiled by the authors based on the Binh Dien Wholesale Market (2018)

Sampling. The study selected three provinces with the largest mud crab output using multi-stage probability sampling based on the criteria of mud crab output of provinces in the Mekong Delta in 2020 after the covid 19 pandemic, mainly Kien Giang, Ca Mau and Bac Lieu. From the distribution structure of mud crab production in districts in three provinces, the study selected three districts from each province. Three communes were selected from each district, and three hamlets were selected from each commune according to the criteria of greatest output to

be the research areas. In Kien Giang province, there were 03 districts (An Minh, An Bien, Vinh Thuan) with the highest mud crab production (accounting for 90% of the entire province). Ca Mau province has 3 districts (Nam Can, Dam Doi, Ngoc Hien) with the output accounting for 60% of the entire province and Bac Lieu province has 3 districts (Gia Rai, Phuoc Long and Dong Hai) with output accounting for 70% of the entire province. Probability sampling was conducted using a systematic sampling method at hamlets based on the list of mud crab farmers (Fig. 1).

**Figure 1.** Map of survey locations

Note: red is the survey area of mud crab farmers (Kien Giang, Ca Mau and Bac Lieu)

Source: compiled by the authors

Therefore, based on consultation with experts and previous studies by T. Trang and N. Toan (2020) the dependent variable is calculated via a scale (%) that is equal to the total output of bucket crabs (poor quality crabs)/(total output of all types of crabs). In detail,

“unqualified bucket crabs” are defined as crabs with defective appendages, soft shells, and not meeting the meat and size ratio. The independent variables are interpreted for meanings and the scale is explained in Table 2.

Table 2. Factors in the production stage influencing the mud crab quality

Independent variable	Symbol	Explanation	Expectation
Quality	Y	The output of bucket crabs/ Total output of crabs (%)	
Experience	X ₁	The number of crab farming years of head of household	
Training	X ₂	Participation in training classes in mud crab farming. A dummy variable (receiving the value 1 in the case of participating in training and otherwise, receiving the value 0)	+
Number of harvests	X ₃	Total times of harvests in a crop (time/crop)	-
The decision to catch crabs Y4	X ₄	Catching Y4 crabs (receiving the value 1 in the case of catching the Y4 crabs and otherwise, receiving the value 0)	-
The number of crab traps	X ₅	The number of crab traps (trap/1000m2).	-
The farming time of crabs.	X ₆	The number of months in a crab farming crop (month/crop).	+
Pond renovation	X ₇	Pond renovation (receiving the value 1 in the case of having pond renovation and otherwise, receiving the value 0.	+
Breeds	X ₈	Breed quarantine (receiving the value 1 in the case of having breed quarantine and otherwise, receiving the value 0)	+

Note: expectation + affects in the same direction as variable Y; - opposite effect on variable Y

Source: compiled by the authors

The sample size of the production stage of mud crab farmers is determined to satisfy the minimum sample size in the sample size calculation formula based on (Yamane, 1967) as follows:

$$n = \frac{N}{(1+N*\varepsilon^2)}, \quad (1)$$

where n is the minimum number of households that need to be surveyed and N is the number of households raising crabs in the research areas. Following the Directorate of Fisheries (2020) of the three provinces of Kien Giang, Bac Lieu and Ca Mau, the total number of households farming mud crabs (N) is 225,167 households. ε is the sampling error, and according to (Subong & Beldia, 2005), ε is 6% (Nam, 2008)

Therefore, according to the formula, the minimum sample number is:

$$n = \frac{225.167}{(1+225.167*0.06^2)} = 277 \text{ household.} \quad (2)$$

However, to ensure high representativeness, 304 mud crab farmers in the three provinces of Kien Giang, Bac Lieu and Ca Mau were interviewed using face-to-face methods and structured questionnaires. All sea farmers provided participation agreements. Research model. A multivariate linear regression model is employed to study the linear relationship between independent factors (independent variables) X_i and a certain factor Y (dependent variable). In particular, X_i affects Y and Y is considered a factor that is dependent on X_i . "The goal of regression analysis is to identify a relationship via a mathematical model that best represents the relationship between X and Y ". The linear regression model is as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n, \quad (3)$$

where Y is a dependent variable that describes the quality of mud crabs in the production stage. Meanwhile, mud crab quality is applied based on the general standards of the seafood industry, which is fresh seafood. When circulating in the market, it must ensure compliance with national standards per food hygiene and safety, chemical residues, and antibiotics (Ministry of Science and Technology, 2016). It is based on a set of criteria including type, size, and quality of mud crabs, as well as gender, the market divides mud crabs into 4 types. The 3 types of which meet quality standards (Y1, Y4, fat crab) and 1 type does not meet quality standards (bucket crab).

RESULTS AND DISCUSSION

Mud crab farming area in the Mekong Delta. In 2016, the mud crab farming area of the Mekong Delta increased by 50% compared to 2012 and the mud crab farming area in this region has been stabilizing from 2016 to 2020 (Table 3). In 2020, the mud crab farming area of the whole Mekong Delta region was nearly 465 thousand hectares. Three provinces of Kien Giang, Ca Mau and Bac Lieu had the highest mud crab farming area in the region, the leading province was Ca Mau province with a total area of nearly 277 thousand hectares, followed by Kien Giang and Bac Lieu with an area of 73 thousand hectares. In recent years, due to high profits from the combined crab farming models, substantial investments in the expansion of their crab farming area and switching to mud crabs were made (Table 3).

Table 3. Mud crab farming area in some provinces in the Mekong Delta in the period of 2016-2020

No.	Province	2016	2017	2018	2019	2020
11	Ca Mau	273,237	271,185	273,463	276,377	276,935
22	Kien Giang	58,755	64,284	64,357	65,544	73,245
33	Bac Lieu	111,929	111,213	98,320	87,213	72,962
44	Ben Tre	26,800	26,976	27,212	27,762	28,654
55	Tra Vinh	10,554	10,987	11,324	12,764	13,000
66	Soc Trang	372	350	142	135	160
Total		481,647	484,995	474,818	469,795	464,956

Note: metric unit: Hectare**Source:** Agricultural Extension Center (2019)

Mud crab output in the Mekong Delta. From 2016 to 2020, farms of mud crabs in the Mekong Delta were regarded as important crab farms in Vietnam (Table 4). Mud crab production accounted for 73% of the country's total crab production. In 2020, the whole region reached 68 thousand tons, an increase of nearly 8.2% of the total output within 5 years. Besides, the average output of mud crabs in 2016 was 143 kg/hectare and in 2020 was 146 kg/hectare. This

determines that the average output of mud crabs was very low. The reason for this was the household's lack of mud crab farming knowledge, which was the object that was raised with forms such as extensive form, improved extensive form, polyculture with shrimp and raised in mangroves. Provinces such as Kien Giang, Ca Mau and Bac Lieu have the highest production, accounting for 80% of the total production in the Mekong Delta.

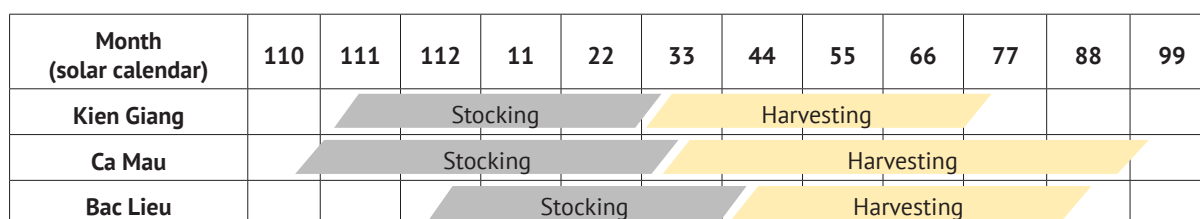
Table 4. Total production of mud crabs in some provinces in the Mekong Delta in the period of 2016-2020

No.	Province	2016	2017	2018	2019	2020
1	Kien Giang	17,675	16,790	17,347	18,759	20,305
2	Ca Mau	17,414	20,086	21,993	22,177	24,027
3	Bac Lieu	14,675	15,792	14,748	12,733	10,214
4	Tra Vinh	8,000	8,130	7,360	7,913	8,100
5	Ben Tre	4,820	4,801	4,898	4,664	5,157
6	Soc Trang	203	190	150	150	165
Total		62,787	65,789	66,496	66,396	67,968

Note: metric unit: tons**Source:** Agricultural Extension Center (2019)

The opinion of H. Dat (2007) on mud crab farming season in the Mekong Delta. The time of raising mud crabs from stocking to harvesting is approximately 3.5-5 months. When crabs reach a weight of over 250 grams/crab, they can be harvested gradually.

Therefore, mud crabs can be raised three times a year. In Kien Giang province, crab farming lasts an average of 8 months, mud crab farmers stock from mid-November and harvest from March to July next year (Fig. 2).

**Figure 2.** Crab farming season in the Mekong Delta**Source:** compiled by the authors

Crab farming season in Ca Mau province lasts 11 months and mud crab farmers stock from mid-October and start harvesting from March to September next year. Ca Mau is a province with a year-round crab-shrimp

farming model. In Bac Lieu province, the farming season lasts an average of 8 months, mud crab farmers start stocking from early December to April next year, and harvest from April to August. In Ca Mau province,

the average crab farming time is 10 months, which is much higher than in Kien Giang and Bac Lieu provinces. Most mud crabs are harvested from March to July, with gradual crab price decrease due to the quantity being higher than the demand. However, in the remaining months of the year, crab harvest levels are very low and thus the price of crabs is often high, peaking in October to December (Fig. 2).

Technical information on crab farming model in the Mekong Delta. The average crab farming area of a household is 2.5 hectares/household. There is a high difference between the lowest household having an area of 0.2 hectares/household and the highest household having an area of 11 hectares/household. Farming areas and farming ponds are also important factors influencing household costs and, thus, the study will focus on analysing these two aspects. The average width of the crab farming ditch is 3.5 meters, the lowest width is only 1 meter, and the highest width is 12 meters. The average depth of the crab farming ditch of extensive farming households in the Mekong Delta is 1.1 meters. The average farming time per crop is from 8 months to 9 months. Because the crab farming model combines shrimp farming and has intercropping with rice farming, there are areas where the farming time is only 6 months, but there are also areas where the farming time lasts 12 months. For the origin of seed crabs, about 90.9% of seed

crabs are not quarantined and only 9.1% of seed crabs are quarantined when farming. Mud crab seed sources purchased by households are mainly produced in the Mekong Delta provinces. Kien Giang and Ca Mau provinces have the largest number of crab seed farms.

For seed size, this is an extensive farming model and thus crab seeds are released many times during the farming season, on average 4 to 5 times/crop. The breed size that farmers choose to release most is pepper crabs, accounting for 78.9% and only 21.1% of watermelon crabs. The average density of mud crab farming via survey results is 0.3 crabs/square meter (m^2). However, there is a high difference between households with the lowest density of 0.03 crabs/ m^2 and the highest density of 25 crabs/ m^2 while the most suitable farming density for mud crabs, according to the recommendations of the Department of Agriculture and Rural Development is 0.05 crabs/ m^2 . Depending on the pond depth and conditions of farmers in terms of production capital, fuel for production, water supply and change systems, shrimp can be raised at a higher density than the recommended density. Meanwhile, the average density of shrimp in polyculture with crabs is 2 shrimps/ m^2 , while the density of extensive shrimp farming in polyculture with crabs is 1 shrimp/ m^2 , according to recommendations of the Department of Agriculture and Rural Development (Table 5)

Table 5. Technical information on the crab and shrimp model in the Mekong Delta

No.	Criteria	Metric unit	Mean	Min	Max	Standard deviation
1	Pond area	1,000 m^2	25.7	2.0	110.0	16.5
2	Width of farming ditch	meter	3.5	1.0	12.0	1.4
3	Depth of farming ditch	meter	1.1	0.5	2.0	0.2
4	Farming time/crop	month	8.8	6.0	12	2.3
5	Seed source	%				
	Traders	%				93.0
	Hatchery	%				6.0
	Catching	%				1.0
	Having quarantine	%				9.1
	No quarantine	%				90.9
6	Seed size					
	Pepper crab	2.18 mm				78.9
	Watermelon crab	3.2 mm				21.1
7	Density of releasing crabs	crab/ m^2	0.3	0.004	4.0	0.4
8	The number of times of releasing crabs/crop	time	4.9	1.0	24	4.2
9	The gap from the last time of releasing crabs to the last time of harvesting the crabs	month	3.0	1.0	6.0	2.5
10	The number of times of releasing shrimps/crop	time	5.6	1.0	24	4.1
11	Density of releasing shrimps	shrimp/ m^2	1.8	0.03	25.0	2.6
12	Developing seed again before releasing					

Table 5. Continued

No.	Criteria	Metric unit	Mean	Min	Max	Standard deviation
	Having developing seed	%				14.0
	No development of seed	%				86.0
13	Distance from pond to main road	km	1.1	0.3	30.0	

Source: compiled by the authors

Due to the nature of the extensive farming model and the aspect of releasing crabs many times in one crop, the size of crabs in the pond is not uniform. Therefore, according to the recommendation of the Agricultural Extension Center (2019), households should develop the crabs within 15 days after catching crabs and before releasing them into ponds. However, only 14% of the total surveyed households develop crabs before releasing them into ponds, the remaining 86% of households release crab seeds directly into ponds. The average distance from the farming pond to the selling place is 1.1 km and the highest distance is up to 30 kilometres (km), with a fairly large standard deviation of 2.3 km. Distance from locations of crab ponds relative to water sources (rivers, canals) and roads are also important factors influencing the efficiency and convenience in the process of transporting crab seeds and harvesting crabs, and water quality (Kha, 2018)

N. Mangun *et al.* (2021) believe that the pond renovation process is substantial. The process of renovating extensive crab farming ponds in the Mekong Delta greatly influences the quality of mud crabs raised in the region. If the pond renovation is done well, a substantial number of natural foods in the mud crab farming ponds will be available, and mud crabs will not attack each other and quickly grow to have crab roe. This will increase the quality of mud crabs in the Mekong Delta at the production stage (Kamaruddin & Baharuddin, 2015). Furthermore, the survey results demonstrated that the household pond renovation process was relatively simple. As a result, only 125 households pumped water out of ponds, with an average pumping time of only 1.7 hours/1,000m²/crop. There

were still households pumping with a very low time of 0.02 hours/1,000m²/crop. These were households that took advantage of the receding tide to release water and thus the amount of water left in the pond was miniscule. For the time of pumping water after renovating the ponds, there were 160 households pumping water out of the ponds in the total number of surveyed households of 308. The remaining households took advantage of the rising tide to get water into the ponds. The average time to pump water was 1.6 hours/1,000m²/crop, which was similar to the time to pump water out of the ponds, only pumping more after taking advantage of the rising tide. Thus, the amount of fuel consumed for water pumping was low, an average of 1.9 litres/1,000m²/crop. Good water pumping time management and the number of pumping cycles will limit environmental contamination in the ponds, thereby ensuring the quality of mud crab products produced.

The extensive crab farming model with trash fish in the ponds was harmful to seed crabs, as it reduced the survival rate, causing high seed costs and low crab output. Therefore, killing trash fish is a requirement in the crab farming process. Currently, according to recommendations from the Department of Agriculture and Rural Development, fish medicine or saponin can be used for fish control, depending on high or low salinity. As a result, 73% of mud crab farming households use fish medicine with an average weight of 0.6kg/1000 m²/crop. However, there is a high difference between households in the region, with some using up to 3.2 kg/1,000m²/crop. Almost 33% of crab farmers use saponin for fish control with an average volume of 0.7 kg/1,000m²/crop (Table 6).

Table 6. Information on the pond renovation process of crab farming model in the Mekong Delta

No.	Features	Unit	Observation (N)	Mean	Min	Max	Standard deviation
1	The water pumping time out of the pond	Hour	125	1.7	0.02	8.0	1.4
2	The water pumping time into the pond	Hour	160	1.6	0.07	10.1	1.4
3	Fuel quantity	Litre	166	1.9	0.06	16.7	1.8
4	The amount of fish medicine	Kg	221	0.6	0.003	3.2	0.4
5	The amount of saponin	Kg	99	0.7	0.05	4.4	0.6
6	Limestone	Kg	193	5.9	0.09	46.0	5.5
7	Calcium lime	Kg	55	9.9	0.8	31.0	5.8
8	Dolomite	Kg	14	5.9	0.20	20.0	5.8
9	Probiotics	Kg	94	0.3	0.08	2.4	0.5

Note: metric unit: 1,000 m²/crop

Source: compiled by the authors

Effectively limestone usage is a decisive factor in the output of the extensive crab farming model in the Mekong Delta. As a result, 63% of mud crab households used limestone in the pond renovation process with an amount of 5.9 kg/1,000m²/crop because the limestone had an alum reduction feature and a bactericidal feature. Thus, it is recommended to use limestone in the pond renovation process. In addition, calcium lime (CaCO₃) was used by 18% of crab farming households with an amount of 9.9 kg/1,000m²/crop, and dolomite lime (CaCO₃ and MgCO₃) was used by 14 households, with an amount of 5.9 kg/1,000m²/crop in both the lime types, had the effect of increasing the pH and alkalinity of water. In addition, 31% of mud crab farmers use probiotics during their pond renovation process with an average amount of 0.3 kg/1,000m²/crop to create a beneficial microbiological environment in the water to reduce pests caused by harmful microorganisms from the environment to ensure the quality of farmed mud crab meat.

Care processes. Good crab care management increases the rate of good-quality crabs and reduces the bucket crab rate. Furthermore, the management process of extensive crab and shrimp farming ponds in the Mekong Delta determines the success as well as the quality of the mud crab farming model. The survey results showed that 44% of mud crab farmers pumped water into ponds, with the average number of pumping times (12 times) and the average pumping time of only 5.1 hours/1,000m²/crop. Thus, on average, each time the water pumping was 0.5 hours, this was consistent with the natural reality in the surveyed provinces in the Mekong Delta region. Each month farmers pumped more water into ponds once a month when the tide was high. However, there were still households that pumped water for a long time, with 50 hours/1,000m²/crop because these households had unstable bank structures and they could not keep the amount of water in the ponds. The average quantity of fuel for water pumping during care was 1.9 litres/1,000m²/crop (Table 7).

Table 7. Information on the care processes of the crab farming model in the Mekong Delta

No.	Criteria	Unit	Observation (N)	Mean	Min	Max	Standard deviation
1	The number of water pumping times	time	134	12	1	121	18
2	The water pumping time into the pond	hour	134	5.1	0.2	50	8.1
3	Fuel quantity	litre	129	1.9	0.04	25	3
4	The amount of fish medicine	kg	53	1.1	0.06	5.4	0.9
5	The amount of saponin	kg	20	2.1	0.2	8.4	2.1
6	Algae remover medicine	kg	31	0.2	0.02	1.1	0.2
7	Limestone	kg	69	10	1.6	67.5	10
8	Calcium lime	kg	30	29	1.2	169	40
9	Dolomite	kg	15	43.5	4.4	200	52.7
10	Zeolite	kg	55	51.7	1.1	402	85.1
11	Probiotics	kg	64	0.8	0.02	7.5	1.4
12	The number of trash fish foods	kg	39	24.7	0.13	142	28
13	The number of snail foods	kg	29	44.7	3	267	63.7

Note: metric unit: 1,000m²/crop

Source: compiled by the authors

Survey results showed that only 17% of mud crab farmers used fish medicine and 20 households used saponin to kill impurities in the ponds with an average usage of 1.1 kg/1,000m²/crop. Limestone is used in limited numbers. Only 69 mud crab farming households used limestone with an average of 10 kg/1,000m²/crop, 10% of the households used calcium limestone with an average amount of 29 kg/1,000m²/crop and 15 households used dolomite limestone with an average amount of 43.5 kg/1,000m²/crop. Zeolite is recommended by the Department of Agriculture and Rural Development to be used in the farming process to help improve the water environment and improve the bottom to create natural foods in extensive crab and shrimp farming ponds.

However, only 55 households used it with an amount of 51.7 kg/1,000m²/crop. Probiotics are also rarely used, with 21% of the mud crab farming households using 0.8 kg/1,000 m²/crop. Foods in the pond are a factor influencing the quality of mud crab products. If mud crabs lack food, the proportion of bucket crabs increases, thereby causing a reduction in the crab quality. According to survey results, the extensive crab and shrimp farming process mainly took advantage of natural food sources. However, when releasing mud crabs with high density, natural foods are not sufficient, and farmers also supplement foods for the crabs. The main foods are trash fish, with 13% of farmers supplementing it with an amount of 24.7 kg/1,000 m²/crop and 10% of

farmers supplementing the snail food with an average amount of 44.7 kg/1,000 m²/crop. The remaining farmers mostly took advantage of natural food sources.

Classification criteria of mud crabs in the Mekong Delta. There are 4 types of crabs in the Mekong Delta based on the criteria used to evaluate the quality of mud crab output at the production stage (such as size, proportion of meat in the body, gender, dirt, parasites clinging to crab, and reflexes). According to the criteria for evaluating Y1 crabs in the Mekong Delta, crabs were larger than 270g in size, did not lose appendages, were free from dirt and parasites, had good reflexes and had a meat percentage of over 70%. According to survey results, the Y1 crab type in the Mekong Delta region accounted for 35%, the highest type in the 4 types of crabs. Y4 crabs were similar to Y1 crabs, which were

meaty, but the size was smaller from 180 to 256 g and accounted for 33% of the entire region. These two types of crab meat accounted for 68% of mud crab output throughout the supply chain. The fat crab was the type of crab that is raised to collect crab roe and was classified according to the roe criteria of 200g or more and must ensure that all parts were intact, free from dirt or parasites, had good reflexes and had dark yellow roe. This fat crab accounted for very low output, only 16% of the total mud crab output in the entire supply chain, while the type of fat crab had a very high economic value compared to other types of mud crabs. Lastly, bucket crab accounted for a large rate of 16% of the total mud crab output throughout the supply chain. Bucket crab was the type of crab that was poor-quality and did not meet the quality criteria of the above crabs (Fig. 3).

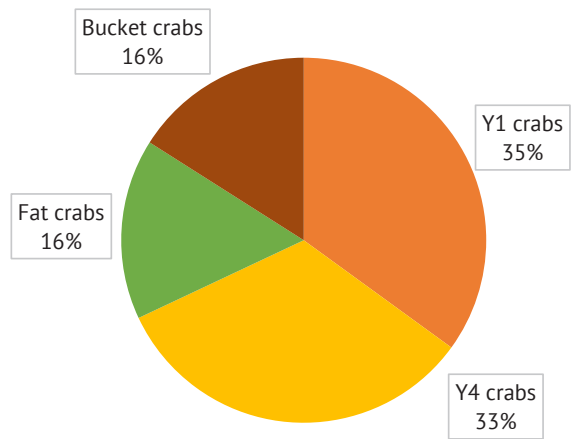


Figure 3. Classification of mud crabs based on criteria of mud crab quality assessment in the Mekong Delta
Source: compiled by the authors

Factors influencing mud crab quality in the production stage in the Mekong Delta. Management of farming techniques will increase the quality of mud crabs in the Mekong Delta, especially the rate of firm-shell crabs and help reduce the rate of bucket crabs. Therefore, the study used an ordinary least squares (OLS) regression model to analyse factors influencing mud crab quality at the production stage, thereby proposing solutions to

help mud crab farming households increase mud crab quality. The dependent variable of the model was bucket crab output in total with harvested crab output (Y) and 8 independent variables consisting of 1) the experience of crab farmers; 2) technical training; 3) the number of harvests in the season; 4) the decision to catch Y4 crab; 5) the number of crab traps; 6) the farming time; 7) pond renovation; and 8) breeds (Fig. 8).

Table 8. Factors influencing mud crab quality in the production stage in the Mekong Delta

Factors	Metric unit	Estimated coefficient	Standard error
Dependent variable	Bucket crab output (poor-quality crabs)/ (Total crab output)		
Independent variable			
Experience	The number of years of farming crabs (year)	-0.0017 ^{ns}	0.0012
Training	(Receiving the value 1 in the case of participating in training and otherwise, receiving the value 0)	-0.0097 ^{ns}	0.016
The harvest times	The number of harvest times in a crop (time/crop)	-0.0009 ^{ns}	0.001
The decision to catch Y4 crabs	(Receiving the value 1 in the case of catching crabs and otherwise, receiving the value 0)	0.0558 ^{***}	0.016
The number of used crab traps	The number of crab traps (trap/1,000m ²)	0.0012 [*]	0.0006

Table 8. Continued

Factors	Metric unit	Estimated coefficient	Standard error
The farming time	The number of months in a farming crop (month/crop)	0.0136 ^{***}	0.0039
Pond renovation	Receiving the value 1 in the case of having pond renovation and otherwise, receiving the value 0	-0.015 ^{ns}	0.0179
Breeds	(Receiving the value 1 in the case of having breed quarantine and otherwise, receiving the value 0)	0.0119 ^{ns}	0.0265
F-statistic	3.940		
Prob (F-statistic)	0.000		
N of valid cases	308		

Note: ***, **, *, and ns are significance levels of 1%, 5%, 10% and no statistical significance respectively.

Source: compiled by the authors

The correlation results show that there is no auto-correlation between each pair of variables at the 0.6 level. The estimated results in the model are statistically significant at the 1% significance level. The variance inflation factor (VIF) of the variables is 1.22 which is much smaller than 10, showing that the variables included in the model do not have multicollinearity. At the same time, the White test result has a p-value coefficient of 0.523 (greater than 5%), showing that the model does not have heteroskedasticity. Prob > Chi-Square (Chi-Square = 0.000) shows that the significance level of the F test of all coefficients of the variables in the model has results that are different from 0 and the model is suitable.

The results of regression analysis coefficients show that 3 statistically significant independent variables are factors influencing the quality of mud crabs in the farming stage in the Mekong Delta. In particular, the variable – the farming time is statistically significant at the 1% significance level and the estimated coefficient of the variable is -0.0136. This means that when the farming time of the household increases to 1 month, the rate of bucket crabs in total crab output decreases by 1.36%. This reduces the number of bucket crabs in the ponds and increases the quality of mud crabs in the model. It could be attributed to the long farming time, with more time for crabs to develop from soft-shell crabs to firm-shell crabs and the rate of fat crabs that are high-quality crabs and have high economic value increases. This is determined by the average time to raise fat crabs being 6 months. In addition, the increased farming time allows farmers to have the opportunity to develop soft-shell crabs into firm-shell crabs, which also increases the output of high-quality crabs in the ponds. This result is similar to previous research by some previous authors (Mojibar *et al.*, 2017; Long, 2019).

For the variable – the decision to catch Y4 crabs, the estimated coefficient is 0.0558 (significant at the 1% significance level), which denotes the moment when crab farmers decide to catch Y4 crabs that are young male crabs, with the proportion of bucket crabs in total mud crab output increasing by 5.58%. This can be explained by the fact that mud crab farming farmers catch Y4 crabs early, which increases the output of bucket

crabs in the total output during the farming process. This means that the output of Y1 crabs and fat crabs will decrease, thereby causing a reduced good-quality crab output. In addition, when farmers decide to catch Y4 crabs, which are young crabs that can easily break claws or sticks during the harvesting process, the output of bucket crabs in the total harvest output also increases. The results of this study are consistent with previous research on mud crab farming techniques (Mojibar *et al.*, 2017; Long, 2019).

For the variable – the number of crab traps, the estimated coefficient of the variable is 0.0012 (significant at the 10% significance level) and this means that when the number of crab traps in a crab farming household increases to 1/1,000 m², the bucket crab rate in total output of the model increases by 0.12%. The diminished yield of bucket crabs in the ponds can be attributed to the strategic placement of crab traps by crab farmers in suitable locations. According to survey results, the average number of crab traps for crab farming households was 4/1,000 m², more than the recommendation of the agricultural extension centre of 2/1,000 m² and studies on crab farming techniques researchers (Mojibar *et al.*, 2017; Long, 2019). This created labour waste because of putting many crab traps, collecting the crabs from the traps, and reducing the quality of mud crabs in the model.

For the non-statistically significant variable of training, previous studies suggest that farmers who undergo extensive technical training and acquire new knowledge can effectively control the quality of farmed mud crabs (Additionally, concerning the variable of experience, farmers with substantial experience are likely to manage crab farming models more efficiently, leading to an improvement in the quality of farmed mud crabs (Mangun *et al.*, 2021; Phong *et al.*, 2021).

The remaining variables of the analytical model including experience, training, the number of harvests, pond renovation, and breeds also influence the quality of mud crabs in the farming stage, however, they are not statistically significant at the 10% significance level. However, in reality, if there are many harvests in one crop, it will increase the output of crabs because many crabs that fall into traps can easily break their claws and sticks. In addition, production experience will help

farmers predict when the crabs will be firm and when the crabs will be shrivelled for a reasonable harvest, helping reduce the bucket crab output and increase the quality of fat crabs in the farming model.

CONCLUSIONS

Mud crabs exhibit favourable traits such as rapid growth, resilience to environmental changes, substantial size, and economic viability. However, the burgeoning mud crab farming industry faces challenges like disrupted plans, increased disease prevalence, and heightened competition leading to falling prices due to an oversupply of seafood products. The Mekong Delta's highest-quality seafood products hail from Ca Mau, Kien Giang, and Bac Lieu provinces. Key factors impacting mud crab quality during farming include farming time, the number of crab traps used, and the decision to catch Y4 crabs. To enhance profitability, farmers should adhere to safe farming practices, abstain from banned chemicals, invest in water retention machinery, avoid harvesting on tidal days, and harvest crabs at night to minimize waiting times. Longer farming periods allow crabs to develop into high-quality specimens with economic value. A conducive farming environment, encompassing water quality control, temperature regulation, and cleanliness, accelerates growth. Adequate and quality food provision, along with pest prevention, is crucial. Regular monitoring using tools and equipment facilitates the adjustment of farming processes.

Survey results show that crab farming households exceed the recommended trap density, necessitating calculation adjustments based on pond area and crab density. Proper trap maintenance and regular checks ensure effective crab capturing. Adjusting trap numbers

based on farming time and conditions is vital for efficiency. The decision to catch Y4 crabs increases the proportion of bucket crabs in the harvest, requiring farmers to choose an appropriate harvesting time and use suitable traps to avoid harm. Catching Y4 crabs too early may lead to small sizes, emphasizing the importance of utilizing traps with small holes for safe capture.

The scientific arguments presented provide a clear understanding of the existence of various factors (such as the decision to catch crabs at the fourth moult stage (Y4), the number of crab traps used, and the duration of the farming period) influencing the quality of farmed mud crabs. Research on these factors is a prerequisite for scientifically explaining the quality of farmed mud crabs in the agricultural domain. However, a limitation of the study is the inability to identify a significant relationship between variables such as experience, training, the number of harvests, pond renovation, and breeds with the quality of farmed mud crabs. Therefore, the focus of the scientific discussion aims to determine the key factors influencing the quality of mud crabs, expanding the scope of the research, and increasing the sample size to uncover factors that have not been investigated in this study.

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CONFLICT OF INTEREST

None.

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Аналіз факторів, що впливають на якість вирощених грязьових крабів у дельті Меконгу, В'єтнам

Дань Ле Нгок

Доктор сільськогосподарської економіки
Університет К'єн Гіанг

320 А, шосе, 61, м. Мінх Ліонг, провінція К'єн Джанг, В'єтнам
<https://orcid.org/0009-0002-8976-9037>

Туан Нго Ань

Доктор сільськогосподарської економіки
Технологічний університет Кантхо
Нгуєн Ван Ку, 256, м. Кантхо, В'єтнам
<https://orcid.org/0009-0009-1537-3823>

Так Нгуєн Ван

Доктор сільськогосподарської економіки
Університет Нам Кантхо
вул. Нгуєн Ван Ку Екст, 168, м. Кантхо, В'єтнам
<https://orcid.org/0009-0002-2522-4628>

Хієн Нгуєн Тхі Біч

Університет К'єн Гіанг
320 А, шосе, 61, м. Мінх Ліонг, провінція К'єн Джанг, В'єтнам
<https://orcid.org/0009-0304-0045-3491>

Анотація. Підвищення якості вирощених грязьових крабів є вирішальним елементом для підвищення продуктивності, якості та економічної ефективності в секторі вирощування грязьових крабів. У цьому дослідженні вивчалися фактори, що впливають на якість вирощених крабів у дельті Меконгу, В'єтнам. У дослідженні використовувались описові статистичні методи та підходи до синтезу для аналізу поточної ситуації в крабовому господарстві в дельті Меконгу, В'єтнам. Крім того, в дослідженні використовується багатовимірний регресійний аналіз для визначення факторів, що впливають на якість вирощеного краба в дельті Меконгу, В'єтнам. Результати опитування 304 крабових фермерів у дельті Меконгу, В'єтнам, показують, що загальна площа дельти Меконгу у В'єтнамі становить 465 тисяч гектарів, а обсяг виробництва – 68 тисяч тонн. Серед провінцій дельти Меконгу, В'єтнам, К'єнжанг, Камау та Бакльєу входять до трійки лідерів за площею та обсягами виробництва. Крім того, при аналізі факторів, що впливають на якість крабової продукції

в процесі вирощування, було виявлено три основні фактори: рішення про вилов крабів на четвертій стадії линьки (Y4), кількість використовуваних крабових пасток і тривалість періоду вирощування. Це дослідження є першим дослідженням якості крабів, вирощених у дельті Меконгу, В'єтнам. Таким чином, отримані результати надають рекомендації щодо поліпшення якості вирощеного краба і слугують основою для подальших досліджень у цій галузі

Ключові слова: рішення про вилов крабів; дельта Меконгу; якість грязьового краба; фермерство



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Building “Porang” processing industry using supply chain management method

Erlyna Wida Riptanti*

Doctor of Agricultural Science, Associated Professor
Sebelas Maret University
57126, 36 Ir. Sutami Str., Surakarta, Indonesia
<https://orcid.org/0000-0001-9275-9265>

Heru Irianto

Doctor of Agricultural Science, Professor
Sebelas Maret University
57126, 36 Ir. Sutami Str., Surakarta, Indonesia
<https://orcid.org/0000-0002-6228-899X>

Mujiyo

Doctor of Soil Science, Professor
Sebelas Maret University
57126, 36 Ir. Sutami Str., Surakarta, Indonesia
<https://orcid.org/0000-0002-6161-7771>

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Abstract. Wonogiri Regency is one of the leading porang producing regions in Indonesia, but the potential of its industry is still largely unexplored. Currently, business actors market coroms and chips in East Java Province, resulting in a multiplier effect. This is because these actors cover only a small portion of the region. Therefore, the purpose of this study was to develop porang processing industry model through Supply Chain Management method. The study employed both quantitative and qualitative methods with Interpretive Structural Modelling (ISM) analysis. The triangulation method was used to cross-examine data at the respondents' level. Based on the results, processed product demand, supplier engagement in the production process, and glucomannan production technology were the sub-criteria with the greatest effect on supply chain management. Furthermore, market access was identified as the major factor influenced by other sub-criteria. The strategies for establishing processing industry based on the 18 sub-criteria were in the linkage quadrant, showing the presence of high effect and interconnection. Variables in this sector must be investigated carefully because the relationship between variables is unstable. Every action on this variable will affect others and the feedback effect can magnify the impact. The strategy implemented must undergo various reviews to obtain best results. The findings of this study can be used as a reference for stakeholders to strengthen competitive advantage and implementing effective strategies

Keywords: sub-criteria; glucomannan; chips; monopsony; oxalic acid

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*Corresponding author

INTRODUCTION

Food is an essential need in the human life cycle, with agriculture as a sector offering various alternative sources. In Indonesia, rice serves as a primary carbohydrate source for the majority of the population, constituting 90% of the overall nutritional content. With technological advance and information development, lifestyle choices, including energy sources, are significantly influenced. This contributes to the interest of the community in more nutritionally dense food. Excessive consumption of white rice can pose a risk of diabetes mellitus. This is attributed to the low nutritional content and high glycaemic index of white rice. One proactive strategy to minimise this risk includes substituting or reducing rice consumption with other food sources (Embling *et al.*, 2020).

Porang (*Amorphophallus muelleri* Blume) is an agricultural commodity sought-after by the export market due to its nutritional content. This plant is categorised as a corm in the *Araceae* family and has been cultivated as export commodities over the past 3 years. In Indonesia, the export demand has significantly increased from 11,721 t in 2019 to 20,476 t in 2020 (Naufali & Putri, 2023). Due to this high demand, the cultivated area for porang has increased from 19,950 ha in 2020 to 47,461 ha in 2021. Indonesian porang has successfully entered markets in China, Japan, South Korea, Australia, Thailand, Malaysia, and Vietnam. Wonogiri Regency is one of the porang producing regions in Indonesia. This plant flourishes in Wonogiri thanks to its favourable geographical conditions. However, porang corms cannot be consumed directly due to their calcium oxalate content which causes throat irritation and large consumption can lead to the formation of stones or crystals in the kidneys (Subagiana *et al.*, 2022; El Habbani *et al.*, 2023).

Porang farming is affected by the National Porang Farmers Association (P3N) community which serves as a platform for farmers to exchange vital information. In practice, this information is still difficult to obtain, specifically during the peak harvest season, since porang market operates as a monopsony (Farhana *et al.*, 2022). A lack of information flow exists within the product supply chain, beginning from farmers, collectors, suppliers, and processing plants, to export readiness. Farmers may not always be aware of the market demands and standards for corms (Shegelman *et al.*, 2020). Frequently, the quality of the product falls short of collectors' expectations in terms of weight, defects, or diseases. This lack of information impacts farmers' understanding of the financial flow, spanning from importers to the payments they receive.

The increasing demand for porang exports each year emphasises the need for robust supply chain management (SCM) planning. X. Zhao *et al.* (2021) uses SCM to determine critical factors in improving product quality. Other research also proves that supply chain

management can be improved by considering the priority of attributes or factors that influence it, as in the study conducted by A. Jokandan *et al.* (2020) on the increase of exports of livestock products. SCM is widely used to determine the most suitable strategy for increasing exports, whether caused by internal or external production factors. The Interpretive Structural Model (ISM) method was chosen in several SCM-related studies, e.g., by H. Amintahmasbi and Y. Zahedan (2022) to identify the best performance factors in the apparel industry and by E. Kusriani *et al.* (2019) to find the key factors that influence the success of MSMEs. S. Kumar *et al.* (2021) aimed to identify barriers to the implementation of Industry 4.0 and the circular economy in the agricultural supply chain. N. Samadi-Foroushani *et al.* (2022) developed models for sustainable entrepreneurial development in Iranian agriculture. N. Durge *et al.* (2021) analysed the factors affecting marketing performance in the fresh mango supply chain.

Bulbotuber supply is exclusively reliant on farmers, but the dependence on intermediaries limits the profit-fulfilling ability. On the other hand, this predicament is exacerbated by the disadvantaged bargaining position of small-scale farmers. According to A. Bidarti *et al.* (2021), farmers with small land holdings may face financial constraints that affect their decision-making process, especially when it comes to prioritising their needs. This dynamic substantially contributes to inefficiencies in the SCM system, specifically in the upstream sector. Farmers readily part with their harvest once a predetermined price offers some semblance of profit, even when it is nominal. Importers, exporters, and large-scale processing industry do not provide price guarantees for each harvest cycle. In the past two years, bulbotuber prices at the farmer level have seen a decline as porang supply from diverse regions inundates processing industry in East Java Province (Naseer *et al.*, 2019). The current situation underscores the motivation behind this study – to develop strategies that will advance the porang processing industry in Wonogiri Regency, enhancing added value and improving the welfare of farmers. The SCM system should focus on simplifying the involvement of intermediaries and enabling farmers to directly engage with the processing industry. In this regard, the purpose of this study was to foster the porang processing industry using the SCM method, thereby strengthening competitive advantage and implementing effective strategies.

MATERIALS AND METHODS

This study was conducted in Wonogiri Regency, a prominent porang producing region in Central Java. It was conducted in May 2023 under the auspices of the Agriculture and Food Department Office. Wonogiri Regency, however, has yet to develop into a central hub for porang processing or trading industry. The study employed an

incorporating Interpretive Structural Modelling (ISM) analysis. This process started by identifying variables relevant to the issues or problems at hand, which were subsequently grouped accordingly (Attri *et al.*, 2013). The discussed issues were divided into criteria and sub-criteria to gain a more profound understanding. The next step was to establish contextual relationships between criteria and sub-criteria with experts through focus group discussions. The results served as data to generate a digraph model illustrating the interconnections between criteria and sub-criteria within the supply chain (Arsiwi & Adi, 2020). Additionally, the triangulation method was applied to cross-examine data across various respondent levels (Fawcett *et al.*, 2008; Mangla *et al.*, 2018).

The steps in developing the ISM model are explained as follows:

1. Determination of Criteria and Sub-criteria. The criteria and sub-criteria were determined through brainstorming, discussions with experts, and a review of relevant literature from earlier studies, such as M. Lim *et al.* (2017), X. Zhao *et al.* (2021), H. Putri *et al.* (2023). The experts were 18 individuals selected based on their insights into the conditions and issues related to the problem under study. These included policymakers (3 people), processing industry actors (7 people), large-scale traders (4 people), the National Porang Farmers Association (P3N) (3 people), and experts from universities (1 person). Sub-criteria functioned as explanatory variables for the established criteria. The criteria and sub-criteria (Table 1) were identified based on the needs and challenges of developing the porang processing industry in Wono-giri Regency. These criteria formed the basis of the questionnaire presented to the experts (Diabat *et al.*, 2014).

Table 1. ISM Criteria and Sub-criteria

Criteria	Sub-criteria
Market	Information access
	Market access
	Processed product demand
	Direct access to processing industry
	Direct access to exporters
Production	Role of suppliers in the production process
	Seedling availability
	Raw material quality
Technology	Oxalic acid removal technology
	Glucomannan production technology
	Postharvest technology (drying)
	Limitations of derivative product technology
HR	Institution
	Partnership network
	Capital access
Partnership	Ease of obtaining buyers for porang chips/flour
Government policies	Policies not yet leading to increasing the added value of porang
Competitive environment	Categorised as a monopsony market

Source: developed by the authors of this study based on E. Riptanti *et al.* (2023)

2. Questionnaire Completion. The questionnaire comprised questions concerning the relationships between sub-criteria. It was administered by an enumerator through interviews and discussions with experts or direct responses. The questions asked how the sub-criteria influence each other, whether one influences the other, or if there is no influence between them.

3. Structural Self-Interaction Matrix (SSIM). The results of the questionnaire completed by the experts, showing the relationships between criteria and sub-criteria, were transformed into the V, A, X, and O filling rules within a matrix (Watson, 1978). Each row (i) and column (j) represent a relationship, and when a sub-criterion in row (i) affects the one in column (j), it is denoted by the letter V. Conversely, when a sub-criterion

in column (j) affects the one in row (i), it is denoted by the letter A. In cases where the sub-criteria in both row (i) and column (j) affect each other, it is denoted by the letter X. The letter O denotes the absence of relationship between the sub-criteria in the respective rows and columns (Raut *et al.*, 2019).

4. Reachability Matrix. The reachability matrix was created based on the output from the SSIM by converting the notations V, A, X, and O in the SSIM into binary symbols 1 and 0 (Lim *et al.*, 2017). When the notation V on the effect of i on j (i, j) is present, it is assigned a value of 1, while in cases where j affects i (j, i), it is assigned a value of 0. The presence of the notation A on the effect of i on j (i, j) is given a value of 0. Meanwhile, the effect of j on i (j, i) is valued to be 1. In cases where

the notation X is present in (i, j) and (j, i), both are assigned a value of 1. When the notation O is present in the relationship between i and j (i, j), it is given a value of 0. Finally, the relationship between j and i (j, i), is valued to be 0.

5. Level Partitioning. Having obtained the reachability results for SSIM, the levels were divided by considering three aspects, namely reachability, antecedent, and intersection set (Kannan & Haq, 2007). The reachability and intersection sets consist of sub-criteria with a relationship value of 1 for i to j (i, j) and j to i (j, i), respectively. The intersection set consists of sub-criteria contained in both reachability and antecedent (Pfohl *et al.*, 2011). The intersecting sub-criteria become

the top elements in the ISM hierarchy. Having identified these top elements, they were separated from other sub-criteria. The same process was repeated to determine the sub-criteria at the next level, continuing until they were all ascertained. These levels help in constructing the ISM diagram and model.

6. ISM Model. The final result from the reachability matrix formed a structural or ISM model. The relationships between two criteria or sub-criteria from i and j were shown by arrows. Furthermore, the hierarchy in the model was divided into 3 categories, namely lower, middle, and upper levels. To obtain the final result of the ISM diagram, transitivity from the initial model was eliminated (Lim *et al.*, 2017).

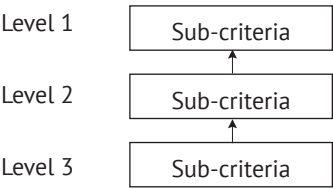


Figure 1. Structure Diagram of the SCM Model

Note: the graphs were created using Exsimpro
Source: W. Rohmah *et al.* (2019)

7. Creating Driver Power and Dependence Diagram. Driver power refers to the aggregate of values from the same row in the matrix, while dependence is the cumulative sum of values from the same column. Both serve as coordinates in constructing a diagram. The driver power value was indicative of the ordinate point on the vertical axis, while the dependence value serves as the abscissa point on the horizontal axis. This diagram was composed of 4 quadrants, each representing distinct clusters, namely autonomous, dependent, linkage, and independent. In quadrant 1, autonomous showed that the elements within it have an effect, albeit with weak dependence. Quadrant 2, labelled as dependent, signifies the elements have a weak effect but high dependence. Quadrant 3, referred to as linkage, implies that the elements have both strong effect and dependence. Finally, quadrant 4, designated as independent, signifies that the elements have a strong effect but weak dependence (Diabat *et al.*, 2014).

RESULTS AND DISCUSSION

1. SCM Model for Establishing Porang Processing Industry. SCM is a coordinated system that integrates activities such as coordination, scheduling, and control from suppliers to consumers. The primary objective is to attain cost efficiency across the entire distribution process until the final delivery to the consumer is achieved. Notably, supply chain does not only connect one party to another or business to business. According to M. Chiu and C. Lin (2022), the mechanisms in the supply chain create relationships across a broader network of multiple businesses. The risk of deviation from the

planned course increases when dealing with a company that manages more than one business entity. SCM is an effort to synchronise activities within and outside the company while maintaining interrelated relationships with the various stakeholders.

The successful implementation of the SCM into business operations is expected to enhance good relationships between upstream and downstream actors, elevate customer satisfaction, and instil trust in the performance of the company. The Global Supply Chain Forum identified 8 core processes that form the essence of the SCM, and they included customer relationship management, customer service management, demand management, demand satisfaction, manufacturing flow management, procurement, product development and marketing, as well as returns (Hazen *et al.*, 2019). Each of these processes forms a strategic plan that can be effectively executed by the company.

The study has yielded an SCM model for porang processing industry as presented in Figure 1 and Table 1. Through level partitioning and subsequent ranking, the relationships within each sub-element were symbolised by arrows. In this case, those at the beginning and end of the arrow represent the causing and resulting events, respectively. Processed product demand (E3), role of suppliers in the production process (E6), and glucomannan production technology (E10) are factors that mostly affect other sub-criteria in each criterion. They are key determinants that initiate events at various levels. Specifically, the level of processed product demand significantly affects the availability of raw materials held by the producers

(Dermoredjo *et al.*, 2021). Therefore, collaboration between producers and raw material suppliers is crucial.

This is because processed product and raw material demands are directly proportional.

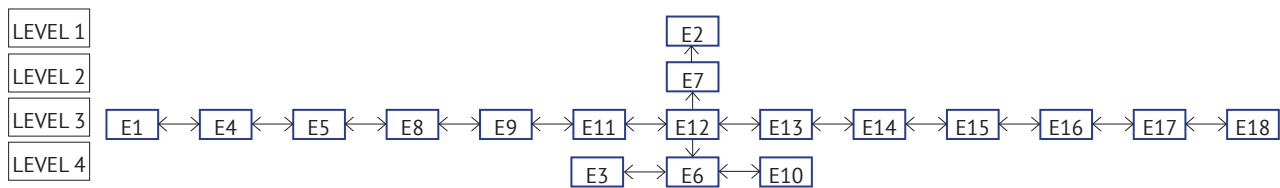


Figure 2. Structure Diagram of SCM Model for porang Processing Industry

Source: data processing results from Exsimpro (2023)

Table 1. Description

E1:	Information Access	E10:	Glucomannan Production Technology
E2:	Market Access	E11:	Postharvest Technology (Drying)
E3:	Processed Product Demand	E12:	Limitations of Derivative Product Technology
E4:	Direct Access to processing Industry	E13:	Institution
E5:	Direct Access to Exporters	E14:	Partnership Network
E6:	Role of suppliers in the Production Process	E15:	Capital Access
E7:	Seedling Availability	E16:	Ease of Obtaining Buyers for Porang Chips/Flour
E8:	Raw Material Quality	E17:	Policies Not Yet Leading to Increasing the Added Value of Porang
E9:	Oxalic Acid Removal Technology	E18:	Classified as Monopsony Market

Source: compiled by the authors of this study

According to K. Kodrat *et al.* (2019), the cooperative relationship between raw material suppliers and processing industry is a crucial issue to be addressed, as their interests often diverge, with each party seeking to maximise its profit. Raw material suppliers aim for the highest possible price to ensure profitability, while processing industry aim for cost maximisation to avoid overbudgeting. In the processed porang production, suppliers play a vital role in providing the desired quality and quantity of raw materials for producers. Conversely, raw material suppliers stand to gain significant profits when demand from producers increases. Effective collaboration establishes a conducive product flow to satisfy market demand, and positive perceptions of consumers are formed when the desired products fulfil expectations in terms of timing, location, quantity, and quality (Milusheva, 2022). These raw materials can be either in their raw or semi-finished state. Porang corms are used as raw materials for other industries once the oxalate acid content, which causes throat irritation and kidney stones in humans, has been eliminated (Chen *et al.*, 2020). Due to its complex and expensive technological requirements, glucomannan production process is typically conducted by major industries.

The demand for processed product (E3), role of supplies in the production process (E6), and glucomannan production technology (E10) are factors that need to be planned before the product flow begins. This underpins the establishment of a sustainable porang processing industry. The fundamental challenge in

this industry was glucomannan production technology (Riptanti *et al.*, 2022; 2023). Therefore, collaboration between the government and study institutions in developing low-cost technology to produce glucomannan is crucial. Government support is needed to facilitate and connect the domestic market for processed porang products. Information flow regarding needs and demand is essential and should be provided by stakeholders. This has a multiplier effect in driving other processing industries. The sustainability of the production process needs to be supported by the continuous availability of raw materials and the role of suppliers. These sub-criteria provide information for business actors.

Business actors require information access (E1) to determine product distribution channels, locate processing industry facilities (E4), and establish direct access to exporters (E5). After the producers have determined the market destination of the product, they proceed to ascertain the raw material quality (E8) and the type of product to be manufactured. Raw materials such as porang corms can only be used after oxalic acid removal, necessitating the corresponding oxalic acid removal technology (E9). Before the removal of this acid, corms are subjected to sun-drying (E11). Not all farmers have the expertise to process porang well due to the limitations in derivative product technology (E12). Furthermore, a structured institution (E13), partnership network (E14), and capital access (E15) are needed to develop processing businesses. Apart from technical considerations, business actors also require ease in

locating buyers for porang chips/flour (E16) to ensure that their products find traction in processed or derivative form. However, this situation is not accompanied by policies geared towards enhancing the value-added aspects of porang (E17). The number of processing industry is still limited, with distribution primarily confined to a select few buyers, resulting in a tendency towards a monopsony market (E18), as observed in

Figures 2 and 3. The conditions and challenges in the porang supply chain, from production to consumption, affect each business actor’s assessment and efforts to improve both the quantity and quality of their products. According to W. Zhang and Q. Su (2020), the information required to support quantity and quality pertains not only to product attributes but also comprises processes, operations, and factors influencing the final products.

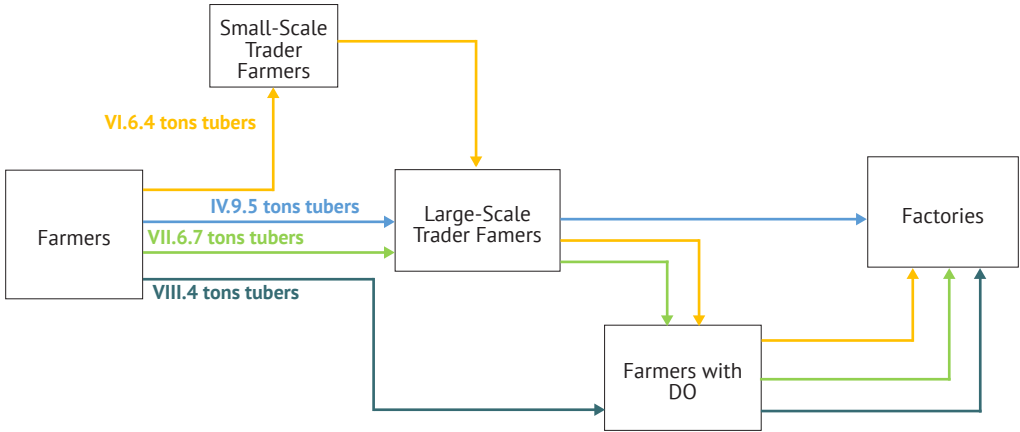


Figure 3. Porang Corms Marketing Channels in the Wonogiri Regency

Source: compiled by the authors of this study

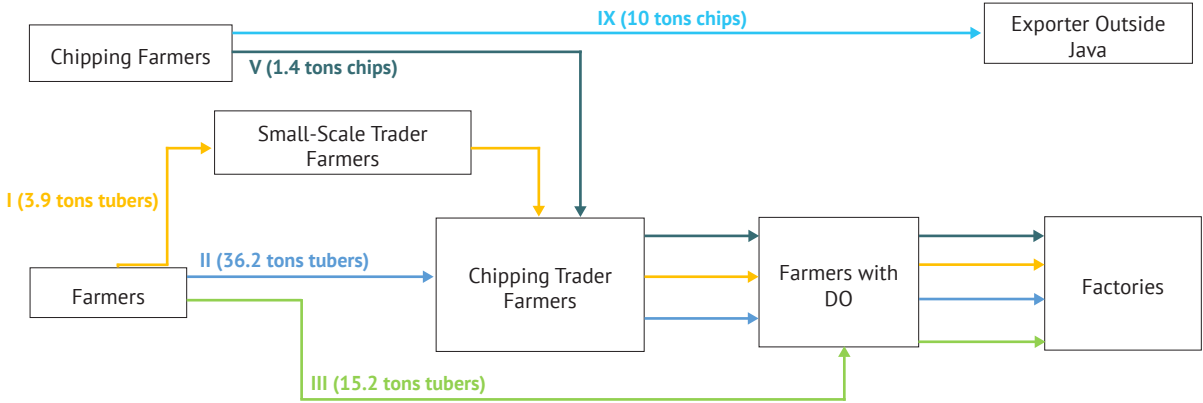


Figure 4. Porang Chips Marketing Channels in the Wonogiri Regency

Source: compiled by the authors of this study

The seedling availability (E7) at level 2 is conditioned by sub-criteria at level 3. Seedlings, sourced from bulbils and corms weighing less than 0.25 kg, play a crucial role in the cultivation process of porang. These seedlings may originate from harvesters, traders, or the National Porang Farmers Association (Riptanti *et al.*, 2022; 2023). A dormancy period can lead to specific planting intervals, as stated by (Harijati & Widoretno, 2019). Farmers strategically plan for the required quantity and source of seedlings in the subsequent year to avert shortages during planting. The last sub-criterion at level 1 is market access, which is the result affected by the preceding sub-criteria. Farmers should have access to information concerning prices, the quantity demanded by processing industry, and the down orders (DO) from processing

industry. The relatively weak bargaining position of farmers needs to be enhanced, and this could be achieved by optimising the function of their institution. The journey of processed porang products begins with the farmers who cultivate them on owned or leased land, while some may operate as permanent labourers. The best harvest age for porang is in the second or third year after planting (Gusmalawati *et al.*, 2022). Harvest timing hinges not only on the maturity of corms but also on market price conditions. When the price is favourable or high, farmers harvest and sell to small-scale traders (Fig. 2 and 3). Most farmers directly sell the corms, and only a small portion engages in chipping (slicing porang thinly and then drying it). Chipping farmers supply their products directly to

small-scale trader farmers. These small-scale traders distribute them to larger-scale counterparts. Corms then move from these larger-scale traders and chipping traders to individuals or traders with DO from processing industry (Fig. 3). However, information regarding prices and required quantities for processing industry is limited. Major industries use oxalic acid removal technology to process corms into glucomannan flour, a critical raw material for various domestic and export industry in China, Japan, and Korea. The results show that not all major industry have this technology. Processing industry exports porang in the form of “krepes”, namely dried and crushed chips.

Supply chain for porang corms, from cultivation to consumption, is quite extensive. The elongated marketing chain allows for the accumulation of significant price transmission biases. Furthermore, it can be attributed to some factors such as distance and monopsony market. According to P. D'Odorico *et al.* (2019), the extended marketing distance can be a result of commodity production being concentrated in specific regions, while consumer areas are relatively scattered within a broader geographical scope. Farmers, as cultivation business actors, face limitations in accessing the market on a wider scale due to the prohibitive costs of processing and the lack of product absorption assurance in the market. A monopoly market limits access to information regarding price, quantity, and the entry of corms into processing industry.

The resolve of farmers in cultivating porang fluctuates in response to uncertainties in market price and demand. According to D. Darmawan *et al.* (2023), a crucial way to build trust and optimism among farmers is to ensure the absorption of the entire product output, either through government policies or by establishing cooperative relationships with various partners. Furthermore, farmers were confronted with the reality that the processed porang products command significantly

higher prices compared to fresh corms. The length of supply chain, technological, and knowledge limitations in corm processing, as well as a lack of market information, create income disparities between farmers acting on farms and processing industry, along with exporters. Therefore, strategic measures are needed to address the challenges inherent in establishing an effective SCM for porang processing industry.

2. Strategies for Establishing Porang Processing Industry. As Figure 5 suggests, market access (E2) has low driver power compared to other sub-criteria and high dependence power. Furthermore, seedling availability (E7) has the same dependence power as market access but higher driver power. These findings are consistent with H. Irianto *et al.* (2021) in the development of “jarak towo” cassava as a raw material for processing industry. Meanwhile, the strongest driving powers were processed product demand (E3), role of supplies in the production process (E6), glucomannan production technology (E10), information access (E1), direct access to processing industry (E4), direct access to exporters (E5), raw material quality (E8), oxalic acid removal technology (E9), post-harvesting technology (E11), limitations of derivative product technology (E12), institution (E13), partnership network (E14), capital access (E15), ease of obtaining buyers for porang chips/flour (E16), policies still not increasing the added value of porang (E17), and categorisation as a monopsony market (E18). All sub-criteria were in the linkage quadrant and have significant strength in affecting and controlling the situation, but they also show strong dependence. Sub-criteria E3, E6, and E10 directly affected E1, E4, E5, E8, E9, E11, E12, E13, E14, E15, E16, E17, and E18. Variables in this quadrant must be investigated carefully because the relationship between variables is unstable. Every action on this variable will have an impact on others and the feedback effect can magnify the impact.

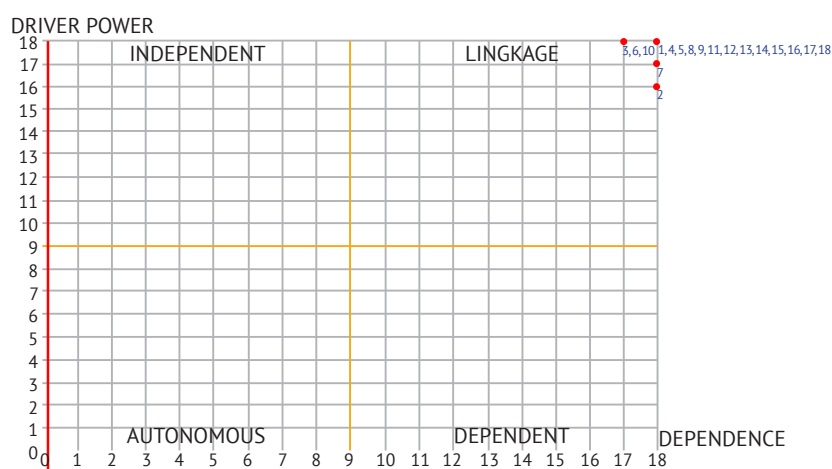


Figure 5. Driver and Dependence Power Diagram (Power Matrix)

Note: all sub-criteria are in the linkage position, sub-criteria with the same position are represented by one red dot

Source: data processing results from Exsimpro (2023)

Barriers and opportunities obtained from these sub-criteria will affect information access (E1), direct access to processing industry (E4), direct access to exporters (E5), raw material quality (E8), oxalic acid removal technology (E9), post-harvest technology (E11), limitations of derivative product technology (E12), institution (E13), partnership network (E14), capital access (E15), ease of obtaining buyers for porang chips/flour (E16), policies still not increasing the added value of porang (E17), and classification as monopsony market (E18). These barriers and opportunities are consistent with the findings of E. Riptanti *et al.* (2023).

Factors in porang distribution process that influence its final product affect input availability at the farmer level. Seedlings also serve as commodities traded at this level. When there is a high demand for seedlings, the price increases. However, when it is produced by the farmers, the price becomes lower. An oversupply of seedlings occurs, as various regions also produce bulbils. The quality of the seedlings declines when they are not used immediately after the dormant period, thereby exhibiting reduced germination and growth rates (Sianturi *et al.*, 2022). With the porang supply chain situation in Wonogiri Regency, market access became the sub-criteria with the lowest driving power but had very strong dependence power. Interactions among these sub-criteria can generate effects and feedback within the SCM system. Each sub-criterion influences the others, and the feedback provided can enhance the effect (Kusrini *et al.*, 2019).

The demand for processed porang products is growing, specifically in the food industry, due to the increasing human awareness of a healthy lifestyle. Glucoman content in porang is excellent for diets, preventing liver and diabetes diseases, lowering cholesterol, and preventing hypertension. Apart from being used as a raw material in the food industry, porang corms are used in cosmetics, adhesives, laboratory raw materials, and pharmaceuticals (Dwiyono & Djauhari, 2021). Therefore, this product has become a sought-after commodity in the export market due to high demand, as it is not cultivated by all countries. Despite being a major producer, Indonesia has not been able to control prices, leading to uncertainties and fluctuations. E. Riptanti *et al.* (2022) attribute this to the absence of sales contracts with exporters or processing industry. Regulatory frameworks surrounding porang are still unclear, leaving farmers in a weak bargaining position. Formalising contracts for bulbotuber procurement with processing industry would benefit both parties, ensuring price stability, quality, and consistent quantities. Such contracts can also address challenges related to raw material procurement and price fluctuations during harvest seasons.

Farmers producing fresh porang corms often complain about price instability set by traders, which tends to decline over time (Arumsari *et al.*, 2023). The long

supply chain results in wider price margins, thereby driving down prices at the farmer level. The findings suggest that porang marketing in the Wonogiri Regency consists of 10 channels that lead to processing industry in East Java and only 1 exporter outside Java (Fig. 2 and 3). Processing industry in East Java and the exporter partner with farmers or traders whom supplier warrants known as DO. According to X. Zhao *et al.* (2021), direct partnership makes it easier for companies to ensure product quality and safety as well as respond quickly to market needs. From the farmers' perspective, partnering directly with exporters provides greater benefits due to the assurance of porang corm distribution. For this export commodity, information about price conditions and demand trends in the global market is crucial for farmers, traders, processing industry, and exporters. The limitation in technology and the prohibitive processing costs have led to a limited number of processors in Indonesia, resulting in a tendency towards monopolistic practices.

Traders, acting as intermediaries between farmers and the processing industry, were relatively few. The monopolistic influence held by farmers or traders with Delivery Order (DO) gives them the flexibility to control purchase prices (Nathan, 2021). Price hikes at processing industry/exporter level did not immediately translate into equivalent increases. This was clear among farmers in the Wonogiri Regency, where a significant price differential exists. Monopolistic power can be established through various means, such as traders collaborating to set purchase prices from farmers, creating barriers to deter other traders from entering the market for particular commodities, and establishing dependence among farmers on marketing their produce exclusively to particular traders. Additionally, there should be a harmonious relationship between farmers and processing industry to establish a more focused and sustainable business framework. Therefore, a suitable SCM model shows that demand for processed products, supplier involvement in the production process, and glucomannan production technology are sub-criteria with the greatest potential to influence SCM of porang processing industry. Collaboration among stakeholders, including processing industry, traders, exporters, government entities, the National Porang Farmers Association, and study institutions, is crucial in advancing the processing industry.

CONCLUSIONS

Processing industry typically relies on a steady supply of raw materials from the surrounding areas. However, despite being a significant porang production region, the Wonogiri Regency lacks processing industry. Even though Wonogiri is a major hub for porang bulbotuber production, this is not in line with the number of industries in it. Farmers in the Wonogiri Regency also lack the supplies to carry out processing, since processing costs are prohibitive and require a complex

technology. Factors such as the length of supply chain, technological and knowledge constraints in bulb tuber processing, and a lack of market information contribute to income disparities between farmers operating at the on-farm level and processing industry, as well as exporters. The proposed porang processing industry model based on the SCM method, hinges on factors such as processed product demand, role of suppliers in the production process, and the application of glucomannan production technology. These sub-criteria are expected to have the greatest impact on the SCM of the porang processing industry. Strategies for implementing this model necessitate close collaboration among the relevant stakeholders, allowing for vital access to essential information. The strategy must ensure that all sub-criteria stay in the linkage quadrant. Variables in this sector must be investigated carefully because the relationship between variables is unstable. Every

action on this variable will have an impact on others and the feedback effect can magnify the impact. This study can be a reference for further research on determining strategies based on the problem characteristics approach using the ISM method.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Побудова переробної промисловості «Поранг» з використанням методу управління ланцюгами поставок

Ерліна Віда Ріптанті

Доктор сільськогосподарських наук, доцент
Університет Себелас Марет
57126, вул. Іра Сутамі, 36, м. Суракарта, Індонезія
<https://orcid.org/0000-0001-9275-9265>

Херу Іріанто

Доктор сільськогосподарських наук, професор
Університет Себелас Марет
57126, вул. Іра Сутамі, 36, м. Суракарта, Індонезія
<https://orcid.org/0000-0002-6228-899X>

Муджійо

Доктор ґрунтознавства, професор
Університет Себелас Марет
57126, вул. Іра Сутамі, 36, м. Суракарта, Індонезія
<https://orcid.org/0000-0002-6161-7771>

Анотація. Регіон Воногірі є одним з провідних регіонів-виробників порангу в Індонезії, але потенціал його галузі залишається значною мірою недослідженим. В даний час бульбоцибулини та чіпси продаються в провінцію Східна Ява, що призводить до мультиплікативного ефекту, оскільки лише невелика частина регіону отримується бізнес-агентами. Тому метою цього дослідження було побудувати модель галузі переробки порангу за допомогою методу управління ланцюгами поставок. Процедури дослідження використовували як кількісні, так і якісні методи з аналізом інтерпретаційного структурного моделювання (ISM). Метод тріангуляції використовувався для перехресної перевірки даних на рівнях респондентів. Згідно з результатами, попит на перероблений продукт, залучення постачальників до виробничого процесу та технологія виробництва глюкоманнану були підкритеріями, що мали найбільший вплив на управління ланцюгами поставок. Крім того, доступ до ринку був визначений як основний фактор, на який впливають інші підкритерії. Стратегії створення переробної промисловості на основі 18 підкритеріїв опинилися у квадранті зв'язку, що свідчить про наявність високого ефекту та взаємозв'язку. Змінні в цьому секторі необхідно ретельно вивчати, оскільки взаємозв'язок між змінними є нестабільним. Кожна дія на одну змінну матиме вплив на інші, а ефект зворотного зв'язку може посилити цей вплив. Впроваджувана стратегія повинна проходити різні перевірки, щоб отримати оптимальні результати. Результати цього дослідження можуть бути використані зацікавленими сторонами як орієнтир для зміцнення конкурентних переваг та впровадження ефективних стратегій.

Ключові слова: підкритерії; глюкоманнан; чіпси; монопсонія; щавлева кислота



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Export competitiveness of Indonesian coffee in the United States market

Gesti Annisa Innayatuhibbah*

Master Student

Universitas Sebelas Maret

57126, 36A Ir. Sutami Str., Surakarta, Indonesia

<https://orcid.org/0000-0002-2329-3876>

Endang Siti Rahayu

Doctor of Agricultural Economics, Professor

Universitas Sebelas Maret

57126, 36A Ir. Sutami Str., Surakarta, Indonesia

<https://orcid.org/0000-0002-4967-0780>

Minar Ferichani

Doctor of Agribusiness, Lecturer

Universitas Sebelas Maret

57126, 36A Ir. Sutami Str., Surakarta, Indonesia

<https://orcid.org/0000-0002-4294-2122>

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Abstract. Indonesia's economic growth has slowed down during the pandemic. Coffee is a foreign exchange-producing agricultural commodity for Indonesia and has become a pillar of the national economy because of its contribution to the gross domestic product. The purpose of this study was to analyse the competitiveness of Indonesian coffee exports in the United States market. The methods employed included the Revealed Comparative Advantage, which can measure export competitiveness comparatively, and the Export Product Dynamic method to analyse and map the position of export competitiveness. The analysis results were also measured to compare the export competitiveness of Vietnamese coffee. The findings showed that Indonesian coffee and Vietnamese coffee in the United States market have a value above 1, proving that both have export competitiveness advantages. The average Revealed Comparative Advantage value of Indonesian coffee is higher than that of Vietnamese coffee, meaning that Indonesian coffee exports are superior to Vietnamese coffee exports to the United States. Meanwhile, the results of the Export Product Dynamic analysis showed that the competitiveness of Indonesian coffee exports is in the position of Lost Opportunity, where the position has shifted from the pre-pandemic position, namely Retreat. Meanwhile, the competitiveness of Vietnamese coffee exports was in the Rising Star position, which shifted from the previous position of Lost Opportunity. Lost

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*Corresponding author

Opportunity of Indonesian coffee shows that the strength of Indonesia's coffee commodity business is weak, but commodity exports are still competitive. The analysis results indicated that even though Indonesia's coffee has export competitiveness, it is not competitive enough in the United States. This study provided an overview of the export competitiveness of Indonesian coffee in the United States, which can be used as a basis for determining strategic steps in an effort to dominate the market to obtain trade benefits

Keywords: competitiveness position; export performance; export potential; international competition; international trade

INTRODUCTION

Indonesia's economic growth has slowed down compared to the period before the pandemic that began to plague Indonesia in 2020. The COVID-19 pandemic has hampered the growth of various economic sectors in Indonesia to negative growth figures. Nevertheless, the agricultural sector grew positively among many other sectors that grew negatively. This sector is one of the sectors that survived the crisis and has a vital role in the Indonesian economy. This is confirmed by the sector's contribution to the national GDP and it has become a pillar of the national economy (Statistics Indonesia, 2022).

The most consumed agricultural product in the world and the most traded commodity globally is coffee (Samoggia *et al.*, 2020). Coffee is a rapidly growing market due to increased consumption in developing countries and strong interest in various coffee product innovations in developed countries. According to A.T. Nugraha *et al.* (2021), coffee is becoming an essential part of the overall economy and a source of foreign income for many developing countries, including Indonesia. Coffee for Indonesia is the largest foreign exchange-producing agricultural commodity after palm oil, rubber, and cocoa, and plays an essential role in the country's development. Coffee has the highest contribution to the plantation sub-sector of Indonesia's economy, with an average coffee export value of USD 1,006.5 million. Through export taxes and export duties, coffee exports also contribute to the international trade surplus, which is the country's source of foreign exchange (Embassy of the Republic of Indonesia, 2020).

Based on N. Rosiana *et al.* (2018), the value of Indonesian coffee exports is predicted to increase in the coming year in line with support from the government to strengthen the competitiveness of Indonesian coffee in the global market. R. Widyantini (2019) shows that Indonesian coffee has strong competitiveness in the international market and has the potential to dominate the market. W. Habibulah *et al.* (2023) analysed the condition of the competitiveness of Indonesian coffee exports in the global market from 2002 to 2017. The findings showed that Indonesia has a strong coffee export competitiveness advantage in the global market, and the forecasting results on the value of coffee exports showed that the export value of Indonesian coffee moved up. A. Rachmaningtyas *et al.* (2019) also observed the same: Indonesian coffee is competitive in

the international market along with coffee from Brazil, Colombia, and Vietnam. As the fourth largest coffee exporter in the world, Indonesia has an excellent opportunity to increase the country's revenue from export activities. Furthermore, Indonesia is the second-largest coffee producer in Asia-Pacific, whose economic stability is highly dependent on the agricultural sector. Indonesia's coffee export destination with the highest export volume is the United States, compared to other export destinations (Indonesian Ministry of Agriculture, 2020).

Coffee in the United States is the most consumed beverage after the drinking water. As many as 63% of Americans consumed a cup of coffee every morning in the past decade. At least 400 million cups of coffee are consumed daily in the United States, and an average of 146 billion cups of coffee are consumed annually (Ismoyowati *et al.*, 2021), making the United States a promising market for coffee exporters. The United States imports at least 28 million of 60 kg bags of coffee per year since 2011 (International Trade Center, 2023). From Indonesia, coffee is imported with an average volume of 61 million kg per year, equivalent to USD 255 million (Statistics Indonesia, 2022). The prominent level of Indonesian coffee exports can indicate the dependence of Indonesia on coffee exports to the United States. However, when the competitiveness of Indonesian coffee exports in the United States market is not competitive and is at a disadvantageous position, Indonesian coffee must exit from the market and look for a more profitable market where Indonesian coffee can compete. For this reason, the purpose of this study was to analyse the competitiveness of Indonesian coffee exports in the United States market and compare the results with Vietnam's indicators as the most significant coffee exporter in Asia.

MATERIALS AND METHODS

The object under study is a coffee commodity with H.S. code 090111 (coffee, not roasted, not decaffeinated). This study used quarterly time series data from 2014 to 2023. The data needed for competitiveness analysis of Indonesian coffee and Vietnamese coffee are data on the export value of coffee commodities from Indonesia to the United States, the export value of all commodities from Indonesia to the United States, the export value of coffee commodities from the world to the United States, and the export value of all commodities

from the world to the United States – data obtained from the International Trade Centre (ITC) via the website (n.d.).

Competitiveness analysis uses a mathematical approach to measure competitiveness from two perspectives: comparative advantage and competitive advantage. Comparative advantage indicates that a country exports a particular goods because it can produce it cheaper than other countries (Pascucci, 2018; Fahmid *et al.*, 2022). Therewith, competitive advantage states that natural conditions are not the only thing that determines a product's competitiveness because excellence can be fought for. A country's superiority depends on the ability of companies within that country to compete in producing products that can compete in the market (Triadinda, 2022). Comparative advantage competitiveness was analysed using the Revealed Comparative Advantage (RCA) method, and competitive advantage competitiveness was analysed according to the Export Product Dynamics (EPD) method.

Revealed Comparative Advantage (RCA). RCA shows the evaluation of the export of a particular commodity in a country's total exports compared to that commodity's share in world trade. "Revealed" means that a country's comparative advantage can be expressed through trade/export patterns (Manalu *et al.*, 2019). The formula of the RCA value is as follows:

$$RCA = \frac{X_{ij}/X_{it}}{W_j/W_t}, \quad (1)$$

where X_{ij} = Indonesian coffee exports to the United States (USD); X_{it} = export of all Indonesian commodities to the United States (USD); W_j = world coffee exports to the United States (USD); W_t = exports of all world commodities to the United States (USD).

If the RCA index value obtained is more than 1 ($RCA > 1$), then Indonesian coffee has a comparative

advantage above the world average, or it can be said that it has solid comparative competitiveness. Conversely, if the RCA index value is less than 1 ($RCA < 1$), then the comparative advantage of Indonesian coffee is below the world average, or it can be said that it has weak comparative competitiveness.

Export Product Dynamics (EPD). Export Product Dynamic (EPD) is used to identify the competitive advantage of Indonesian coffee and analyse the dynamic of Indonesian coffee in export trade flows in the destination country of the United States (Yustian *et al.*, 2019). The market position can be found because this method uses the total export share (X) and commodity export share (Y) as variables. Mathematically, the business strength/market share (X-axis) of a product is formulated as follows:

$$X = \frac{\sum_{t=1}^n \left(\frac{X_i}{W_t} \right) \times 100\% - \sum_{t=1}^n \left(\frac{X_i}{W_t} \right)_{t-1} \times 100\%}{T} \quad (2)$$

Whereas market attractiveness (Y-axis) is mathematically formulated as follows:

$$Y = \frac{\sum_{t=1}^n \left(\frac{X_t}{W_t} \right) \times 100\% - \sum_{t=1}^n \left(\frac{X_t}{W_t} \right)_{t-1} \times 100\%}{T}, \quad (3)$$

where X_i = Indonesian coffee exports to the United States (USD); W_i = world coffee exports to the United States (USD); X_t = export of all Indonesian commodities to the United States (USD); W_t = exports of all world commodities to the United States (USD); T = number of analysis periods; $t-1$ = previous year.

The results of the stages of the EPD method can present the business strength (X-axis) and market attractiveness (Y-axis) of a product in quadrant form as follows (Fig. 1):

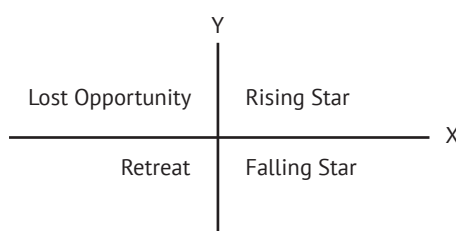


Figure 1. Competitiveness Position Matrix

Source: Esterhuizent (2006)

The EPD method comprises a matrix that places the analysed commodities into four categories. The analysis performed on a particular commodity will occupy a position on one of the quadrants of the EPD matrix. Rising Star is a quadrant that indicates the ideal market position in which the trade gains market share in a fast-growing commodity. Lost Opportunity indicates a decrease in the share of the competitive export market; products in this quadrant lose their dynamic, but the

market still demands the product. This position indicates an increase in the share of the commodity market in world trade, but a decrease in the share of the export market from the source country.

Unlike the Lost Opportunity, in the Falling Star position, an increase in a country's export market share for certain products but a decrease in commodity market share in world trade shows that commodities in importing countries are no longer dynamic. The Retreat

position shows a decrease in the export market share of the source country and the market share of the coffee commodity in world trade. It means that commodities are no longer desirable in the market, and therefore efforts must be made to move stagnant products towards dynamic products.

RESULTS AND DISCUSSION

World coffee consumption. According to Figure 2, world coffee consumption has an increasing trend in 2017-2022 except for 2019, where world coffee consumption decreased to 167,592 thsd of 60 kg bags from the

total consumption of the previous year, 170,876 thsd of 60 kg bags. However, consumption increased again in 2020 to 168,569 thsd of 60 kg bags and continued to increase until 2022, with as many as 178,534 thsd of 60 kg bags of coffee consumed worldwide. The increase in coffee consumption continues until 2023 to reach 179,159 thsd of 60 kg bags. The continent with the most coffee consumption is the United States, whose total consumption exceeds 60 million of 60 kg bags. The next continent with high coffee consumption is Europe, Asia-Pacific, and the least is Africa continent (International Coffee Organization, 2023).

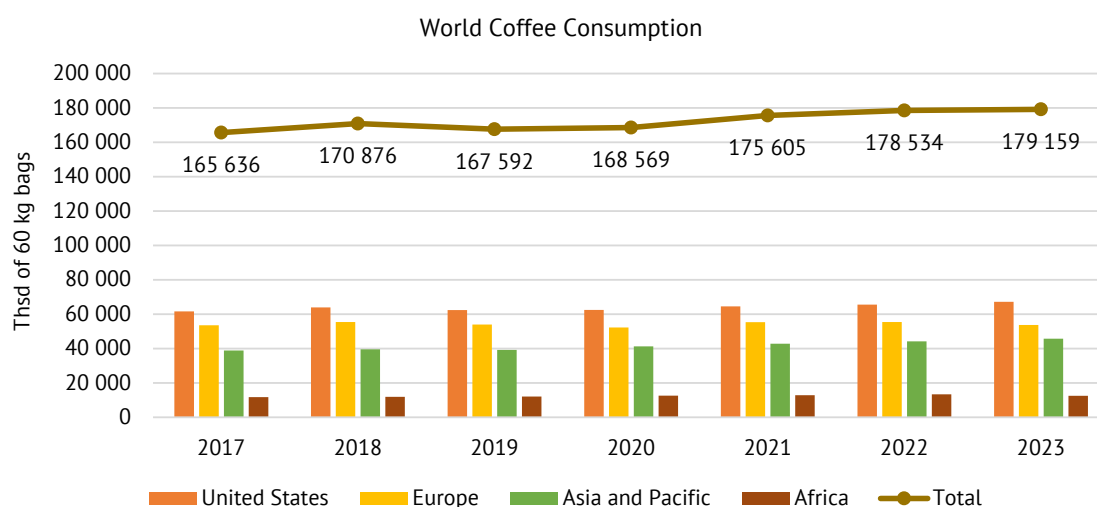


Figure 2. World coffee consumption (2017-2023)

Source: compiled by the authors of this study based on data from the International Coffee Organization, 2023

Indonesian coffee production. Indonesian coffee production fluctuates with an increasing trend from 2014 to 2023 (Fig. 3). The decline in Indonesian coffee production occurred in 2015, and subsequently, Indonesian coffee production increased or stagnated until 2022. The highest coffee production in 2022 was 795 thsd t,

and the lowest in 2015 was 639 thsd t. The decline in coffee production in 2015 was caused by a lousy climate that happened not only in Indonesia but in the entire world. The decline in Indonesian coffee production occurred again in 2023 to 786 thsd t caused by the long drought due to the El Nino disaster.



Figure 3. Indonesian Coffee Production (2014-2023)

Source: compiled by the authors of this study based on data from Statistics Indonesia (2022) and International Coffee Organization (2023)

Indonesian coffee exports. Figure 4 shows the destination of Indonesian coffee exports based on the highest export volume to five central destination countries: the United States, Malaysia, Germany, Italy, and Japan. The United States is the destination country with the highest export volume for the last ten years (from 2014 to 2023), with export volume above 50,000 t. The most volatile export development to Germany

can be observed from the significant increase and decrease in export volume, with a sharp decline in export volume in 2018 to <10,000 t from the previous year, which reached >40,000 t, and a sharp increase in 2022 to <40,000 t from >20,000 t in 2021. Meanwhile, coffee exports to Malaysia, Germany, and Italy showed stable development from 2014 to 2023 within 20,000-40,000 t (Statistics Indonesia, 2022).

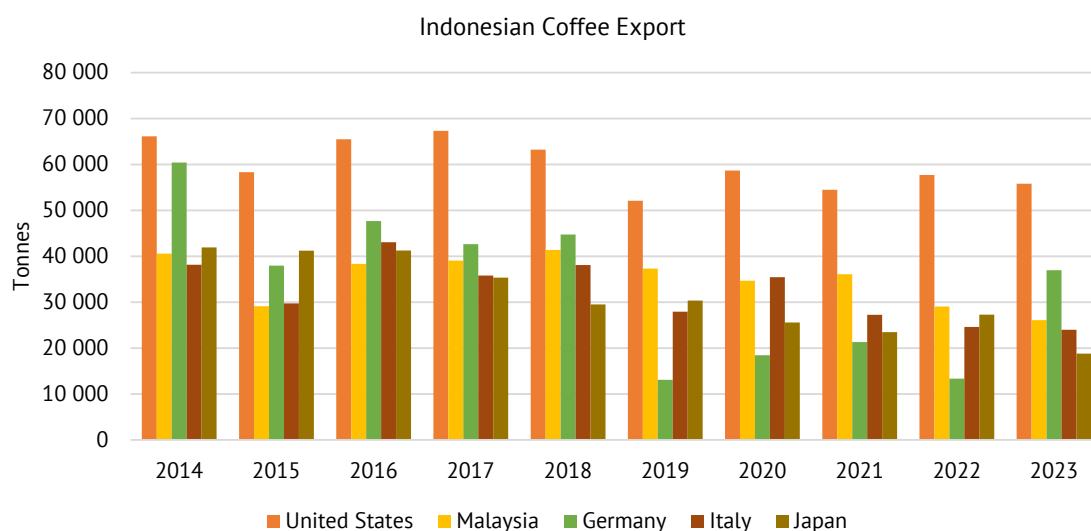


Figure 4. Indonesian coffee exports by main destination countries (2014-2023)

Source: compiled by the authors of this study based on data from Statistics Indonesia (2022) and Indonesia Information Portal (2023)

United States coffee imports. The United States is the second-largest importer of coffee beans (USDA, 2023) – the development of United States coffee import presented in Figure 5. Over the years, the total coffee import has been increasing and reached its peak in 2022. The United States imports coffee from many countries. For more

than a decade, Brazil, Colombia, and Vietnam have been the top-3 suppliers, with more than 10% of total annual U.S. coffee imports (USDA, 2023). In contrast, coffee imports from Indonesia are within 5% per year. Therewith, the value of Indonesian coffee exports continued to increase, until its value was surpassed by Vietnam in 2022.

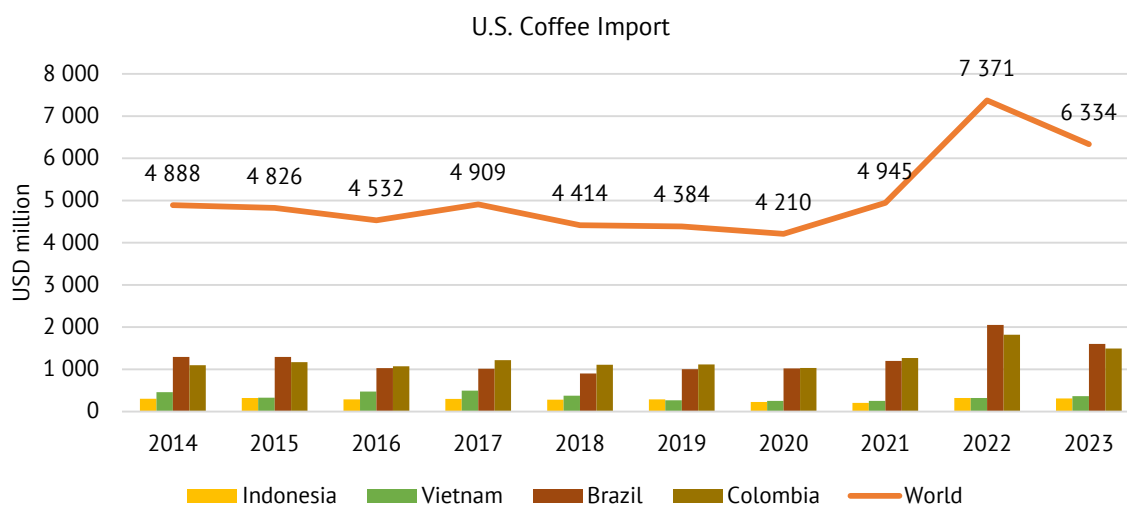


Figure 5. United States coffee imports 2014-2023

Source: compiled by the authors of this study based on data from International Trade Center, 2023

Competitiveness of Indonesian coffee. Comparative competitiveness or comparative advantage of exports is measured by Revealed Comparative Advantage (RCA), which measures the share of a country's exports in the same industry group as other exporting countries, and therefore it is used to measure comparative advantage (Jalata, 2021). This analysis obtained the RCA value of Indonesian coffee products exported to the United States.

The results of the RCA analysis of Indonesian coffee in United States export destination countries showed RCA values of more than 1 in all quarters, as presented

in Figure 6. Indonesian coffee has been competitive in the United States export destination countries for the last decade (2014-2023). Commodities with comparative advantages can be argued to have economic efficiency (Long, 2021). It means that Indonesian coffee exports to the United States have created economic efficiency that gives an edge to Indonesian coffee commodities in the United States market. After quarter 3 of 2014, the RCA value of Indonesian coffee was consistently higher than that of Vietnamese coffee, despite Vietnam being the leading competitor in Asia and the United States being the export destination country.

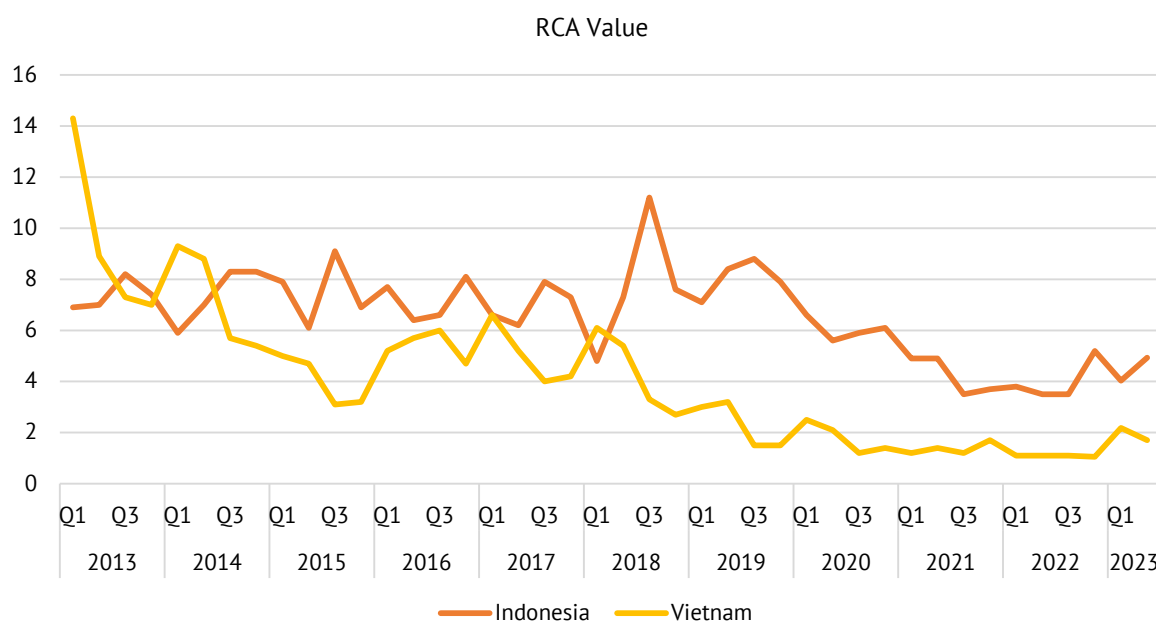


Figure 6. Development of RCA value of Indonesian coffee and Vietnamese coffee in the United States in 2013-2023
Source: compiled by the authors of this study

These results differ from the findings of D. Pratita and R. Budiarto (2021), who noted that the RCA value of Vietnamese coffee has a downward trend, while the RCA value of Indonesian coffee tends to increase little by little. The decline in the RCA value of Vietnamese coffee was caused by increasing domestic consumption in Vietnam, resulting in reduced export volumes. However, it is still classified as low (2 kg/person/year) (Nguyen, 2023). On the other hand, for Indonesia, the United States is the leading destination for Indonesian coffee exports, with an average export volume that exceeds 50 thsd t each year. The volume of coffee exported to the United States is always the largest compared to other countries that export destinations of Indonesian coffee commodities. Despite the decline in production, coffee exports to the United States are still high (Directorate General of Estates, 2021).

The high and low level of competitiveness in RCA analysis is positively influenced by the value of coffee exports from exporting countries (Pascucci, 2018).

Based on Purnomo *et al.* (2021), the volume of Indonesian coffee exports is lower than that of Vietnamese coffee exports to the United States, which causes the RCA value of Indonesian coffee in 2001-2019 to be lower than the RCA value of Vietnamese coffee. However, Indonesian coffee has an advantage in import prices over the United States, which causes the value of Indonesian coffee exports to be high.

Regarding the price of coffee imported by the United States, Brazilian coffee is the most expensive, followed by Columbia, Indonesia, and the lowest is Vietnamese coffee (Nguyen, 2023). D. Sari and S. Utomo (2021) stated that world coffee prices are very influential on the value of coffee exports the higher the world coffee price. The higher the price, the higher the value of coffee exports (Azhari *et al.*, 2022). The decline in Vietnamese coffee exports caused the decline of both the value of Vietnamese coffee exports and the RCA value. On the other hand, the price of Indonesian coffee is higher than that of Vietnamese coffee, which causes the RCA value

of Indonesian coffee to slowly outperform the RCA value of Vietnamese coffee in the United States.

In terms of the price, W. Hadiwardoyo (2020) states that the relationship between Indonesian coffee and Brazilian coffee or Columbian coffee is a substitution. This means that when there is an increase in the price of Brazilian and Colombian coffee, the United States will import coffee from Indonesia. Meanwhile, Indonesian coffee and Vietnamese coffee have a complementary relationship in the United States, and therefore the increase in prices of Indonesian coffee will affect the import of United States coffee from Vietnam and vice versa.

Although Vietnam is the second largest coffee exporter in the world, its impact on the world coffee market is not commensurate with its position. Innovation and certification are the weaknesses of Vietnamese coffee. This statement is supported by (Nguyen, 2023), who argues that Vietnam needs innovation to improve its coffee export competitiveness. In this case, Indonesia excels thanks to government support in enforcing quality standards and certification of export coffee. Furthermore, the Indonesian government has applied Good Agriculture Practices Technical Guidelines in coffee cultivation to improve the quality of coffee produced (Kansrini *et al.*, 2020; Wakhid & Suciati, 2020). This guideline has been implemented by some Indonesian

coffee farmers, which has an impact on maintaining the quality of coffee beans produced by Indonesia for the selling price to compete with Vietnamese coffee.

Position of Indonesian coffee competitiveness. The competitiveness position of Indonesian coffee is analysed using the Export Product Dynamic (EPD) method, which also measures the competitive advantage of Indonesian coffee. EPD can indicate the dynamic performance or absence of a product. Furthermore, the analysis results were compared with the conditions before the pandemic and during the pandemic to observe differences in competitiveness positions affected by the COVID-19 outbreak.

Based on EPD analysis results presented in Table 1, the study found the position of Indonesian coffee commodities in the United States export destination countries. Before the pandemic in Indonesia (2017-2019), the competitiveness of Indonesian coffee in the destination country of the United States was in the Retreat position, where the X-axis and Y-axis were both negative. This position indicates uncompetitive business strength and stagnant market appeal. The value of coffee exports and Indonesia's total commodity exports to the United States experienced declining/negative growth per quarter. If this continues, Indonesian coffee must exit the market because it cannot compete.

Table 1. Market position of Indonesian coffee and Vietnamese coffee in The United States

	Indonesia		Vietnam	
	2017-2019	2020-2023	2017-2019	2020-2023
X-axis	-0.00025 (Non-Competitive)	-0.0019 (Non-Competitive)	-0.0041 (Non-Competitive)	0.0012 (Competitive)
Y-axis	-0.00001 (Stagnant)	0.0000 (Dynamic)	0.0008 (Dynamic)	0.0005 (Dynamic)
Position	Retreat	Lost Opportunity	Lost Opportunity	Rising Star

Source: compiled by the authors of this study

During the pandemic period and afterwards (2020-2023), the competitiveness of Indonesian coffee in the United States market was in the Lost Opportunity position when the X-axis is negative, and the Y-axis is positive. Market attractiveness has grown positively even though the strength of the business has not been competitive. It means that the value of Indonesia's commodity exports is increasing. However, unfortunately, the growth of the value of Indonesian coffee exports to the United States is still negative. The strength of the Indonesian coffee business in the United States is weak and not competitive. This study has the same findings as B. Rahardjo *et al.* (2020): Indonesian coffee's competitiveness position is in the Lost Opportunity position. Lost Opportunity is not the ideal position of trade, and therefore strategic innovation is needed to increase competitiveness and win the coffee market in the United States. Vietnam's coffee position was advantageous during a pandemic. Previously,

during 2017-2019, the position of Vietnamese coffee in the destination country of the United States was Lost Opportunity, where the X-axis was negative, and the Y-axis was positive. The X-axis, which represents the strength of the business, is negative, meaning that Vietnamese coffee in the United States market is not competitive due to a decrease in market share in the dynamic product market. It shows a negative growth in coffee exports, but the total export of all Vietnamese commodities to the United States is increasing. In 2020-2023, during the pandemic, the position of Vietnamese coffee in the United States shifted to the Rising Star position, when the X and Y axes are both positive values. Rising Star is an ideal condition of trade, where there is an increase in the share of Vietnamese coffee and the share of Vietnamese exports in the United States. Vietnam's coffee products are highly competitive, and Vietnam's export share is in a dynamic or fast-growing state.

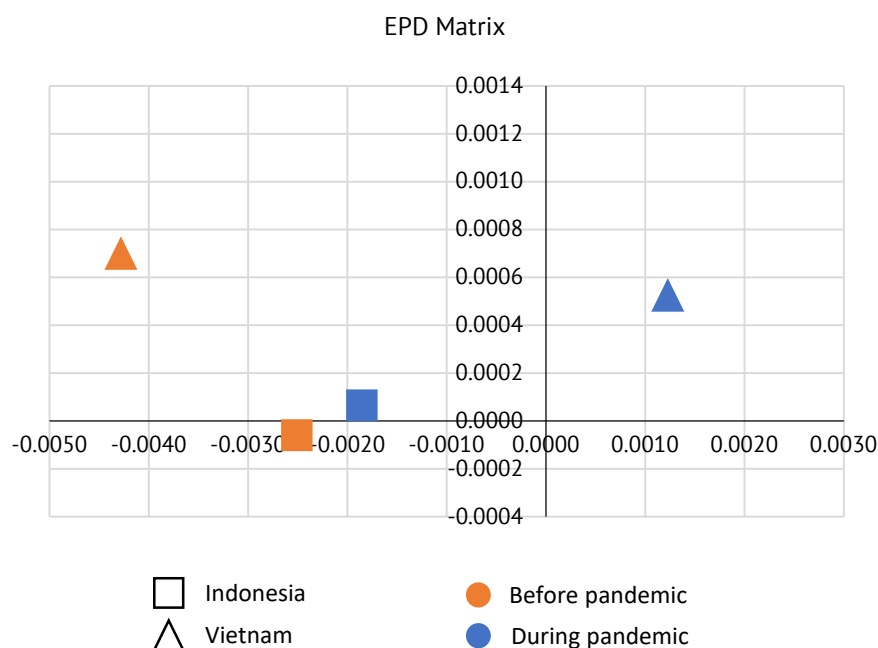


Figure 7. The position of Indonesian coffee and Vietnamese coffee in the United States in 2013-2023

Source: compiled by the authors of this study

The swift position change during the pandemic is caused by the high demand for coffee in the United States. Coffee consumption has surged to its highest level in two decades, increasing 14% since January 2021 (Castellana *et al.*, 2021). It is triggered by social restrictions that make people work from home without leaving the habit of drinking coffee even more. The pandemic has created a new routine where 84% of coffee connoisseurs drink their coffee at home. According to (Rehm *et al.*, 2020), the most popular ways to enjoy coffee are using a drip coffee maker and a single-serving coffee machine. Thus, there has been an increase in coffee beans consumption rather than instant coffee. Instant coffee is only consumed by about 5% annually (Datta, 2020).

The United States is the suitable market for coffee export destinations due to the development of coffee demand that continues to rise even during the pandemic. Regrettably, the analysis results show that Indonesian coffee lacks competitive advantage. The weak strength of Indonesian coffee was caused by the negative growth in the value of Indonesian coffee exports due to the decreasing volume of coffee exports per quarter. It is caused by the increasing domestic demand since 2017 and falling world coffee prices during the pandemic (Hanadian, 2022). Meanwhile, according to E. Nurjati (2021), the coffee supply chain caused a decline in export value, hampered by lockdowns that closed coffee marketing channels. D. Manalu *et al.* (2019) argue that the competitiveness of Indonesian coffee exports is weak due to the quality of Indonesian coffee that does not meet the standards of export destination countries.

Indonesian coffee faces many problems related to the quality of coffee beans exported. According to

(Sitanini *et al.*, 2020), most of Indonesia's coffee is produced by smallholder plantations with old coffee plants, which results in the low quality of coffee beans produced by farmers. M. Mahyud *et al.* (2018) added that most Indonesian coffee farmers are small farmers with limited capabilities, which makes most Indonesian coffee farmers harvest coffee beans while still green, causing the low quality of Indonesian coffee. Non-tariff barriers often hold back Indonesian coffee and must undergo various food safety tests by the importing countries (Manalu *et al.*, 2019).

M. Ibnu and L. Marlina (2019) proposed that non-tariff barriers can be solved by implementing the correct certification mechanism and the Good Agriculture Practice already underway. Certification is essential for Americans whose awareness of health and environmental issues is increasing. Therefore, certified coffee products are the choice for coffee lovers in the United States. It needs to be a concern not only about internal factors (related to production and product quality) but also about external factors (related to market conditions) to be able to win the market so that Indonesian coffee commodities are in the most advantageous position in the United States.

CONCLUSIONS

Indonesian coffee commodities in the United States Market have comparative competitiveness indicated by a *Revealed Comparative Advantage* (RCA) value of more than 1 in all quarters. The RCA of Vietnamese coffee also has a value of more than 1 in all quarters, meaning Vietnamese coffee has comparative competitiveness, but the values are lower than Indonesian coffee's RCA value

in almost all quarters, indicating that Indonesian coffee competitiveness is superior to Vietnamese coffee. The high RCA value of Indonesian coffee can be influenced by the value of Indonesian coffee exports, which is influenced by export volumes and world coffee prices. This condition inferred that the export of Indonesian coffee has an economic efficiency influenced by the high price of Indonesian coffee imported by the United States. This situation needs to be maintained by maintaining the quality of Indonesian coffee bean exports so that they stay highly valued in the international market.

Meanwhile, the results of the EPD analysis show a shift in the competitive competitiveness position of Indonesian coffee and Vietnamese coffee in the United States. During the pandemic, the position of competitiveness shifted towards a better direction from Retreat (-0.00025, -0.00001) to Lost Opportunity (-0.0019, 0.0000) for Indonesian coffee, and from Lost Opportunity (-0.0041, 0.0008) to Rising Star (0.0012, 0.0005) for Vietnamese coffee. However, Indonesian coffee has not yet reached the ideal position of a trade, namely the Rising Star (a position where both the X-axis and Y-axis values are positive). Indonesia needs to strengthen its business strategy to win the coffee market in the

United States. Some of the problems Indonesia faces are export volume, quality, and certification issues of coffee commodities exported, which are still low or do not meet standards. Strategies and government intervention are needed to maintain comparative advantage and achieve the ideal trading position in the United States market. The role of the government in supporting all pre-export stages is indispensable for Indonesian coffee to reach its destination countries. Furthermore, maintaining good bilateral relations between the two countries is also essential for the smooth trade.

The findings of this study have shown the competitive superiority of Indonesian coffee in the export destination country of the United States. Further research is expected to analyse the factors that affect the competitiveness of Indonesian coffee exports in the United States to provide an overview of the right strategy for marketing Indonesian coffee.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Експортна конкурентоспроможність індонезійської кави на ринку США

Гесті Анніса Іннатахіббах

Магістр

Університет Себелас Марет

57126, вул. Іра Сутамі, 36А, м. Суракарта, Індонезія

<https://orcid.org/0000-0002-2329-3876>

Енданг Сіті Рахаю

Доктор сільськогосподарської економіки, професор

Університет Себелас Марет

57126, вул. Іра Сутамі, 36А, м. Суракарта, Індонезія

<https://orcid.org/0000-0002-4967-0780>

Мінар Ферічані

Доктор агробізнесу, викладач

Університет Себелас Марет

57126, вул. Іра Сутамі, 36А, м. Суракарта, Індонезія

<https://orcid.org/0000-0002-4294-2122>

Анотація. Економічне зростання Індонезії сповільнилося під час пандемії. Кава є валютоутворюючим сільськогосподарським товаром для Індонезії і стала основою національної економіки завдяки своєму внеску у валовий внутрішній продукт. Метою дослідження було проаналізувати конкурентоспроможність індонезійського експорту кави на ринку США. Були використані такі методи, як метод виявлених порівняльних переваг, який дозволяє порівняно виміряти експортну конкурентоспроможність, та метод динаміки експортної продукції для аналізу та картографування позиції експортної конкурентоспроможності. Результати аналізу також були виміряні для порівняння експортної конкурентоспроможності в'єтнамської кави. Результати показують, що індонезійська та в'єтнамська кава на ринку США мають значення вище 1, що свідчить про те, що обидва види кави мають переваги в експортній конкурентоспроможності. Середнє значення виявлених порівняльних переваг індонезійської кави вище, ніж в'єтнамської, а це означає, що експорт індонезійської кави перевершує експорт в'єтнамської кави до США. Водночас результати аналізу динаміки експортної продукції показують, що конкурентоспроможність індонезійського експорту кави перебуває в позиції «Втрачені можливості», де позиція змістилася з допандемічної позиції, а саме – «Відступ». Тим часом конкурентоспроможність в'єтнамського експорту кави опинилася в позиції «Висхідна зірка», яка змістилася з попередньої позиції «Втрачена можливість». Втрачені можливості індонезійської кави свідчать про те, що сильні сторони індонезійського товарного кавового бізнесу є слабкими, але експорт кави залишається конкурентоспроможним. Результати аналізу свідчать про те, що хоча індонезійська кава має експортну конкурентоспроможність, вона недостатньо конкурентоспроможна в США. Це дослідження надає огляд експортної конкурентоспроможності індонезійської кави в США, який може бути використаний як основа для визначення стратегічних кроків у прагненні домінувати на ринку для отримання торговельних переваг

Ключові слова: конкурентна позиція; експортні показники; експортний потенціал; міжнародна конкуренція; міжнародна торгівля



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Innovative tools for risk management of the production activities of agricultural enterprises in an institutional environment

Bohdan Khakhula*

PhD in Economics, Associate Professor
Bila Tserkva National Agrarian University
09117, 8/1 Soborna Sq., Bila Tserkva, Ukraine
<https://orcid.org/0000-0003-4286-2381>

Oksana Kostyuk

Graduate Student
Bila Tserkva National Agrarian University
09117, 8/1 Soborna Sq., Bila Tserkva, Ukraine
<https://orcid.org/0000-0003-4924-5140>

Oleksii Lanchenko

Graduate Student
Bila Tserkva National Agrarian University
09117, 8/1 Soborna Sq., Bila Tserkva, Ukraine
<https://orcid.org/0000-0003-1476-7133>

Hanna Antonyuk

Graduate Student
Bila Tserkva National Agrarian University
09117, 8/1 Soborna Sq., Bila Tserkva, Ukraine
<http://orcid.org/0000-0003-3704-7008>

Oleksandr Homon

Graduate Student
Bila Tserkva National Agrarian University
09117, 8/1 Soborna Sq., Bila Tserkva, Ukraine
<https://orcid.org/0000-0003-3773-7391>

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Abstract. The security of agricultural sector development is increasingly becoming a relevant issue in modern conditions, as the agricultural sector determines the sustainability of the economy and ensures the country's food security. Growing risks, such as climate change, economic difficulties and geopolitical conflicts, highlight the need to improve the security of agricultural production systems,

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*Corresponding author

which makes the study relevant. The study aimed to conduct a systematic analysis of innovative tools for managing the risks of production activities of agricultural enterprises and their impact on the formation of secure development in the agricultural sector of Ukraine. To achieve this goal, the method of analysis and modelling was used. Study results indicate that innovative approaches to risk management have a decisive impact on the sustainability and efficiency of agricultural production systems. To achieve a stable level of safe development of agricultural enterprises, it is necessary to focus on the implementation of key areas, such as maintaining the food base at a level sufficient to ensure a healthy diet, ensuring an adequate level of effective demand for the population and eliminating dependence on imports, aimed at protecting the interests of domestic producers of agricultural raw materials. The study demonstrated that institutional factors of risk management form an integrated system, where efficiency depends on the implementation of specific strategies for the development of agricultural institutions aimed at introducing innovative tools into the production cycle of agricultural enterprises. Furthermore, due to the accumulation of large amounts of production, financial, logistical and innovative potential of agricultural enterprises in the Forest-Steppe and Polissia regions, the level of safe development of agricultural enterprises is significantly increasing. This indicates their high capacity for sustainable reproduction of production and making a significant contribution to the agricultural sector of Ukraine. The study is of practical importance for agricultural enterprises, research institutions and government agencies, which can use the results to improve risk management strategies and increase the level of security of agricultural sector development

Keywords: risks of the production activity; business efficiency; financial stability; technological development; production system; institutes of agriculture

INTRODUCTION

In the modern economic landscape, where agriculture plays a strategic role in ensuring food security and economic resilience, the relevance of exploring innovative risk management tools for agricultural enterprises in an institutional context is becoming urgent. The growing instability of the economic and social environment, combined with the effects of climate change and global market fluctuations, causes a wide range of risks that directly affect agriculture. In this context, the study of innovative risk management tools becomes a strategically important task, as it aims to develop effective strategies and tools to help agricultural enterprises adapt to unpredictability and ensure stability in production processes.

An analysis of existing research indicates the need to improve risk management methods in agriculture. As such, A. Sudip and A. Khanal (2022) emphasised the importance of innovative approaches in addressing risk in agriculture. The authors emphasise that innovative tools can effectively contribute to the management of various aspects of production risks.

F. Capitanio (2022) highlights the need to consider risks in agriculture as a complex and multidimensional phenomenon. Innovative approaches address different dimensions of risk and develop comprehensive risk management strategies. S. Bai and X. Jia (2022) argue that innovative tools can serve as an effective tool for making informed decisions in risk management. This is especially important in the context of uncertainty and volatility in agriculture. S.V. Jansi Rani *et al.* (2022) believe that innovative methods can contribute to increasing the resilience of agriculture to various threats,

such as climate change, market fluctuations and economic difficulties.

L.R. Deng *et al.* (2022) note a definite impact of innovative risk management approaches on the agricultural sector. These approaches stimulate technological development, which plays a key role in increasing the productivity and competitiveness of agriculture. The authors emphasise that the integration of the latest technologies, the development of agricultural technologies and the increase in production accuracy are the result of the implementation of innovative risk management strategies. This contributes not only to increasing the efficiency of farming but also to creating a sustainable agricultural sector that can withstand various external influences.

Furthermore, Z. Gao (2022) notes that the introduction of risk management innovations stimulates research and development activity, contributing to the emergence of new technological solutions and techniques. In general, innovative approaches not only improve current risk management methods in the agricultural sector but also create prospects for the future development of agriculture. According to O. Stashchuk *et al.* (2021), in the field of Ukrainian agriculture risk management, innovative approaches are important to stimulate technological development. Modern innovations can play a key role in increasing the productivity and competitiveness of agriculture in Ukraine.

In particular, M.P. Sychevskiy (2019) argues that the integration of the latest technologies, the use of modern agrotechnical solutions, as well as the application

of innovative risk management methods, can help improve the quality of production and optimise agricultural processes. This, in turn, will contribute to increased farming efficiency, higher farmers' incomes, and the competitiveness of Ukrainian agricultural enterprises on the international market. Understanding and active implementation of innovative risk management solutions is becoming a strategically important element for the sustainable development of Ukraine's agricultural sector.

D. Tretiak *et al.* (2023) also note that in the context of uncertainty and military conflict in Ukraine, a weakening of agricultural institutions is evident, which leads to alarming trends in the functional state of the production system. The modernisation of technological innovations in agriculture in the pre-war period showed that the interaction between the areas of innovative development of agricultural enterprises and the institutional platform for economic development of agriculture was manifested through the introduction of state regulatory instruments aimed at ensuring the result of the process of creating, disseminating and applying new rules and forms of production activities of enterprises and aimed at implementing innovative solutions for managing risks.

The institutional approach of the pre-war period was used to reduce threats and risks in the production system of agricultural enterprises through the processes of integration and unification of organisational technologies that ensured the transition from a disordered state to an orderly one through the joint, cooperative (synchronous) action of many subsystems (Shahini *et al.*, 2024). This is especially true for agricultural enterprises whose production conditions did not meet the norms and standards of the European market.

M. Sychevskiy (2019), O. Yatsukh *et al.* (2021) note that the production system unites agricultural institutions and determines the rules of their relations, as well as generates an innovative infrastructure of technologies in the production and technological process. However, the effectiveness of agricultural enterprises' development under the significant impact of martial law depends on innovative tools for managing the risks of production activities, which determine the pace of renewal, modernisation of fixed assets, creation and implementation of basic, improvement of technical, technological and information innovations to increase the competitiveness of products and guarantee the country's food security. However, reasonable steps are not always taken to address the problem of risk minimisation, and in some cases, they are limited by national guidelines for the economic development of agricultural enterprises. Therefore, conceptually, the institutional environment for innovation in agriculture should be implemented in a clear and unified systematic approach to risk management of agricultural

enterprises' production activities with the identification of priority instruments for innovative development at the territorial level.

The solution to this problem is a complex multi-criteria task, which actualises the directions of overcoming various obstacles to the production activity of agricultural enterprises at different levels. Considering the existing research gaps, this study aims to review and develop innovative approaches to risk management of agricultural enterprises in the institutional environment. This approach will contribute to improving the sustainability of agriculture and ensure more effective risk management in this important sector of the economy.

MATERIALS AND METHODS

After a high-quality assessment of the risks of production activity, a quantitative study of its magnitude is considered; the numerical values of unit risks are calculated, taking into account the probable loss of the volume of production or resources; the final stage is the formation of a system of anti-risk events and the calculation of the value equivalent of risk in the institutional environment of safe development of agricultural enterprises. The quantitative measurement of the risk of production activity of agricultural enterprises is determined by the following indicators: the absolute level of losses (the amount of possible losses in material or cost form); relative to the level of losses (risk factor, K_r) (1):

$$K_r = \frac{E_p}{E_l}, \quad (1)$$

where K_r – risk coefficient; E_p – expected profit; E_l – expected profit.

The first method is presented in Table 1, and the second method of calculation (K_r) can be used to calculate how much income per 1 USD of loss. The most relevant today are the models that, based on the results of qualitative dispersion analysis, allow for mathematically evaluating the effectiveness of innovative tools for risk management of the production activities (ITMRPA) of agricultural enterprises. As one of the methods of quantitative assessment of the efficiency of ITMRPA of agricultural enterprises, an approach based on the analysis of Net Present Value (NPV) as a whole for a set of innovative production programs (projects), taking into account their changes depending on the functioning of ITMRPA of agricultural enterprises, is proposed. In the process of implementing innovative production programs (projects), agricultural enterprises face systematic and unsystematic risks. Non-systematic risks are those risks whose impact agricultural production entities can independently reduce by introducing more effective innovative tools for managing the risks of production activity.

Table 1. Methods of calculating the risk factor of production activity

Calculation algorithm	Note
	<i>If there are losses</i>
$K_r^L = PL/NV$	PL – possible losses; NV – normative value of the coefficient
$K_r^L = (EP-AP)/NV$	EP – estimated profit; FP – actual profit
	<i>If there is a profit</i>
$K_r^P = EP/NV$	EP – excess profit
$K_r^P = (AP-EP)/NV$	AP – actual profit (loss)

Source: constructed by the authors

Let's consider the stages of ITMRPA assessment of agricultural enterprises, which are based on the analysis of Net Present Value (NPV).

Stage 1. The following analysis is performed for unsystematic risks:

- calculation of the risks of the innovative production program (project) in the absence of ITMRPA – P_i ;
- calculation of new values of each type of risk P_i after the implementation of ITMRPA, which allows to reduce the risks of the innovative production program (project), i.e. $P_i > P_i^*$;

– calculation of the initial and final risk of the innovative production program (project) taking into account the weighting factors according to formulas (2-3):

$$P = P_1 \times K_1 + P_2 \times K_2 + P_3 \times K_3 + P_4 \times K_4, \quad (2)$$

$$P^* = P_1^* \times K_1 + P_2^* \times K_2 + P_3^* \times K_3 + P_4^* \times K_4, \quad (3)$$

where K_1, K_2, K_3, K_4 – coefficients.

The content of the constituent (2-3), as well as the general structure of the assessment of unsystematic risks, are shown in Table 2.

Table 2. Estimates of unsystematic risks of production activity

The type of risk of an innovative project of production activity	Risk assessment in the absence of ITMRPA	Control procedures	Risk assessment of the innovation program (project) of production activity when using ITMRPA	Conditions for the effectiveness of procedures
1	P_1	Procedure 1.1 Procedure 1.2	P_1^*	$P_i > P_i^*$
2	P_2	Procedure 2.1 Procedure 2.2	P_2^*	
3	P_3	Procedure 3.1 Procedure 3.2	P_3^*	
4	P_4	Procedure 4.1 Procedure 4.2	P_4^*	
n	P	-	P^*	

Source: constructed by the authors

Stage 2. Experts assess systematic risks ($P_{systemic}$), which form the environment for the operation of an innovative production program (project) and are not amenable to the management of agricultural enterprises. Systematic risks are determined by many factors (macroeconomic, legal and political), which have an equal level of influence on the implementation of an innovative production project.

Stage 3. According to the proposed approach to evaluating the effectiveness of the implementation of innovative tools for risk management of the production activity, the overall risk of the innovative production program (project) is determined (r). The total risk consists of the sum of unsystematic and systematic risk (4-5):

$$r = K_p \times P + K_{p_{systemic}} \times P_{systemic}, \quad (4)$$

$$r^* = K_p \times P^* + K_{p_{systemic}} \times P_{systemic}, \quad (5)$$

where P – the initial unsystematic risk of the innovative production program (project); P^* – final non-systematic risk of an innovative production program (project); K_p – specific weight of non-systematic risk of innovative production program (project); $K_{p_{systemic}}$ – the specific weight of the systematic risk of the innovation program (project) of production; $P_{systemic}$ – systematic risk of the innovative production program (project); r – the initial total risk of the innovative production program (project); r^* – the final total risk of the innovative production program (project).

Stage 4. Modernize for purposes the calculation of the net present value of the innovative production program (project) (NPV) – the formula (6):

$$NPV = -I + \sum \frac{cf_t}{(1+d)^t}, \quad (6)$$

where NPV – Net Present Value of the innovative production program (project); I – the amount of investment in innovative production programs (projects); cf_t – the value of possible cash flow streams; d – discount rate; t – a period of time.

Stage 5. At the next stage, propose to calculate two discount rates: the first takes into account the risk of an innovative production program (project) in the absence of ITMRPA, and the second takes into account the implemented and functioning ITMRPA. A model CAMP (Capital Assets Pricing Model) is used to calculate the discount rate. According to CAMP, the discount rate (d) is calculated using the following formula (7):

$$d = R_f + \beta \times (R_m - R_f), \quad (7)$$

where R_f – the risk-free rate of return; β – the coefficient that determines changes in the price of assets of agricultural enterprises in comparison with changes in the prices of assets for all subjects of agricultural production in this market segment – the risk coefficient; $(R_m - R_f)$ – risk premium; R_m – average market rates of return on the stock market.

$$Q = (W, E), V = \{F_{zm1}, F_{zm2}, F_{zm3}, F_{zm4}, F_{zm5}, F_{zm6}, F_{zm7}, F_{zm8}, F_{zm9}, F_{zm10}, F_{zm11}, F_{zm12}, F_{zm13}\}, \quad (12)$$

where W – set of peaks of innovative tools that correspond to external and internal risk factors of production activity of agricultural enterprises; E – sets of arcs reflecting the direct influence of risk scenarios of the institutional environment on the parameters of the safe development of agricultural enterprises.

The interrelationship of parameters involves the construction of matrices of acceleration (deceleration) of leverage factors of innovative tools for risk managing of production activities, which have certain characteristics (Table 3). Thus, at a value of (+1), there is an increase (decrease) in the factor (F_{zmi}, F_{imi}), which leads to an increase (decrease) (F_{zmj}, F_{imj}); at a value of (-1), there is an increase (decrease) of the factor (F_{zmi}, F_{imi}), which leads to a decrease (increase) (F_{zmj}, F_{imj}); with the value (0), there is a weak or completely absent connection between the factors (F_{zmi}, F_{imi}) and (F_{zmj}, F_{imj}).

The intensity of the interaction is assessed on a point scale: 0.1 – no direct impact; 0.5 – weak influence; 1.0 – medium impact; 2.0 is a strong influence. Among the active peaks, lever factors of innovative tools for management of the risks of production activity were identified, which affect the safe development of

It should be noted that the considered coefficient (β) is a measure of ITMRPA, and if assume that the obtained result of the ITMRPA qualitative assessment reflects the main risks of the innovative production program (project), then it can assume the following calculation of the coefficient (β) according to the formulas (8-9):

$$\beta = \frac{r}{100+1}, \quad (8)$$

$$\beta^* = \frac{r}{100+1}, \quad (9)$$

where r – total project risk is obtained as a result of the analysis.

Stage 6. Let's calculate two scenarios for calculating net present value with and without ITMRPA, using (6) and (10):

$$NPV^* = -I + \sum \frac{cf_t}{(1+d)^t}. \quad (10)$$

Stage 7. Finding the efficiency of ITMRPA implementation as the difference between the flows NPV and NPV^* be the formula (11):

$$ITMRPA_{ef} = NPV^* - NPV. \quad (11)$$

The simulation model "Activation of innovative tools for risk management of the production activity in the institutional environment of safe development of agricultural enterprises" is carried out according to the formula (12):

agricultural enterprises, namely: F_{zm1} – Government programs for creating buffer stocks of agricultural raw materials of the grain and oil group; F_{zm2} – Futures contracts, F_{zm3} – Spots prices for agricultural products; F_{zm4} – manufacturing outsourcing; F_{zm6} – Blockchain, F_{zm7} – Digital financial technologies; F_{im8} – Contract farming.

RESULTS

The reason for the ambiguous perception of the methodology of risk management of production activity lies in the conservative approach of agricultural production entities to ensure the safety of the production cycle. The inability to quickly evaluate alternative scenarios of risk-indicative management of production resources limits agricultural enterprises in innovative development and accelerated economic processes following established innovative regulatory instruments, rules, and norms of agricultural institutes, which regulate the right to own land plots for agricultural land and ensure stable production cycle Summarizing the above, let's highlight the key institutional determinants of risk management of production activities of agricultural enterprises (Fig. 1).

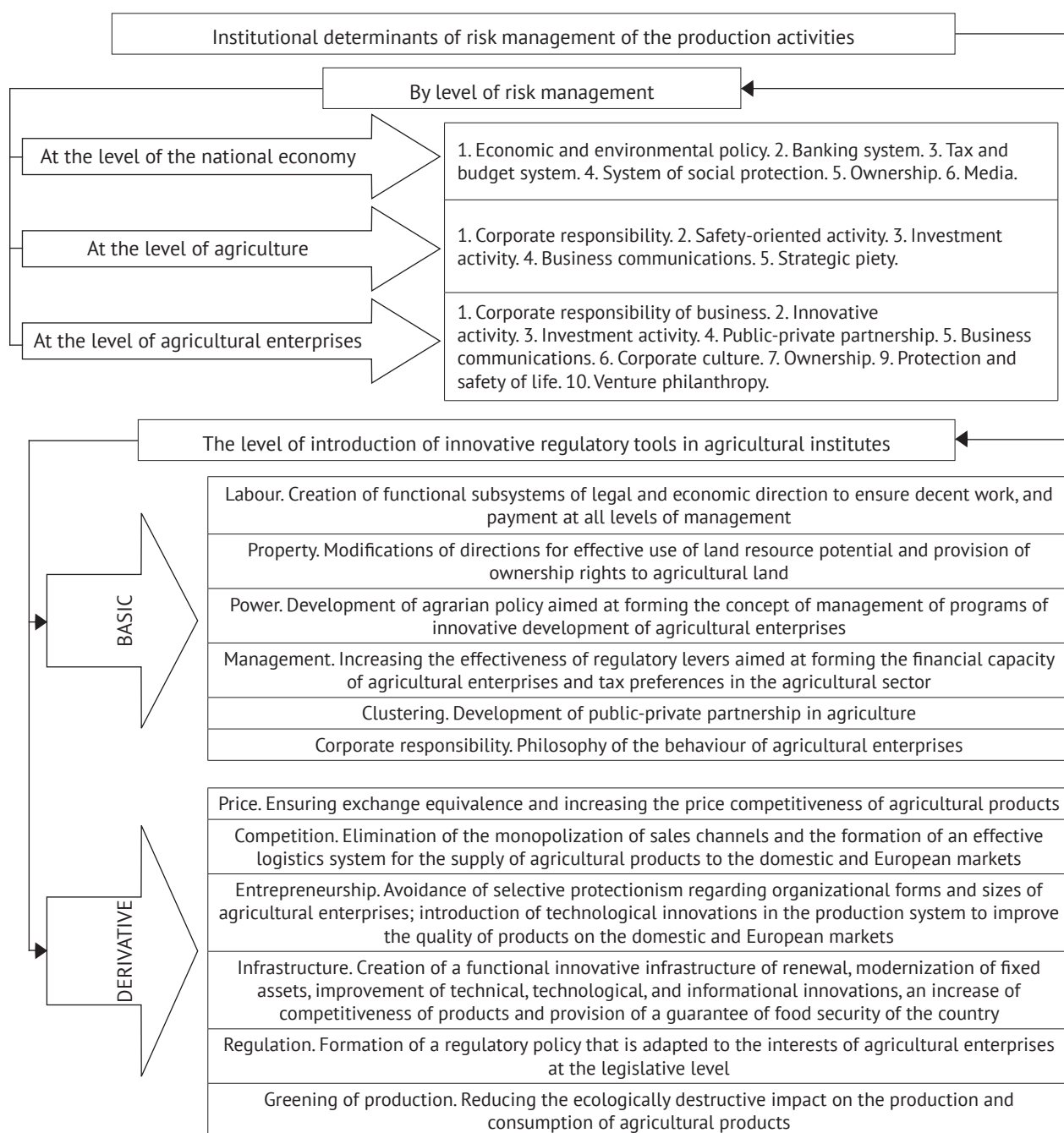


Figure 1. Institutional determinants of risk management of the production activities of agricultural enterprises

Source: constructed by the authors

Institutional determinants for risk management of the production activity of agricultural enterprises form a system, the effectiveness of which depends on the implementation of the relevant directions of development of agricultural institutes, focused on the introduction of innovative tools in the production cycle of agricultural enterprises. At the same time, the managerial function of neutralizing the risks of production activity is determined by the institutional and economic ability of agricultural enterprises to acquire, maintain and expand their share of product markets through the levers of market influence. The institutional and economic

importance of agricultural enterprises in the national economy determines the need to increase their competitiveness in the country.

Valuable ideas for the formation of a new conceptual approach to risk-indicative management of the production activities of agricultural enterprises to activate the process of safe development in the institutional environment were offered by R. Zięba, who conducted an analysis of the breakdown of the quality standards of agricultural raw materials in the self-regulating contractual markets of various states. In his opinion, risks in agriculture occur due to the levelling of the interests of

subjects of agricultural production. These considerations are supported by the arguments of the new institutional theory, which is based on a set of elements that form the core of an innovative approach to the risk-oriented

management of production activities of agricultural enterprises in the structure of an integral production system of agriculture with certain form-forming components of the institutional environment (Fig. 2).

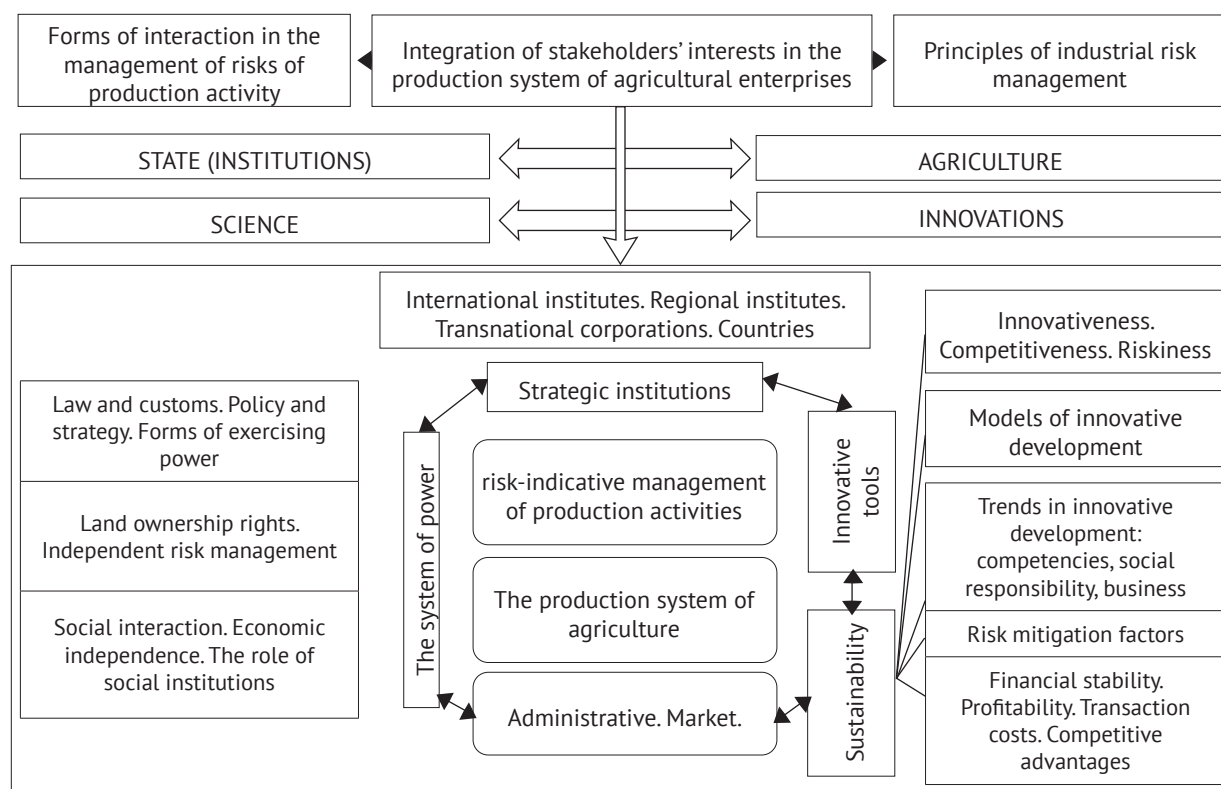


Figure 2. Innovative approach to risk-indicative management of production activities of agricultural enterprises in an institutional environment

Source: constructed by the authors

One of the directions for evaluating the effectiveness of the implementation of innovative tools for risk management of the production activity is the creation of a single model that would combine both qualitative and quantitative approaches for the calculation of various quantitative indicators. Therefore, the main ways of preventing the risks of production activity at the level of safe development of agricultural enterprises can be a justified approach to the choice of the sales market and the development of a price strategy based on spot prices for agricultural products, as a quickly realized asset of agricultural enterprises; compliance with the principles of fair competition and the main provisions of multilateral trade agreements, as well as government programs for the creation of buffer stocks of agricultural raw materials of the grain and oil group; thought out own marketing policy, taking into account strategic innovation programs and production projects, determining the behaviour of competitors on the market. Their prevention is also facilitated by systematic monitoring of factors and areas of production risk formation in trade contracts and contracts for consumer production of agricultural products. Therefore, the

stimulus for predicting potential risks of production activity, as well as an effective innovative management of them, are model scenarios of excitation (activation) of innovative tools. At the same time, scenarios for the activation of innovative tools for managing the risks of production activity are aimed at the safe development of agricultural enterprises in an institutional environment with numerical impulses that strengthen the vertices of the simulation model and determine changes in the values of the vertices at the corresponding steps of the simulation model.

Institutional form-forming components within the framework of study single out key innovative tools for neutralizing the risks of production activity of agricultural enterprises, which, with a certain amplitude of resource provision of subjects of agricultural production, accelerate the protective functions of the production system of agriculture from uncoordinated decisions of formal and informal institutions, the principle actions of which are the structuring of mutual relations between subjects of agricultural production and stakeholders. Misalignment of their interests in making management decisions provokes dynamic fluctuations in the

production system of agriculture and discourages the coordination of production cycles of agricultural enterprises at different levels of influence of risk scenarios in the innovative development of agricultural production. This happens through a philosophical approach to riskology, as a science that singles out several axioms (axioms of generality, axioms of acceptability and axioms of non-repeatability).

The dialectic of the theory of development and the theory of systems provides an opportunity to consider

innovations in the context of tools for the renewed development of production cycles of agricultural enterprises that change the production system of agriculture to a qualitatively new level. On this basis, the uncertainty and unrepeatability of production cycles of agricultural enterprises in the institutional environment are considered as a stage of using innovations as tools for managing the risks of production activity, as a percolation of the stages of the transition of the production system of agriculture from one state to another (Fig. 3).

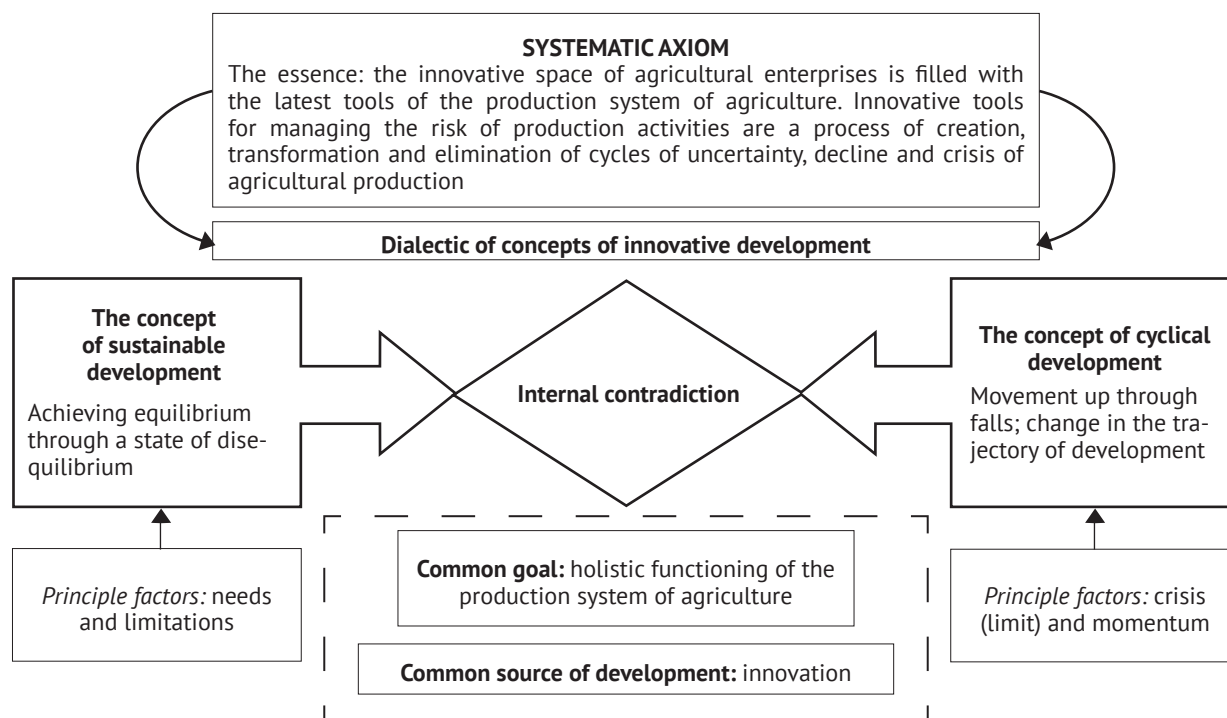


Figure 3. Structural dialectical connection of the concept of innovative development of agricultural enterprises with sustainable and cyclical development of the production system of agriculture

Source: constructed by the authors

Ensuring the innovative development of agricultural enterprises involves the activation of the functioning of the production system of agriculture in a new quality, while preserving its structural and functional integrity. This is possible only under the condition of the formation of a mechanism for the activation of innovative tools for risk management of the production activity, which can ensure a transformational transition to the safe development of agricultural enterprises and a new qualitative level of their capabilities in the institutional environment in the presence of innovative potential of enterprises, risk-oriented behaviour of enterprises; formation of a favourable innovation climate; modification of the structure of enterprise assets, in the direction of resource provision.

Strategic innovation programs and innovation projects at the level of the state and agricultural institutes belong to the innovative tools for risk management of agricultural enterprises. These programs and projects

are formed with the help of levers and regulators (futures (option) contracts; spot prices for agricultural products; production outsourcing; agricultural technologies; blockchain; digital financial technologies; contract farming; trading contracts; government programs for creating buffer stocks of agricultural raw materials and oil group; insurance of agricultural raw materials; hedging and diversification of production systems, contracts of consumer production), which are implemented at the territorial level, and on which the dynamic fluctuation of production cycles of agricultural enterprises depends.

Production cycles are subject to the influence of different levels of risk, and their partial neutralization (minimization) depends on the time lag in the provision and use of resources for the production needs of agricultural enterprises. Processes of evaluation of factor events in addition to typical and repeated situations in the production activity of agricultural enterprises have

a limited number of possible results. At the same time, dynamic forecasting methods provide a more reliable result than static regularities and simple extrapolation dependencies. Extrapolation allows obtaining only a partial forecast, which reflects changes in individual components of the safe development of agricultural enterprises. Therefore, individual security parameters that do not have system properties are replaced by system-non-forecasting based on simulation technologies. It should be noted that the factors of change (modification) of the parameters of the macro- and microenvironment of agricultural enterprises in the institutional environment act as a risk factor.

For agricultural enterprises, the danger of production activity risks is particularly significant, since the impact of macroeconomic fluctuations leads to a crisis of the microeconomic genesis of agricultural production. Accordingly, the combined influence of factors of the macro- and microenvironment of agricultural enterprises strengthens or weakens the process mechanism of assessing the safe development of agricultural production entities in the institutional environment. A comprehensive understanding of risk, as an element of managing the production activities of agricultural enterprises, contains an effective component – economic losses that threaten their competitiveness and the corresponding consequences for the country's agriculture. Risk, as “a deviation of a parameter of the production system of agriculture from a given target value by an amount that does not exceed the permissible deviation of this parameter”, allows, on the one hand, to realize

the economic interests of agricultural enterprises, and on the other – “to single out a threat to their competitiveness”. From the point of view of the implementation of the integrated value of safety in the macro- and microenvironment of agricultural enterprises, risk factors in the system of protection of production activities are associated with the problems of implementing innovative tools for resource management and ensuring the needs of agricultural enterprises in the future.

Assessment of risk factors allows: first, to generalize threats and activate the safe development of agricultural enterprises; secondly, to identify risk events and develop optimal management solutions. Therefore, an important task is to identify and systematize the relationship between the manifestation of risk factors and their impact on the results of production activities of agricultural enterprises. The stages of evaluating the effectiveness of the implementation of innovative tools for risk management of industrial activity (ITMRPA) involve the determination of their number and the causes of danger. An important stage of innovative risk management of production activity is the assessment of the level of safety according to qualitative and quantitative parameters. Qualitative analysis of risks allows to determine in advance the sources of their occurrence. The advantage of this approach is that already at the initial stages, it is possible to assess the degree of risk when carrying out a particular activity. The matrix of acceleration and deceleration of innovative tools for risk management of production activities of agricultural enterprises was used (Table 3, 4).

Table 3. Matrix of acceleration of the action of innovative tools for risk management of production activities of agricultural enterprises

	F _{zm1}	F _{zm2}	F _{zm3}	F _{zm4}	F _{zm5}	F _{zm6}	F _{zm7}	F _{im8}	F _{im9}	F _{im10}	F _{im11}	F _{im12}	F _{im13}	Degree of interaction
F _{zm1}		1	1	1	0.5	1	1	0.5	0.5	0.5	0.5	0.5	0.5	126.4
F _{zm2}	1		0.5	0.1	1	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	128.8
F _{zm3}	1	0.1		2	1	1	0.5	2	2	1	0.1	0.1	2	161.6
F _{zm4}	1	1	0.5		1	0.5	0.5	0.5	0.5	0.5	1	0.1	0.1	149.9
F _{zm5}	1	1	0.5	0.1		0.5	1	0.5	0.1	0.1	0.5	0.5	1	62.3
F _{zm6}	0.5	2	0.5	1	2		2	1	1	2	2	0.5	0.1	155.8
F _{zm7}	0.5	0.5	0.5	0.5	1	1		1	1	1	1	0.5	0.5	162.4
F _{im8}	0.5	1	2	1	2	2	0.5		2	2	2	0.5	0.1	167.3
F _{im9}	0.1	1	0.5	0.1	1	2	1	2		2	2	0.1	0.5	136.1
F _{im10}	0.1	2	2	0.1	1	0.5	0.5	0.5	1		2	0.1	0.5	47.9
F _{im11}	0.1	2	0.1	0.1	1	1	1	0.5	0.5	1		0.5	0.1	28.5
F _{im12}	2	1	0.1	0.1	0.5	0.1	0.1	0.1	0.5	0.5	0.5		2	29.6
F _{im13}	0.5	2	0.5	0.5	1	0.5	0.5	0.1	2	1	1	0.5		95.6
Degree of activity	1	0.5	1.1	1.9	0.7	1.4	0.5	1.9	1.2	0.9	0.5	2.2	1.5	

Source: constructed by the authors

Table 4. Matrix of slowing down the effect of innovative tools for risk management of production activity of agricultural enterprises

	F _{zm1}	F _{zm2}	F _{zm3}	F _{zm4}	F _{zm5}	F _{zm6}	F _{zm7}	F _{im8}	F _{im9}	F _{im10}	F _{im11}	F _{im12}	F _{im13}	Degree of interaction
F _{zm1}		1	1	0.5	1	0.5	0.5	0.1	0.1	0.1	1	0.1	1	55.5
F _{zm2}	1		1	1	1	0.5	1	0.5	0.5	0.5	1	0.1	0.5	127.7
F _{zm3}	0.1	0.1		0.1	0.1	0.1	0.1	0.5	1	1	1	0.1	0.1	33
F _{zm4}	0.5	0.5	0.1		0.5	0.5	0.5	0.5	0.1	0.5	1	0.1	0.1	31.5
F _{zm5}	1	1	1	0.5		0.1	0.1	0.1	0.1	0.5	0.5	0.1	1	62.3
F _{zm6}	0.1	0.1	0.1	0.1	0.5		0.5	0.5	0.5	1	0.5	0.1	0.5	36.5
F _{zm7}	0.1	0.5	0.1	0.1	0.5	0.5		0.5	0.1	0.5	0.5	0.1	0.5	34.7
F _{im8}	0.1	0.1	0.1	0.5	0.1	0.5	0.5		1	1	0.5	0.1	0.1	43.7
F _{im9}	0.1	1	0.5	0.5	0.1	1	0.5	1		2	1	0.1	0.5	95.9
F _{im10}	0.5	1	0.5	0.5	0.1	1	0.5	2	2		2	0.5	0.5	152.5
F _{im11}	0.5	2	2	1	1	1	1	2	2	2		0.5	1	221
F _{im12}	2	2	0.5	0.5	2	0.1	1	0.1	0.5	0.5	1		2	26.7
F _{im13}	0.5	2	0.1	0.5	1	0.5	0.5	0.5	1	1	1	0.1		85.4
Degree of activity	1.1	0.7	0.6	0.8	0.8	0.7	0.6	0.5	0.8	1	1.3	2	1.1	

Source: constructed by the authors

Behaviour models of innovative tools for risk management of the production activities provide optimal and positive interaction of factors according to scenarios 1 and 12, which demonstrate the best results of safe development of agricultural enterprises. Important components in this process are public-private partnerships, as well as the presence of a regulatory price policy. Thus, according to Scenario 1, the momentum of the

interaction of the factors of innovative tools for managing the risks of production activity is carried out in three vertices – $F_{im8}=1$, $F_{zm4}=1$, $F_{zm2}=1$ (the improvement of contract farming increases the level of production outsourcing and expands the volume of futures contracts). At the same time, there is an increase in all weighted coefficients of safe development of agricultural enterprises in the institutional environment (Fig. 4).

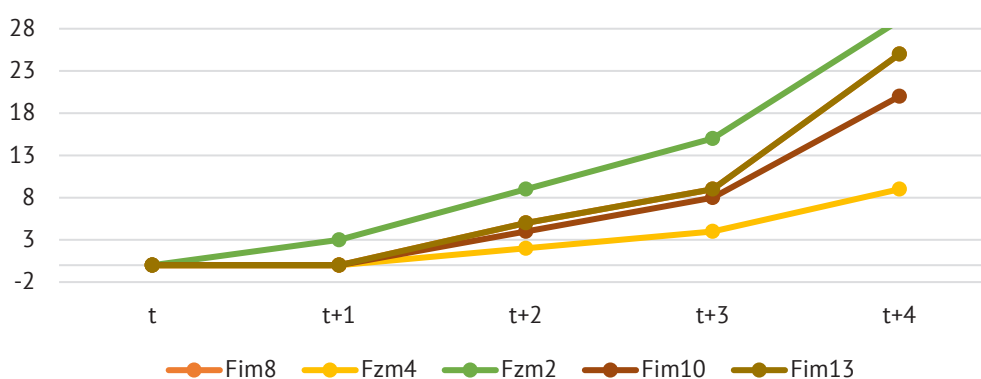


Figure 4. Simulation model of the behaviour of innovative tools for risk management of the production activities of agricultural enterprises (Scenario 1)

Note: Scenario 1. The momentum of the interaction of innovative tools for managing the risks of production activities improves contract farming, increases the level of production outsourcing, and expands the volume of futures contracts to fix prices for agricultural enterprises. There is an increase in all weighted coefficients of safe development of agricultural enterprises in the institutional environment

Source: constructed by the authors

Scenario 12 demonstrates the momentum of the interaction of four vertices – $F_{im8}=1$, $F_{zm3}=1$, $F_{zm6}=1$, $F_{zm7}=1$, (the state policy of regulating spot prices for

agricultural products under rational contract farming, improves the logistics infrastructure of the agricultural market with the help of Blockchain technologies and

digital financial technologies). Sensing the state's interest in the implementation of modern digital innovations allows to optimize innovation potential, rationally

use the available resource potential of agricultural enterprises and ensure their safe development based on public-private partnership (Fig. 5).

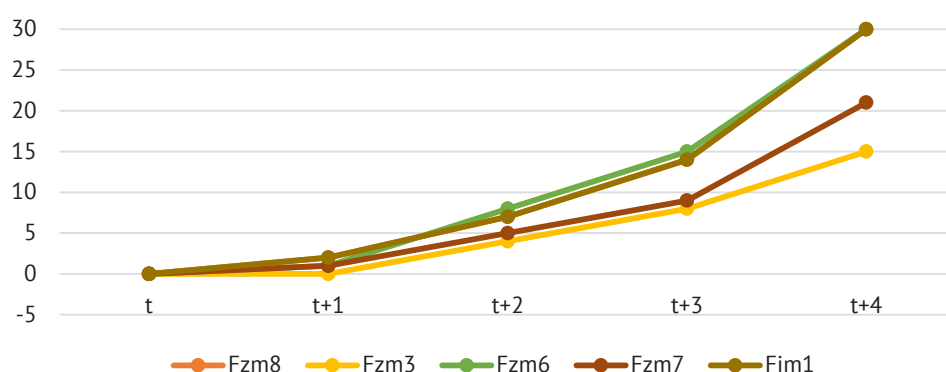


Figure 5. Simulation model of the behaviour of innovative tools for risk management of the production activities of agricultural enterprises (Scenario 12)

Note: Scenario 12. The impetus for the interaction of innovative tools for managing the risks of production activity is provided by the state policy of regulating spot prices for agricultural products in rational contract farming; and improves the logistics infrastructure of the agricultural market with the help of Blockchain technologies and digital financial technologies; innovative and resource potential is optimized; the level of safe development of agricultural enterprises based on public-private partnership is increasing

Source: constructed by the authors

Taking into account the state of war in Ukraine and the consequences of the destabilization of the production system of agriculture in the country (the extent of the loss of resources and production potential of agricultural production), based on the results of simulation modelling, the integral index of the safe development of agricultural enterprises was calculated (the calculation was made on average per subject of agricultural production) certain agroclimatic zone. The activation of strategic innovative tools for managing the risks of production activity in the institutional environment made it possible to single out the structural coefficients of the index of safe development of agricultural enterprises, which are listed in Table 5. Thus, the calculations revealed that a high level of coefficients of production, financial, logistical

and innovative components of safe development per agricultural enterprise was recorded in the agro-climatic forest-steppe zone, Polissia zone and Western Zone. This is due to the rapid acceleration in the institutional environment of production outsourcing and futures contracts based on the government's creation of reserves of agricultural raw materials of the grain and oil group under food security programs in the country. The peculiarity of this trend was felt in 2021 and, despite hostilities in most regions of Ukraine, in 2022. During this period, the diversification of the production system of agriculture was intensified, and agricultural technologies, Blockchain and protective financial technologies were introduced, which have a huge potential for increasing productivity, income and food security in Ukraine.

Table 5. Integrated index of safe agricultural development enterprises in agro-climatic zones of Ukraine for 2018-2023 (on average for one subject of agricultural production)

Structural coefficients of safe development agricultural enterprises	Steppe zone	Forest-steppe zone	Polissia zone	Western zone
The level of costs per 1 ha of agricultural land	4.41	4.09	3.07	2.54
Fund return	0.22	0.94	0.97	0.62
Product profitability level	4.5	55.7	43.8	38.1
Productivity	51.6	123.5	148.2	111.7
Coefficient of production component of safe development	15.009	162.624	139.031	81.874
Solvency ratio	0.12	0.87	0.71	0.46
Liquidity ratio	0.171	0.358	0.347	0.342
Turnover ratio of current assets	0.19	0.58	0.54	0.41
Financial leverage ratio	0.06	0.76	0.74	0.58

Table 5. Continued

Structural coefficients of safe development agricultural enterprises	Steppe zone	Forest-steppe zone	Polissia zone	Western zone
Coefficient of the financial component of safe development	0.015	0.371	0.314	0.193
Profitability of sales	1.5	34.5	33.6	9.2
Product quality factor	8.1	12.1	13.1	12.4
The level of the ratio of the spot price to the sale price on the domestic market	1.07	1.86	1.2	1.13
Coefficient of stimulation of the logistics process	0.04	0.44	0.32	0.77
Coefficient of the logistic component of safe development	0.721	18.484	13.001	9.963
The volume of innovations in production	0.15	0.87	0.51	0.77
Innovative potential	19.6	90.1	38.04	39.4
The level of innovation	0.41	10.9	7.9	1.75
Coefficient of innovative component of safe development	1.097	29.234	12.399	7.286
Integral Index of Safe Development (Isd)	0.422	180.56	83.888	33.871
Interpretation of the level of safe development	Critical level	High level	High level	Sufficient level

Source: constructed by the authors

By 2022, the steppe zone of Ukraine had a high level of safe development of agricultural enterprises. However, due to the occupation of a significant territory of this zone by the aggressor country during 2022, a clearly expressed risk scenario of lost opportunities for agricultural production entities due to the deterioration of a significant amount of resources and assets is being followed. This affected the slowdown of the index of the production component of the safe development of agricultural enterprises. Such distribution increases the need for the reproduction of the process of activation of innovative tools for managing the risks of production activity after agricultural enterprises after the liberation of the occupied territory of the Steppe zone, and the reconstruction of residential, industrial, transport, logistic and agricultural infrastructure. In 2022, most of the agricultural enterprises of the Steppe zone, which is not occupied by the aggressor country, have limited resource capabilities, and face large fluctuations in yield and agricultural production due to the high frequency and probability of climatic shocks, which also increase the pressure on high food prices. In turn, this increases vulnerability and reinforces the importance of supporting supply so that markets can adjust to price fluctuations. It is believed that the implementation of agricultural technologies is critical for overcoming the risks of production activities.

In 2023, agricultural enterprises in the Steppe zone of Ukraine continue to face challenges arising from the difficulties associated with the loss of part of the territory due to the occupation, as well as from natural and economic factors. The occupation significantly limits the possibilities of restoring agricultural infrastructure and resources, which requires great efforts to restore and modernise them. The vulnerability of agricultural enterprises in this area is also increased by unstable climatic conditions that affect production. In particular, insufficient rainfall and rising temperatures are factors

that increase the risk to productivity. In addition, climate change introduces uncertainty into crop production and can lead to difficulties in making decisions about technology choices. In the context of high food prices and price fluctuations, it is necessary to support the agricultural market and implement measures that contribute to its stabilisation. The introduction of agricultural technologies and innovative risk management methods remains a key element of the strategy to adapt to new conditions and maintain the competitiveness of agriculture in the face of the challenges of 2023. Indeed, the increase in climate variability in the Steppe zone of Ukraine, which has seen a decrease in annual precipitation and an increase in temperature over the past two decades, is harming agricultural productivity. In turn, factors such as yield variability and the risk of failure affect technology adoption decisions, especially in low-income contract farming. This limits agriculture in innovative approaches, and even more so, in increasing investment in agricultural technologies.

Ukraine's strong political commitment to strengthening agrarian policy and agricultural institutions will allow in the future (in the post-conflict period) restore the deployment of Blockchain-technology agricultural enterprises throughout the territory, to restore the infrastructure of logistical supplies of agricultural raw materials to the market under future contracts, and to improve the adaptation of agricultural technologies to farming in the conditions of climate change, to mitigate shocks and stability of the production system of agriculture. For example, improved varieties give higher and more stable yields, resistant to numerous stresses, and new technological packages (drought-resistant varieties of wheat and barley) in combination with integrated pest control increase yields and reduce production costs. Research centres in Ukraine are developing new varieties of crops and production technologies that help to ensure sustainable growth in the productivity of

the crop production industry in agricultural enterprises. In underdeveloped farms, taking into account the high variability of profits and (average) productivity growth of this industry is not a sufficient condition for the attraction and implementation of new technologies and agricultural innovations. New technologies must bring adequate and stable profits from year to year. There is no reason to introduce new technologies if small agricultural enterprises are exposed to price uncertainty due to the high volatility of food prices and frequent changes in agricultural policy, which increases uncertainty.

The main task of agricultural institutions is to ensure proper investment in agricultural research, and this is possible only with a favourable institutional environment, as well as increased government support for the introduction of technologies in small farms. The comprehensive reform of the irrigation system in Ukraine, which includes a new contractual land use of agricultural enterprises, provides a guarantee of ownership of agricultural land plots and full compensation of costs for innovative projects, which, with the help of production outsourcing, allow managing the

production system of agriculture at the institutional level. This leads to an increase in the production and yield of crops of the grain and oil group, as export-oriented agricultural raw materials on the domestic and world markets. Under such conditions, there is a need to review the current production activity of agricultural enterprises, and, first of all, introduce diversification of the logistics component to ensure the safe development, stability and independence of agricultural production entities. This will make it possible to develop one's production base and fulfil relevant economic obligations to neutralize risks based on the above-mentioned innovative tools. At the same time, the predictive stability of the level of safe development of agricultural enterprises is of scientific and practical interest, which is determined comprehensively by calculating this index in the dynamic trend of the entire set of coefficients (production, financial, logistical and innovative components) in the long term. The calculated forecast integral index of safe development of agricultural enterprises by agro-climatic zones of Ukraine for 2024-2027 is presented in Figure 6.

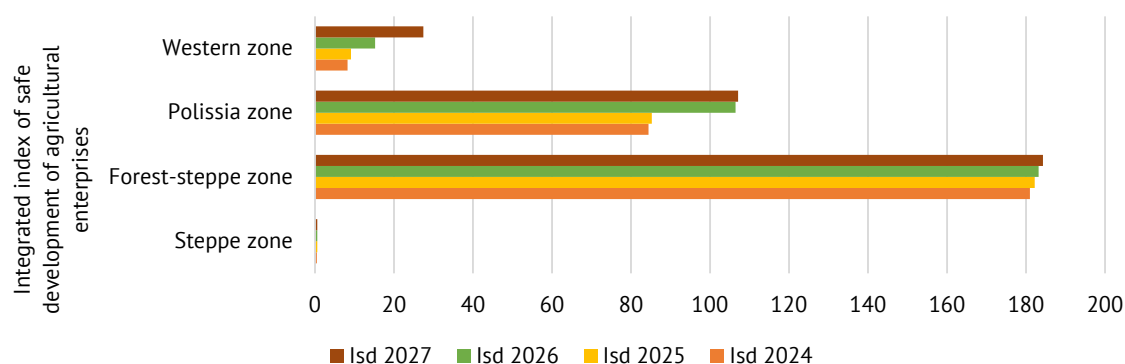


Figure 6. Forecast of the integrated index of safe development of agricultural enterprises for 2024-2027

Source: constructed by the authors

Thus, it is worth emphasising that the study results demonstrate that the accumulation of significant production, financial, logistics and innovation potential improves the secure development of agricultural enterprises in the Forest-Steppe and Polissia zones. This indicates their ability to reproduce the agricultural production system in Ukraine and determines their sustainability and competitiveness in the industry. By emphasising the importance of optimising production, financial and innovation capabilities, it is possible not only to support the sustainability of agricultural enterprises but also to contribute to the efficiency of the national economy. These results emphasise the importance of business and innovation potential for the development of the agricultural sector. Accumulation of production and financial resources, improvement of logistics processes and introduction of innovative approaches become catalysts for sustainable agricultural development.

DISCUSSION

Studies by many scholars, including A. Sudip and A. Khanal (2022) as well as the present study, confirm that the production activities of agricultural enterprises, unlike other areas of their activity, are closely related to the processes of their safe development in the institutional environment. Comparing the statements of scientists, it should be noted that the institutional environment of agriculture embodies formal and informal institutions that develop normative regulations for the production cycle of agricultural enterprises, enhance their economic opportunities and minimise potential risks in the market. The main formal institutions of agriculture that influence the resource provision and intensification of the production cycle of agricultural enterprises include the property institute, the state regulation institute, the entrepreneurship institute, the contract institute, the competition institute, and the knowledge institute. Informal institutions include peasant customs, traditions,

religious preferences, moral principles and views, stereotypes of agricultural behaviour. The basis of informal rules for the development of institutions is the cultural traditions and values of rural areas, which determine the worldview and behaviour of rural people. In rural areas, personalised exchange dominates (economic actors in agribusiness know each other directly), and therefore contractual agreements are often concluded formally; the resolution of conflicts over the provision and use of resources in the production activities of agricultural actors is often based more on local customs than on legal norms (Belgibayeva *et al.*, 2022).

At the same time, some authors may approach risk management from different perspectives and emphasise technological innovations. Thus, according to X. Liu (2022), Z. Ali *et al.* (2019) and others, in the process of managing the risks of agricultural enterprises' production activities, informal rules of conduct of economic agents interacting with agricultural institutions emerge. This is not just a part, but the most important component of risk-based resource management in the agricultural production system, which depends on a formalised description of innovative tools for risk-based management of agricultural enterprise development. While formal rules for managing the risks of agricultural enterprises' production activities change rapidly, informal rules tend to change gradually, but they set the vector for neutralising threats in production activities for innovative regulatory instruments (Horbal & Makarova, 2023). Informal rules and norms of risk management of agricultural enterprises are not created by the authorities, they often develop spontaneously and generate misuse of resources in the production system of the actors.

Scholars also identify different key risks for agricultural enterprises. Some may emphasise environmental risks, while others may focus on financial aspects. In this regard, X. Huang *et al.* (2021) argue that the imperfect institutional vector of agricultural institutions does not allow to fully neutralise risks in the production, social, human, financial and resource capital of agricultural enterprises, which is also highlighted in the present study. Accordingly, a new conceptual approach to the development of innovative tools for risk-oriented management of production activities of agricultural enterprises is needed to intensify the processes of their safe development in the institutional environment. On the one hand, it is necessary to constantly support the development of relevant agricultural institutions in the required direction (including their formation, development and training), and on the other hand, to stimulate this process by persuading agricultural producers to move forward and look for new standards of risk management of production activities, their systematisation in the organisational structure of risk management (Sinaj & Vela, 2022).

According to many authors, including B.M. Leybert *et al.* (2023) and O. Kotykova *et al.* (2020), social justice and trust institutions play a special role, being the main

agro-industrial complex coordinators in the institutional environment. They establish conventional norms for all agricultural actors, formed from economic culture, value systems, habits and traditions, and mentality, and influence their behaviour when applying risk-based management in the institutional environment. They include the rules of social justice; mechanisms for ensuring and implementing the rules of social responsibility; and norms of behaviour of agricultural enterprises in economic relations.

M. Van Bergen *et al.* (2019), N.V. Trusova *et al.* (2019) and others, define that the norms of informal relations of agricultural enterprises in the institutional environment are based on economic relations and have a long period of development and are based on previous intergenerational relations in rural areas. They depend on the effective functioning of formal institutions (state, property, democracy, human rights, rule of law) and informal institutions (culture, religion, respect, traditions, customs, moral attitudes). In agriculture, these institutions are distorted, and the lack of stakeholder confidence in reducing transaction costs in the development of innovative tools for managing the risks of agricultural production leads to negative consequences and development risks, as demonstrated in the study. A key characteristic of the safe development of agricultural enterprises is the availability, possibility, stability and use of the production system, which largely depends not only on the growth of agricultural production but also on trade policy and the development of trade relations in agricultural raw materials markets, which can enhance these characteristics to a positive level (Penkova & Kharenko, 2023).

Another confirmation of the study is the results of R. Sharma *et al.* (2020), X. Li and Y. Sun (2022) as authors emphasise that the problem of introducing innovative tools for managing the risks of agricultural enterprises' production activities and ensuring the state's food security on their basis is complex and complex, and therefore its solution should be considered from the point of view of different hierarchical levels. The basis for the allocation and classification of such levels can be considered the specific interests of a separate group of subjects of each particular level. J. Gascón and K. Mamani (2022) and R. Finger (2023) also note that the national level of implementation of innovative tools for managing the risks of agricultural production activities and ensuring the food security of the state on their basis is achieved through government programmes that create a sustainable potential of the agricultural production system and determine the directions for improving the quality of grain and oilseed products.

A similar opinion is expressed by D.K. Deng-Kui Sii *et al.* (2021), according to which the specifics of managing production risks with the help of innovative tools at the national level are determined by the differences in economic and political interests of countries.

Thus, countries with the most developed agricultural production have natural advantages in agricultural production. However, F. Ciccullo *et al.* (2019) believe that countries with less favourable natural conditions for agricultural production are limited by agricultural production resources or emerging markets. The concept of innovative development of agricultural enterprises at the national level does not mean self-sufficiency in food. It implies that a country should produce enough food for its needs, but if comparative advantages prevent this, innovative tools for strengthening the food supply should be introduced.

Balancing the quantitative and qualitative parameters of agricultural production, and defining the criteria for neutralising risks at the local level allows for ensuring food security of the territories where the grain and oilseeds group entities are located. Consumer demand growth due to the intensification of production cycles leads to an increase in the potential of the agricultural production system, which, in turn, stimulates the introduction of innovations in the supply chain of grain and oil products through Blockchain technologies. It is worth noting that technological innovations in the agricultural production system ensure the reproduction process of territories and support the food security of the region (country) as a whole (Salo *et al.*, 2023). This approach expands the range of products of the grain and oilseed group following the scale of their consumption with differentiation of pricing policy and within the regional market, stimulating international trade and export of agricultural products. This is confirmed by W. Bai *et al.* (2022) and A. Burliai *et al.* (2021), who emphasise that the highest guarantee of food security of the state, with the sustainable introduction of innovations in the production system, requires spot prices for agricultural products, which in the logistics chain of planning and forecasting transport services for grain and oilseed products ensure the availability of long-term agreements for the supply of agricultural raw materials to countries of the world on mutually beneficial terms.

Thus, the discussion on the research of various authors in the field of innovative tools for managing the risks of production activities of agricultural enterprises in the institutional environment and the results of the study confirms that the study of innovative tools for managing the risks of production activities of agricultural enterprises and their impact on development security indicates the importance of both formal and informal institutions in agriculture. A key characteristic of the safe development of agricultural enterprises is the existence of a sustainable and efficient production system, which depends on a comprehensive approach to risk management. Technological innovations, such as the introduction of Blockchain and other advanced technologies in supply chains, play a key role in maintaining food security by stimulating international trade and securing long-term agreements for the supply of

agricultural raw materials. In summary, innovative approaches to risk management are a necessary element for achieving sustainable and secure agricultural development in an institutional environment.

CONCLUSIONS

Sustainable development of agricultural enterprises in the institutional environment is focused on ensuring agro-economic capabilities without resorting to food imports from other countries. Achieving a stable level of sustainable development of agricultural enterprises should involve the implementation of the following key areas: maintaining the food supply at a level sufficient for a healthy diet; ensuring an adequate level of effective demand of the population; eliminating dependence on imports and protecting the interests of domestic producers of agricultural raw materials.

From the perspective of the sectoral approach, Blockchain technologies as innovative tools for managing the risks of production activities in cities where agricultural enterprises are located and located at short distances from logistics centres should be fully involved in the model of safe operations. Development of agricultural production entities to expand the segment of agricultural raw materials on the world market. The introduction of innovative tools for managing production risks should be transferred to a new doctrine of innovation of production and technological standards of regional development. This will facilitate the state's influence on the reproduction of an integral agricultural production system, creating the sustainability of the production system's potential in the country, ensuring the greening of agricultural raw materials and shaping the new behaviour of agricultural enterprises to ensure the security of their production system. This approach promotes cooperation with other stakeholders, strengthening interaction in the field of agricultural policy and ensuring national security in the production of agricultural raw materials.

The study results indicate a high level of security for the development of agricultural enterprises in Ukraine, which was assessed using integral indices. These indices consist of various components that reflect the level of costs, financial stability, productivity, logistics and innovation in the agricultural sector. Based on the calculations, the study found that the agro-climatic zones of the Forest-Steppe, Polissia and Western zones have the highest level of security in agriculture. Despite the difficult conditions of the military conflict in Ukraine, these regions have shown resilience and adaptability through the successful implementation of innovations such as blockchain technologies and financial instruments. The practical implications of the findings are that successful strategies for sustainable agricultural development include maintaining the food supply, creating an adequate level of effective demand, and eliminating dependence on imports. It is also important to use innovative tools, such as blockchain technologies,

which provide risk management and increase the resilience of production systems. Further studies should detail specific measures to implement innovations, as well as study the impact of such strategies on the socio-economic development of regions.

None.

None.

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CONFLICT OF INTEREST

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Інноваційні інструменти управління ризиками виробничої діяльності сільськогосподарських підприємств в інституційному середовищі

Богдан Валерійович Хахула

Кандидат економічних наук, доцент

Білоцерківський національний аграрний університет

09117, Соборна площа, 8/1, м. Біла Церква, Україна

<https://orcid.org/0000-0003-4286-2381>

Оксана Миколаївна Костюк

Аспірант

Білоцерківський національний аграрний університет

09117, Соборна площа, 8/1, м. Біла Церква, Україна

<https://orcid.org/0000-0003-4924-5140>

Олексій Васильович Ланченко

Аспірант

Білоцерківський національний аграрний університет

09117, Соборна площа, 8/1, м. Біла Церква, Україна

<https://orcid.org/0000-0003-1476-7133>

Ганна Олександрівна Антонюк

Аспірант

Білоцерківський національний аграрний університет

09117, Соборна площа, 8/1, м. Біла Церква, Україна

<http://orcid.org/0000-0003-3704-7008>

Олександр Вікторович Гомон

Аспірант

Білоцерківський національний аграрний університет

09117, Соборна площа, 8/1, м. Біла Церква, Україна

<https://orcid.org/0000-0003-3773-7391>

Анотація. Безпека розвитку аграрного сектору стає все більш актуальним питанням в сучасних умовах, оскільки аграрний сектор визначає стійкість економіки та забезпечує продовольчу безпеку країни. Зростаючі ризики, такі як зміна клімату, економічні труднощі та геополітичні конфлікти, підкреслюють необхідність підвищення безпеки систем сільськогосподарського виробництва, що обумовлює актуальність дослідження. Метою дослідження було проведення системного аналізу інноваційних інструментів управління ризиками виробничої діяльності сільськогосподарських підприємств та їх впливу на формування безпечного розвитку в аграрному секторі України. Для досягнення поставленої мети використано метод аналізу та моделювання. Результати дослідження свідчать, що інноваційні підходи до управління ризиками мають вирішальний вплив на стійкість та ефективність систем сільськогосподарського виробництва. Для досягнення стабільного рівня безпечного розвитку сільськогосподарських підприємств необхідно зосередитися на реалізації ключових напрямів, таких як підтримання продовольчої бази на рівні, достатньому для забезпечення здорового харчування, забезпечення належного рівня платоспроможного попиту населення та усунення залежності від імпорту, спрямованих на захист інтересів вітчизняних виробників сільськогосподарської сировини. Дослідження показало, що інституційні чинники управління ризиками формують інтегровану систему, ефективність якої залежить від реалізації конкретних стратегій розвитку аграрних інституцій, спрямованих на впровадження інноваційних інструментів у виробничий цикл сільськогосподарських підприємств. Крім того, завдяки накопиченню значних обсягів виробничого, фінансового, матеріально-технічного та інноваційного потенціалу сільськогосподарських підприємств Лісостепу та Полісся, рівень безпечного розвитку сільськогосподарських підприємств значно підвищується. Це свідчить про їх високу спроможність до сталого відтворення виробництва та здійснення вагомго внеску в аграрний сектор України. Дослідження має практичне значення для сільськогосподарських підприємств, науково-дослідних установ та державних органів, які можуть використовувати отримані результати для вдосконалення стратегій управління ризиками та підвищення рівня безпеки розвитку аграрного сектору

Ключові слова: ризики виробничої діяльності; ефективність бізнесу; фінансова стійкість; технологічний розвиток; виробнича система; інститути сільського господарства



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Status and prospects of beef and veal production in Ukraine in the context of international economic integration

Oleksandr Nechyporenko

Doctor of Economic Sciences, Associate Professor
National Scientific Centre "Institute of Agrarian Economics"
03127, 10 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0002-1080-5633>

Nadiia Kryvenko*

PhD in Economic Sciences, Senior Researcher
National Scientific Centre "Institute of Agrarian Economics"
03127, 10 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0003-3439-432X>

Dmytro Liudvenko

Doctor of Economic Sciences, Senior Researcher
National Scientific Centre "Institute of Agrarian Economics"
03127, 10 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0002-7908-5718>

Victoria Rud

PhD in Economic Sciences, Senior Researcher
The Institute of Vegetable and Melon Growing of the National Academy of Agrarian Sciences of Ukraine
62478, 1 Instytutaska Str., Selection Village, Ukraine
<https://orcid.org/0000-0002-2464-0200>

Yurii Nosenko

PhD in Agricultural Sciences
Presidium of the National Academy of Agrarian Sciences of Ukraine
01010, 9 Mykhailo Omelyanovych-Pavlenko Str., Kyiv, Ukraine
<https://orcid.org/0000-0002-6648-3418>

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Abstract. Beef production is driven by the need to ensure the country's food security, meet the processing industry's demand for raw materials, and increase state budget revenues from exports. The purpose of this study was to highlight the status and trends of production in the world and Ukraine, to identify issues and find areas of development considering international economic integration. The methods employed were analysis, synthesis, generalisation, specification, mathematical, and graphical.

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*Corresponding author

The study identified the upward trend in global beef and veal production, the main producing countries and the predominant stability of their share in global volumes over the decades. It was found that higher beef production does not always allow for higher per capita volumes (e.g., in China and India). Ukraine's production and its share in global volumes are declining, although production exceeds consumption. It was found that the share of agricultural enterprises in the structure of economic entities is decreasing, and there is a direct dependence between their number and production volumes, which prevails in the Forest-Steppe zone. The largest producing regions (Kyiv, Vinnytsia, Kharkiv, Lviv) were highlighted, where priority development in the post-war period is expected. The study found the principal reasons for the decline in production, including a decrease in livestock numbers, negative profitability, and rising average consumer prices. Based on the SWOT analysis results, development prospects were identified and relevant proposals were developed. The reasons for the decrease in the number of cattle and beef were proposed to be distinguished as general, and those related to enterprises and household farms; their interdependence was emphasised. The study stressed the need for state support for producers by providing food aid to the population free of charge and by purchasing products at lower prices depending on their financial level. International economic integration was singled out as one of the key factors of increasing beef production. The study emphasised the need to consider the consumer preferences of the population of countries with which regional trade agreements have been concluded, specifically regarding the production of kosher and halal products. The findings of this study and the proposals provided can be used to develop government measures to increase beef production and increase its share in world exports

Keywords: production volumes; beef; national policy; regional trade agreements; profitability; food aid

INTRODUCTION

The significance of beef production is driven by the need to provide food for the population, while it is also important for raw materials for processing companies in various industries, state budget revenues, job creation, exports, etc. In Ukraine, this development is facilitated by natural and climatic conditions, production of considerable volumes of crops, including fodder crops, the availability of relevant government programmes, the benefits of regional trade agreements, etc. Considering the importance of meeting the beef consumption needs of the majority of the population of Ukraine and the world, and the fact that cattle breeding and processing produce meat products, as well as such vital products as milk, cheese, leather, gelatin, etc., there is a need to expand research into the economic aspects of its production.

Investigating the state of livestock production in Ukraine, O. Izhboldina *et al.* (2021) note negative trends in the industry, addressing the decrease in livestock numbers, a considerable decrease in livestock productivity and the quality of products. V. Lavruk *et al.* (2021) argue that the development and gradual revival of livestock production will mainly depend on the effective efficiency of the economic mechanism, as well as the interdependence of its principal components, namely, the establishment of price, credit and tax policies, state regulation, investment, and innovation processes. N. Lialina (2018) notes that one of the key trends at the current stage of development of livestock production in Ukraine is a decrease in the concentration of production for most enterprises, which leads to a decrease in the role of the industry in their economy. This causes a noticeable decline in the efficiency of the industry.

According to O. Kravchenko (2019; 2020), the economic interests of an agricultural producer as a

business entity are not satisfied by its share in the retail price structure, which for beef and milk amounted to 30.5% and 32.8%, respectively (January 2019), which is confirmed by the decrease in production. Furthermore, the issue of food security should be considered, and research on food policy in Ukraine and the world is becoming increasingly relevant (Kuts & Boki, 2020). Studies on foreign trade in meat are essential as well (Kovalenko, 2021), as it also considerably affects its production.

The authors of this study believe in the importance of the key areas proposed by K. Andriushchenko *et al.* (2021), namely, modernisation of the technical and technological base and production processes according to export priorities, research, training, and implementation of the best world practices in product processing and farming, improvement of innovative developments at food enterprises, development of modern strategies for innovative development of agricultural enterprises, etc.

The issue of efficient beef production is vital not only for Ukraine, as evidenced by research by foreign scientists. B. Abebe *et al.* (2022) note the significance of cattle breeding in Ethiopia as the main source of meat-producing animals for internal and external markets. Compared to other African countries, Ethiopia has a massive number of cattle (about 65 million heads), but the quality and quantity of beef consumed per capita is rather low (8.4 kg per year). It is expected that the increase in production will be driven by population growth, high demand in the internal and external markets, etc. While investigating the issue of beef production, P. Greenwood (2021) notes that beef is a high-quality source of protein, and demand for it in the

global market is growing. It is noted that digital and other technologies that allow for the rapid collection and use of data on the environment and cattle productivity should increase the productivity, efficiency, and welfare of animals.

When investigating beef production, researchers also paid attention to the specifics of its quality and consumption. D. Magalhaes *et al.* (2023) studied changes in beef consumption and consumer behaviour trends in Brazil, Spain, and Turkey. The study analysed the impact of economic factors, aspects of trust, health concerns, lifestyle influences on beef consumption, and purchase decision factors. Furthermore, C. Whetton *et al.* (2021) investigated meat consumption in a number of countries, considering gross domestic product. One of the crucial factors that changed consumer behaviour and led to a decline in consumption, mainly among Brazilian and Turkish consumers, was the availability of products. It was presented that lifestyle factors, such as eating out, availability of time for cooking, etc., change consumption patterns and should be carefully considered by the industry, factoring in the cultural differences and consumer needs.

V.-B. Hoa *et al.* (2023) paid particular attention to the product quality factor. They conducted research on the quality, taste, and flavour of meat from Korean cattle of various breeds. It was found that under identical feeding conditions, the breed has a considerable impact on the nutritional quality of beef. The issue of increasing beef production is being raised more often, which requires finding the reasons that hinder it and the factors that will facilitate its development. This determines the relevance of the present study. The purpose of this study was to highlight the status and investigate the trends in beef production in the world and Ukraine, to identify the principal problems and determine the areas of its effective development. It is advisable to determine the prospects for beef production in Ukraine based on the current situation, global trends, and participation in integration processes.

MATERIALS AND METHODS

The study employed the methods of analysis and synthesis to assess the development of beef production in Ukraine and the world, the abstract and logical method to draw conclusions, generalization and concretization to develop proposals, mathematical and graphical methods to investigate and display trends and the state of production. The information base of the study included the scientific papers of Ukrainian and foreign scientists, statistical data of the international organisation

FAO (n.d.) and the State Statistics Service of Ukraine (n.d.). The study focused on global production volumes, i.e., in total and by country, imports, consumption, production by category of farms, considering zones and regions of Ukraine, production profitability, average consumer prices, population, cattle supply to processing enterprises, etc.

To assess the place and prospects of Ukraine in world production and the world market, the FAO period from 1961 to 2021 was used for all countries, and for Ukraine from 1992. Considering the significance and impact of the hostilities in Ukraine, the pre- and post-war periods were used. This study used the main statistical data on beef production in Ukraine and its regions, as well as global and country-specific data for comparison.

To determine beef self-sufficiency, the study employed the approach to food self-sufficiency proposed by B. Paskhaver (2018). The study analysed the trends and status of beef production in the world and Ukraine, considering the largest producers, natural and climatic conditions, and population. Calculations were made on the supply of live animals to processing enterprises, considering military operations. The study examined the production of beef and veal depending on the structure of producers, specific regional features, factoring in the profitability of production, the dynamics of average consumer prices, population, including migration processes. The prospects of post-war recovery are identified as a result of the analysis of production in recent years. A SWOT-analysis of the prospects for beef production in Ukraine was carried out, the reasons for the decline in cattle numbers were analysed and classified, and recommendations for the development of production were developed.

RESULTS AND DISCUSSION

The significance of agri-food production is becoming increasingly important, specifically due to population growth and climate change. The second of the UN Sustainable Development Goals is to end hunger, and it calls for less food to be thrown into landfills and more support for farmers, as a third of the world's food is wasted, while 821 million people are malnourished (Sustainable Development Goals, n.d.). Ukraine is one of the world's largest producers of wheat, corn, soybeans, sunflower seeds, and other crops, i.e., it produces mainly crop products. Global production of all types of meat reached 361 million tonnes (slaughter weight equivalent) in 2022, up 1.4% in 2022, although slower than the 4.5% growth in 2021 compared to 2020 (Table 1).

Table 1. Dynamics of the global meat market, million tonnes, 2020, 2021, 2022

Indicators	years			changes 2022 to 2021	
	2020	2021	2022	+, -	in %
Production, total	340.3	355.5	360.5	5	1.4

Table 1. Continued

Indicators	years			changes 2022 to 2021	
	2020	2021	2022	+, -	in %
beef	72.0	72.5	73.2	0.7	1.0
poultry meat	136.0	137.8	138.8	1	0.7
lamb	16.1	16.4	16.6	0.2	1.2
Trade, total	41.7	42.1	42.3	0.2	0.5
beef	11.7	12.1	12.4	0.3	2.5
poultry meat	15.5	15.8	16.2	0.4	2.5
lamb	1.1	1.1	1.16	0.06	5.5
Per capita consumption, world (kg/year)	43.4	44.9	45.1	0.2	0.4
Trade – share of production (%)	12.3	11.8	11.7	-0.1	-0.8

Source: calculated based on FAO (2022)

The expansion was mainly driven by the rapid growth in meat production in China and strong growth in Brazil, Australia, and Vietnam. At the same time, the relative static nature of global production was partially offset by a drop in production in the EU, the US, Canada, Iran, and Argentina. Total meat production in China increased to 96 million tonnes, up 4.4% year-on-year. Global trade in meat and meat products reached 42 million tonnes (in slaughter weight equivalent).

At the same time, global beef production (fresh or frozen) increased by 2.6 times from 1961 to 2022, from 27.7 to 73.2 million tonnes, and the growth was quite steady. Therewith, the share of beef in global

meat production in 2022 was 20.1%, although in some years it was much higher, e.g., in 1961 it was 38.8% (in 2000 it was 24.3%). Notably, poultry meat was produced the most, with a share of 34%. Major beef producers include countries in North and South America, Asia, and Australia. The top 10 producers accounted for 63.1% of global production in 2022, and the share of each country did not fall below 2%, unlike other countries. More than a third of beef is produced in the US and Brazil, with a share of 17.7% and 13.2% respectively. It is advisable to consider the change in the share of production of the main producing countries in world volumes (Table 2).

Table 2. Share of the largest beef producers in the world, 1961, 2000, 2010, 2022

1961			2000			2010			2022		
Countries	%		Countries	%		Countries	%		Countries	%	
1 USA	26.8	1	USA	21.2	1	USA	18.2	1	USA	17.7	
2 USSR	10.3	2	Brazil	11.6	2	Brazil	14.0	2	Brazil	13.2	
3 Argentina	7.7	3	China	8.2	3	China	8.7	3	China	9.7	
4 France	5.2	4	Argentina	4.8	4	India	4.8	4	India	4.1	
5 Brazil	4.9	5	Australia	3.5	5	Argentina	4.1	5	Argentina	3.3	
6 Germany	4.7	6	Russia	3.4	6	Australia	3.3	6	Mexico	3.2	
7 United Kingdom	3.3	7	France	2.7	7	Mexico	2.7	7	Australia	3.9	
8 Italy	2.4	8	India	2.7	8	Russia	2.7	8	Russia	2.7	
9 Canada	2.4	9	Mexico	2.5	9	France	2.4	9	Turkey	2.3	
10 Australia	2.3	10	Germany	2.3	10	Canada	1.9	10	France	2.2	
11 India	1.8	11	Canada	2.2	11	Germany	1.9	11	Canada	2.1	
12 South Africa	1.4	12	Italy	2.0	12	Italy	1.6	12	Pakistan	2.0	
13 Mexico	1.3	13	Ukraine	1.3	13	United Kingdom	1.4	13	Germany	1.7	
14 Poland	1.2	14	Colombia	1.3	14	South Africa	1.4	14	South Africa	1.5	
15 Colombia	1.2	15	United Kingdom	1.2	15	Colombia	1.2	15	Uzbekistan	1.6	
16 Uruguay	1.0	16	Spain	1.1	16	Pakistan	1.1	16	United Kingdom	1.4	
17 New Zealand	0.9	17	Zimbabwe	1.1	17	Uzbekistan	1.0	17	Zimbabwe	1.2	
18 Netherlands	0.8	18	New Zealand	1.0	18	Zimbabwe	1.0	18	Colombia	1.2	
19 Ethiopia	0.8	19	South Africa	1.0	19	Turkey	1.0	19	New Zealand	1.1	
20 Belgium-Luxembourg	0.8	20	Ireland	1.0	20	New Zealand	1.0	20	Italy	1.0	

Source: calculated based on FAO (2022)

Table 1 shows that the US share in world production was the highest in 2022, as well as in 1961, 2000 and 2010, although it has been declining. In 1961, the USSR held the second position (10.3%), but later, among its republics, only Russia entered the top ten largest producers, while Ukraine (from 2020) and Uzbekistan (2010 and 2021) entered the top twenty. Thus, the largest producers have largely stayed so for decades, including the United States, Russia, Brazil, and Argentina, although their positions have shifted somewhat, for instance, with Brazil's growing and Argentina's declining. The shares of Canada, the UK, Germany, and Italy decreased, but they stayed among the top 20 producers. China, India, and Mexico have seen noticeable increases in beef production, and although the latter two countries were among the 15 largest producers in 1961, the opposite is true of China, which has been among the top ten in the following years. This suggests that countries can considerably increase their exports, although the top positions in the global market have been held by the main supplier countries for decades. This, admittedly, also applies to Ukraine, but special attention should be paid to product quality.

Among the largest beef producers are countries with which Ukraine has regional trade agreements, including Turkey, France, Germany, Italy, the UK, and Canada. Considering the benefits of international economic integration, including liberalisation of foreign trade, access to innovative technologies, and foreign direct investment, it is expected that production and trade in these products will increase. Among the EU countries, France and Germany produce the most beef, but their share in global volumes in 2022 was 2.2% and 1.7%, respectively, and 9.3% in the EU-27 as a whole. However, it is also decreasing for them, specifically, in 1961 and 2000, for the countries that became members of the grouping, it was 19.4% and 13.6%, respectively. Italy, Spain, and Poland are also among the largest producers in the association, but each of them has a share of less than 1%.

In 2022, the EU produced 6.7 million tonnes of beef, down 1.1% from 2021. At the same time, with a decline in production in 2022, the EU increased exports of fresh and frozen beef by 1.4% compared to 2021, to 463 thous. t. Around 50% of this beef comes from the UK, with volumes largely unchanged from 2021. Deliveries grew to Asian markets, including Hong Kong and Japan, as well as to North American markets such as Canada and the United States. This and growth in other markets were enough to outweigh losses in other export destinations, especially Algeria.

On the other hand, imports dropped by 21% year-on-year to 236.4 thous t. Volumes declined from all key suppliers, but primarily from the UK and Brazil. Figure 1 shows the main beef producing countries, which accounted for 85% of total EU beef production. This is likely to be caused by the pandemic or a disruption in

demand for food services in the EU. Beef production in the EU in the first half of 2023 was 4.5% lower than in the same period a year ago, with cattle slaughter in key producing countries limited. The largest drop in production in the EU was observed in Italy (-23%), followed by key producers France, Spain, Ireland, and Poland. The only major producers to see growth were Germany and the Netherlands. However, in Germany, this growth is contrary to the overall long-term trend of declining production. Overall, the total slaughter of adult cattle (bulls, steers, heifers, and cows) was 8.1 million heads, down 3.6% from the same period a year ago. Therewith, the total slaughter of cows was just under 3 million head, down 3.7% year-on-year.

In several countries, including France, Poland, and Spain, there has been a marked decline in cow slaughter rates. Cow production in France is currently at its lowest level in at least five years, which is likely to contribute to the continued high French prices on the EU market. Livestock slaughter in Poland is also historically low. Conversely, cow slaughter in Spain has increased in recent years and, although lower than last year's record level, stays historically high in 2023. Cow slaughter in Germany stayed relatively stable year-on-year after several years of decline.

Meanwhile, cow slaughter in the Netherlands has increased considerably compared to last year (+13%) and will stay at the same level as in 2021. From a market balance standpoint, considering the reduction in production and trade, it is shown that stocks available for consumption are lower across the bloc. Price inflation is affecting beef consumption in the EU, as it does in the UK, with consumption and retail data from key EU countries pointing to a fall in demand. Domestic consumption of beef in France fell by 2.5% in the first half of 2023, while average prices increased by 9.1%. Purchases of beef by households in Germany from January to July (inclusive) decreased by 6.2% year-on-year, while the average price increased by 6.9%, and the level of meat consumption is declining. Purchases of Italian beef fell by 4% year-on-year in the first half of 2023, to a level that is also lower than in the previous two years. Beef consumption in Spain is also declining.

Cattle prices in the EU have generally been on the decline since March. However, in recent weeks, cattle prices in a range of countries have shown an upward trend. Prices for young bulls and cows in Ireland, France, Germany, and Poland increased, while the price of young bulls also rose in Spain. In other countries, cattle prices continue to be lower, e.g., in the Netherlands and Italy. Ukraine's share of global production has declined significantly, from a high of 3.1% in 1992 to a low of 0.4% in 2022 and has not even reached 1% since 2005. Ukraine ranked 39th among beef producers in the world in 2022, behind even African countries such as Nigeria, Sudan, Ethiopia, Chad, and Zimbabwe, which are not the largest producers of grain and, accordingly,

feed. Notably, Ukraine's beef production decreased by 5.3 times between 1992 and 2022, from 1.7 to 0.3 million tonnes, while global production increased by 1.4 times, from 53.3 to 72.4 million tonnes (Fig. 1).

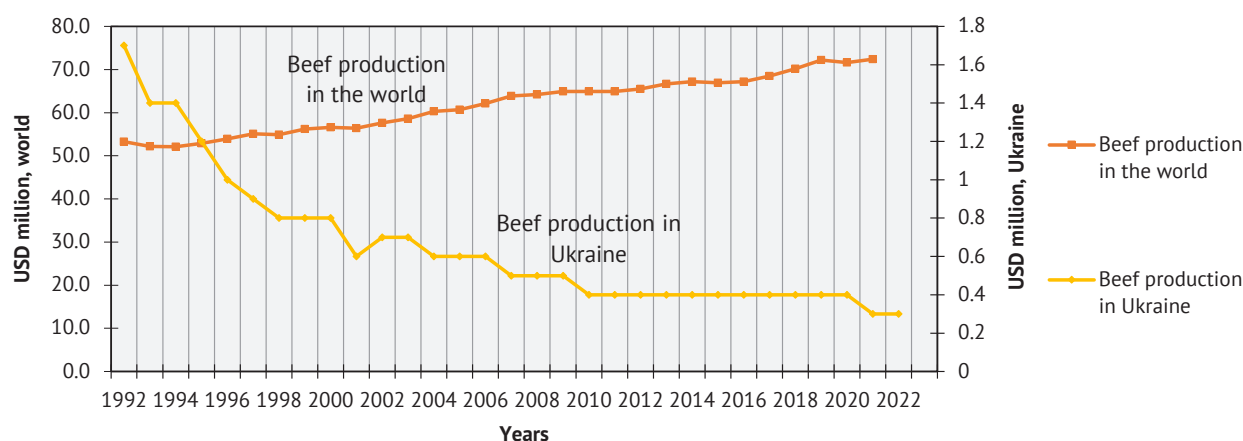


Figure 1. Beef production in the world and Ukraine, 1992-2022

Source: calculated based on FAO (2022)

The figure suggests that the trend of beef production in the world and Ukraine is markedly different, but the presence of demand in the Ukrainian and global markets and consumption by the majority of the population should stimulate an increase in beef production in Ukraine. In 2022, the Ukrainian meat market increased beef imports to 2.8 thousand tonnes, up 34.2% from 2021. In 2022, Ukraine supplied 22.4 thous. t of frozen cattle meat worth USD 78.5 million. The main deliveries were made to China – 4.9 thous. t for USD 21.9 million (28%); Azerbaijan – 4.6 thous. t for USD 17.6 million (22.5%); Kazakhstan – 3.2 thous. t for USD 11.2 million (14.9%). Specifically, Ukraine exported 5.7 thous. t of fresh or chilled cattle meat worth USD 5.3 million. Ukraine was supplied with 1.9 thous. t of frozen meat (70% of total supplies).

The world's largest beef producers are the US, Brazil, China, and the EU. In 2022, they produced 50.1% of the world's beef. Brazil, the US, India, and Australia were the largest exporters of beef, accounting for 54.2% of all beef exports. The average level of beef consumption in 2022 was 9.2 kg per person. Argentina consumed 5.1 times more beef per person than the global average, while the US and Brazil consumed 4.3 times more. In India, this figure is 8.7 times lower, while in China it is almost 30% lower. The key exporters of beef to Ukraine were Brazil – 2327 thous. t, the USA – 1637 thous. t; India – 1336 thous. t, Australia – 1245 thous. t, and EU countries – a total of 901 thous. t (Table 3).

It is essential to know how much beef the main producing countries produce per capita (Table 4).

Table 3. Beef production, consumption and exports in 2022

Main producing countries	Production		Consumption per 1 person per year, kg	Main exporting countries	Export	
	thous. t	%			thous. t	%
USA	12961.4	17.7	37.7	Brazil	2327	19.3
Brazil	9666.1	13.2	37.1	USA	1637	13.5
China	7103.1	9.7	6.3	India	1336	11.1
EU	6956.7	9.5	14.1	Australia	1245	10.3
Argentina	2416.5	3.3	48.9	EU total	901	7.5
India	732.3	4.1	1.0	Argentina	750	6.2
Pakistan	1464.6	2.0	9.0	New Zealand	651	5.4
Mexico	2343.3	3.2	14.9	Canada	589	4.9
Australia	2855.9	3.9	26.1	Uruguay	528	4.4
Russia	1977.2	2.7	13.2	Mexico	368	3.0
Other countries	23140.0	31.6	X	Other countries	1751	14.5
Total	100.0	100	9.0	Total	12083	100

Source: calculated based on FAO (2022)

Table 4. Comparison of the shares of beef production in global volumes and per capita in the main beef producing countries, 2022

Countries	Share in global production, %	Produced per capita, kg
USA	17.7	37.6
Brazil	13.2	44.5
China	9.7	4.8
India	4.1	3.1
Argentina	3.3	65.8
Mexico	3.2	16.5
Australia	3.9	74.4
Russia	2.7	10.2
Turkey	2.3	17.1
France	2.2	22.0
Canada	1.9	36.5
Pakistan	2.0	5.1
Germany	1.7	13.1
South Africa	1.5	17.5
Uzbekistan	1.6	29.2
United Kingdom	1.4	13.1
Zimbabwe	1.2	50.4
Colombia	1.2	14.7
New Zealand	1.1	145.8
Italy	1.0	12.3

Source: calculated based on FAO (2022)

Notably, the highest beef production per capita among the top 20 producers is observed in New Zealand, which ranked only 19th in terms of total volumes, as well as Australia, Argentina, even Zimbabwe, Brazil, and the United States. However, such prominent producers as China and India, which occupy the third and fourth positions, produce the least per capita of the countries represented – only 4.9 kg and 3.0 kg, respectively, and slightly more Pakistan – 5.3 kg. This is the basis for researching demand in this market and increasing

exports. For Ukraine, the situation is still better, with 7.1 kg of beef produced per capita.

In the context of declining global food security, a special place is occupied by Ukraine, where agriculture is currently the leading sector of the economy. Active Russian hostilities have had a direct impact on global food and agricultural markets. Despite a steady decline in the industry's performance, Ukraine stays an active player in the global beef and veal production market (Table 5).

Table 5. Supply of live cattle to processing enterprises in the pre-war and post-war years (2021 and 2022)

	Farms of all categories			Enterprises			Household farms		
	2022	2021	2022 in % to 2021	2022	2021	2022 in % to 2021	2022	2021	2022 in % to 2021
Live weight of farm animals, thous. t	2954.8	3329.8	88.7	2182.4	2359.3	92.5	772.4	970.5	79.6
cattle	388.4	475.1	81.8	127.4	134.7	94.6	261.0	340.4	76.7
market share, %	13.1	14.3	92.1	5.8	5.7	102.2	33.8	35.1	96.3
Live weight of farm animals sold for slaughter, thous. t	3059.1	3391.2	90.2	2166.0	2318.5	93.4	893.1	1072.7	83.3
cattle	455.1	526.8	86.4	135.2	131.9	102.5	319.9	394.9	81.0
market share, %	14.9	15.5	95.8	6.2	5.7	109.7	35.8	36.8	97.3
Average live weight of cattle sold for slaughter, kg	284	293	96.9	377	380	99.2	257	273	94.1
Slaughter weight of farm animals sold for slaughter, thous. t	2206.7	2438.3	90.5	1608.1	1720.4	93.5	598.6	717.9	83.4

Table 5. Continued

	Farms of all categories			Enterprises			Household farms		
	2022	2021	2022 in % to 2021	2022	2021	2022 in % to 2021	2022	2021	2022 in % to 2021
beef and veal	268.4	310.5	86.4	80.7	77.9	103.6	187.7	232.6	80.7
market share, %	14.9	15.5	95.8	6.2	5.7	109.7	35.8	36.8	97.3

Source: calculated according to data from the State Statistics Service of Ukraine (n.d.)

The calculations suggest that the downward trend continues: the total supply of live animals to processing enterprises in the post-war year was 7.9% lower, while the supply to enterprises increased by 2.2% and to household farms decreased by 3.7%. The catastrophic drop in the purchase of animals from the population is linked to the active military operations, which suggests that people are abandoning animals to cover their own

beef needs. Positive dynamics is observed only in calves under 1 year of age by 6.4% (by 1.8% in enterprises); bulls over 2 years of age by 12.5% in enterprises and heifers over 2 years of age in the population by 23.4%. An interesting fact is the positive dynamics of changes in the average live weight of one head of cattle purchased by processing enterprises in the pre-war and post-war years (Table 6).

Table 6. Dynamics of changes in the average live weight of one head of cattle purchased by processing enterprises in the pre-war and post-war years

	Purchased – total				incl. in							
	heads	average weight per head, kg			heads	enterprises			heads	population		
		2021	2022	2022 to 2021, %		2021	2022	2022 to 2021, %		2021	2022	2022 to 2021, %
Cattle	135568	469	470	100.2	120201	470	476	101.3	15367	464	438	94.4
of which:												
cows	81058	512	507	99.0	73121	514	526	102.3	7937	490	428	87.3
calves up to 1 year of age	6206	102	150	147.1	5810	94	142	151.1	396	216	203	94.0
bulls aged 1-2 years	27217	442	425	96.2	22583	436	415	95.2	4634	469	495	105.5
bulls older than 2 years	10037	453	451	99.6	9361	456	451	98.9	676	416	452	108.7
heifers aged 1-2 years	5621	440	457	103.9	5312	444	453	102.0	309	373	524	140.5
heifers older than 2 years	5429	455	465	102.2	4014	469	475	101.3	1415	414	414	100.0

Source: calculated according to data from the State Statistics Service of Ukraine (n.d.)

Studies have shown that the average live weight of one head of cattle purchased by processing companies increased by 0.2% in the post-war year. This is especially true for calves under 1 year of age by 47.1%. An increase is also observed in heifers aged 1-2 years and heifers over 2 years old (by 3.9% and 2.2%, respectively). In enterprises, there was a 1.3% increase in cows, a 2.3% increase in calves under 1 year old, a 51.1% increase in calves under 1 year old, and a 2.0% and 1.3% increase

in heifers over 2 years old, respectively. In household farms, the study observed an increase of 5.5% in bulls aged 1-2 years, 8.7% in bulls over 2 years, and 40.5% in heifers aged over 2 years. The positive dynamics in beef and veal production amid the hostilities gives hope for an accelerated post-war recovery in the livestock sector and the livestock industry as a whole. It is also advisable to determine the level of self-sufficiency of Ukraine in beef and veal (Table 7).

Table 7. Level of self-sufficiency in beef and veal in Ukraine

	Years							
	2016	2017	2018	2019	2020	2021	2022	2023*
Production, thous. t	376	364	359	370	345	311	268	247
Imports, thous. t	16	13	14	14	18	17	12	11
Consumption fund, thous. t	346	318	318	324	337	299	264	235

Table 7. Continued

	Years							
	2016	2017	2018	2019	2020	2021	2022	2023*
Per 1 person, kg	8.1	7.5	7.5	7.7	8.1	7.3	7.7	6.5
Consumption to production, %	92.0	87.4	88.6	87.6	97.7	96.1	98.4	95.1
Imports to consumption, %	4.6	4.1	4.4	4.3	5.3	5.7	4.5	4.6

Note: *For 11 months of 2023

Source: calculated according to data from the State Statistics Service of Ukraine (n.d.)

The above data shows that in 2016-2023, production of beef and veal exceeded consumption at all times. There was a 2.5% decrease in initial stocks and

a 16.0% decrease in beef production in 2021 compared to 2019. The war years only intensified the downward trend (Table 8).

Table 8. Annual balance of beef meat in Ukraine in 2019-2023, thous. t

Indicators	2019	2020	2021	2022	2023*
Initial stocks	41	42	40	39	38
Import	14	18	17	12	11
Production	370	345	311	268	247
incl. agricultural enterprises	102	85	78	81	80
household farms	268	261	233	188	167
General offer	383	365	329	281	259
Export	58	27	29	16	23
Consumption	324	337	299	265	235
Losses	1	1	1	1	1
Ending stocks	42	40	39	38	37
Total demand	383	365	329	281	259
Consumption per capita	7.7	8.1	7.3	7.7	6.5
Consumption to production, %	87.6	97.7	96.1	98.4	95.1

Source: calculated according to data from the State Statistics Service of Ukraine (n.d.)

Imports of these products in the pre-war period (from 2019 to 2021) increased by 21.4%, while exports dropped by half. In 2022, the decline in these indicators will only intensify. Beef meat consumption per capita also decreased by 0.8 kg (13.11%) in 2021 compared to 2016, and in 2023 the figure became critical – 6.5 kg. The above data shows that in 2016-2023, production of beef and veal consistently exceeded consumption. Notably, beef and veal production in Ukraine in 2021 decreased by 2.3 times compared to 1961, and by 6.4 times since 1990, with a 7.2 times and 23.2 times increase in enterprises, respectively, but a 1.5 times and 1.3 times increase in household farms, but then decreased in comparison to 2000, 2010, 2015, 2019-2021.

Active hostilities have also substantially reduced this figure, almost by half.

Although livestock keeping in household farms appears to be less costly, and its decline was smaller than in enterprises, it is worth considering the processes of migration, urbanisation, and the keeping of small numbers of livestock by household farms. However, under difficult conditions, livestock preservation by household farms is more likely than by large-scale enterprises, and therefore the activities of all categories of farms are significant. It is interesting to observe the production of beef and veal in the pre-war and post-war periods, in the context of further post-war recovery and Ukraine's place in the world market (Table 9).

Table 9. Livestock production (all categories of farms)

	Year				average for 2018- 2021	average growth rate	2022	2022 in % to:	
	2018	2019	2020	2021				2021	average for 2018-2021
Livestock (end of year), thous. heads									
Cattle in total	3333	3092	2874	2644	2986	0.926	2312	87.4	77.4
including:									
cows	1919	1789	1673	1544	1731	0.930	1341	86.9	77.5

Table 1. Continued

	Year				average for 2018- 2021	average growth rate	2022	2022 in % to:	
	2018	2019	2020	2021				2021	average for 2018-2021
Cattle in fattening	1414	1304	1201	1100	1255	0.920	971	88.2	77.4
Production, thous. t									
Meat – total (slaughter weight)	2354.9	2492	2478	2438	2441	1.012	2169	88.9	88.8
including: beef and veal	358.9	370	345	310.5	346	0.953	242	78.1	70.0

Source: calculated according to data from the State Statistics Service of Ukraine (n.d.)

The overall growth rate of meat production (in total) in the pre-war period was 1.2%, which is clearly insufficient for both post-war recovery and the livestock sector's recovery from the protracted crisis. Today, overcoming current challenges and risks is becoming an arduous task for Ukraine. Beef production is largely dependent on the support of European and global partners, and

it will also determine our country's place in the global market. Notably, the structure of beef and veal producers has changed considerably (Table 10), with the share of enterprises ranging from 91.1% (1990) to 30.1% (in 2022), while it increased for farm enterprises – from a minimum of 0.8% (1990) to a maximum of 2.3% (2022), and household farms (69.9% in 2022).

Table 10. Beef and veal production by category of farms, %

Farm category	Years										
	1960	1970	1980	1990	2000	2010	2015	2019	2020	2021	2022
Enterprises	78.3	85.1	85.4	91.1	40.5	24.5	24.4	27.5	24.6	25.1	30.1
incl. Farm enterprises	-	-	-	-	0.8	1.4	2.1	2.3	2.2	2.3	2.3
Household farms	21.7	14.9	14.6	8.9	59.5	75.5	75.6	72.5	75.4	74.9	69.9

Source: calculated according to data from the State Statistics Service of Ukraine (n.d.)

However, the authors of this study do not consider this to be a basis for positive conclusions, as output has

been declining, with a direct dependence on the share of enterprises in production (Fig. 2).

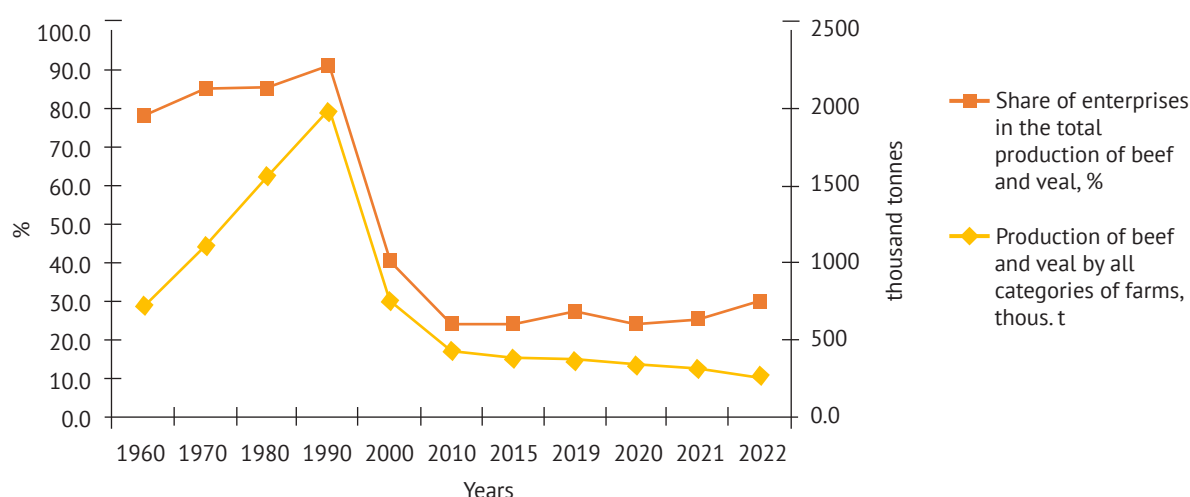


Figure 2. Beef and veal production and share of enterprises in total production, 1960-2022

Source: calculated according to data from the State Statistics Service of Ukraine (n.d.)

Beef production by enterprises is more efficient due to greater opportunities to apply modern technologies and technical equipment, attracting highly qualified personnel, etc. At the regional level, beef and veal production in Ukraine is mainly concentrated in

the Forest-Steppe zone (Table 11), accounting for more than 43% of the total in 2019-2022. In 2010, 2015, and 2022, the lowest production was in the Steppe, but the value did not fall below 22.1%, and in 1990 and 2000 – in Polissia.

Table 11. Zonal distribution and main beef and veal producing regions in Ukraine, %

Years	Region			Region's largest producers
	Steppe, %	Forest-Steppe, %	Polissia, %	
1990	30.1	41.1	25.4	Kyiv (130.2 thous. t), Vinnytsia (118.0 thous. t), Chernihiv (110.2 thous. t)
2000	29.2	40.4	27.1	Khmelnyskyi (43.6 thous. t), Kyiv (41.8 thous. t), Chernihiv (37.1 thous. t)
2010	24.9	37.3	32.6	Lviv (33.1 thous. t), Ivano-Frankivsk (26.7 thous. t), Vinnytsia (23.1 thous. t)
2015	25.8	42.1	32.1	Lviv (27.9 thous. t), Ivano-Frankivsk (27.8 thous. t), Kharkiv (23.2 thous. t)
2019	24.8	44.2	31.0	Ivano-Frankivsk (26.3 thous. t), Kharkiv (25.0 thous. t), Lviv (24.9 thous. t)
2020	24.3	43.7	32.0	Ivano-Frankivsk (25.9 thous. t), Kyiv (24.4 thous. t), Lviv (24.4 thous. t)
2021	23.9	43.6	32.5	Ivano-Frankivsk (25.7 thous. t), Kyiv (22.9 thous. t), Lviv (21.6 thous. t)
2022	22.1	43.3	34.6	Ivano-Frankivsk (25.8 thous. t), Kyiv (21.6 thous. t), Lviv (22.4 thous. t)

Source: calculated according to data from the State Statistics Service of Ukraine (n.d.)

The largest producers are Lviv and Ivano-Frankivsk regions, especially in 2010, 2015, 2022, as well as Kyiv, Vinnytsia, Kharkiv, etc. Therewith, production volumes in these areas are declining. This decline in beef and veal production is conditioned by a decrease in the number of cattle, specifically, as of 1 January 2022, compared to 1961 and 1991, by 6.7 times and 9.3 times, respectively. The largest number was in Khmelnytskyi, Odesa, and Zakarpattia regions, accounting for 24% of the total in Ukraine, as well as in Lviv, Zhytomyr, Vinnytsia, and Ivano-Frankivsk regions. That is, almost half of the country's livestock (49.7%, 815.5 thous. heads) is kept in 7 regions. Moreover, the share of enterprises fell to 38.0% (2022), although in 1991 it was 85.6%.

As for the level of profitability of cattle meat production, with the exception of 1990-1994 and 2017, it was negative, and in 2020 it was -24.2% (Level of profitability of agricultural production in enterprises), which was also primarily caused by a decrease in production volumes. It is also worth noting the increase in average consumer prices (Consumer Price Indices for 2021. Statistical Collection) for beef in Ukraine, specifically, while in January 2021 the price was 149.51 UAH/kg, in December it was 182.84 UAH/kg. In December, the highest prices were in Kyiv, Kyiv, and Kirovohrad regions, at

206.54 UAH/kg, 200.48 UAH/kg, and 193.80 UAH/kg, respectively, and the lowest in Chernihiv, Chernivtsi, and Poltava regions, at 169.38 UAH/kg, 168.98 UAH/kg, and 168.08 UAH/kg, respectively, which does not contribute to the increase in demand.

When investigating self-sufficiency, it is also worth considering the change in the population, because while in 2000 it was 49 million people, in 2020 it was about 42 million people, and this trend continues. Thus, in 2020, the population in Ukraine decreased by 314,062 people, with a decrease in all regions except Kyiv, where the growth was 7,486 people. Each region experienced a natural population decline. At the same time, in 2020, despite the overall population decline, there was a migration increase, specifically in Kyiv, Ivano-Frankivsk, Kharkiv, Odesa, Lviv, Poltava, and Khmelnytskyi regions. The ongoing hostilities in Ukraine have also led to migration processes, and thus, when reviving livestock production, it is essential to focus mainly on regions with a prospect of growing demand for agri-food products, including livestock. As a result of this study, a SWOT analysis (Table 12) was also carried out on the prospects for further beef production in Ukraine, which helped to identify its strengths and weaknesses.

Table 12. SWOT analysis of beef and veal production in Ukraine

Strengths	Weaknesses
Availability of suitable natural conditions for raising livestock; Favourable natural and climatic conditions for growing crops, including fodder; historically established system of cattle breeding in all regions of Ukraine; availability of highly qualified personnel; availability of state development programmes; consumption of products by the majority of the Ukrainian population	Insufficient and unbalanced fattening of cows; insufficient technological and technical equipment; lack of breeding work with the herd; simple reproduction of the industry due to low profitability of production; insufficient competitiveness of enterprises; deformed production structure (dominated by individual farms); curtailment of production; insignificant share in meat consumption by the population
Opportunities	Threats
Introduction of a system for monitoring prices for products; favourable pricing policy for producers, coordination of standard production costs, price levels and incomes; attraction of foreign investment; preferential lending to producers; leasing for equipment supply; introduction of new technologies in the production of feed and livestock products; intensification of integration processes between meat producers and processors; creation of cooperative associations; increase in exports to the world market; improvement of customs and tariff protection of Ukrainian producers	High cost of fodder and other material and technical resources; lack of support for the promotion of meat products on the foreign market; weak commercial and integration processes; and reduced competitiveness; growth of low-quality imports; bankruptcy of enterprises; military operations

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

Thus, it is necessary to promote the increase in production, considering the identified strengths, weaknesses, opportunities, and threats. It is advisable to take appropriate actions by the state through direct and indirect influence on production, including through direct

payments, the tax system, etc. Considering the significant decline in beef and veal production due to a decrease in the number of cattle, the reasons for this are identified, which are proposed to be distinguished as general and those related to enterprises and household farms (Fig. 3).

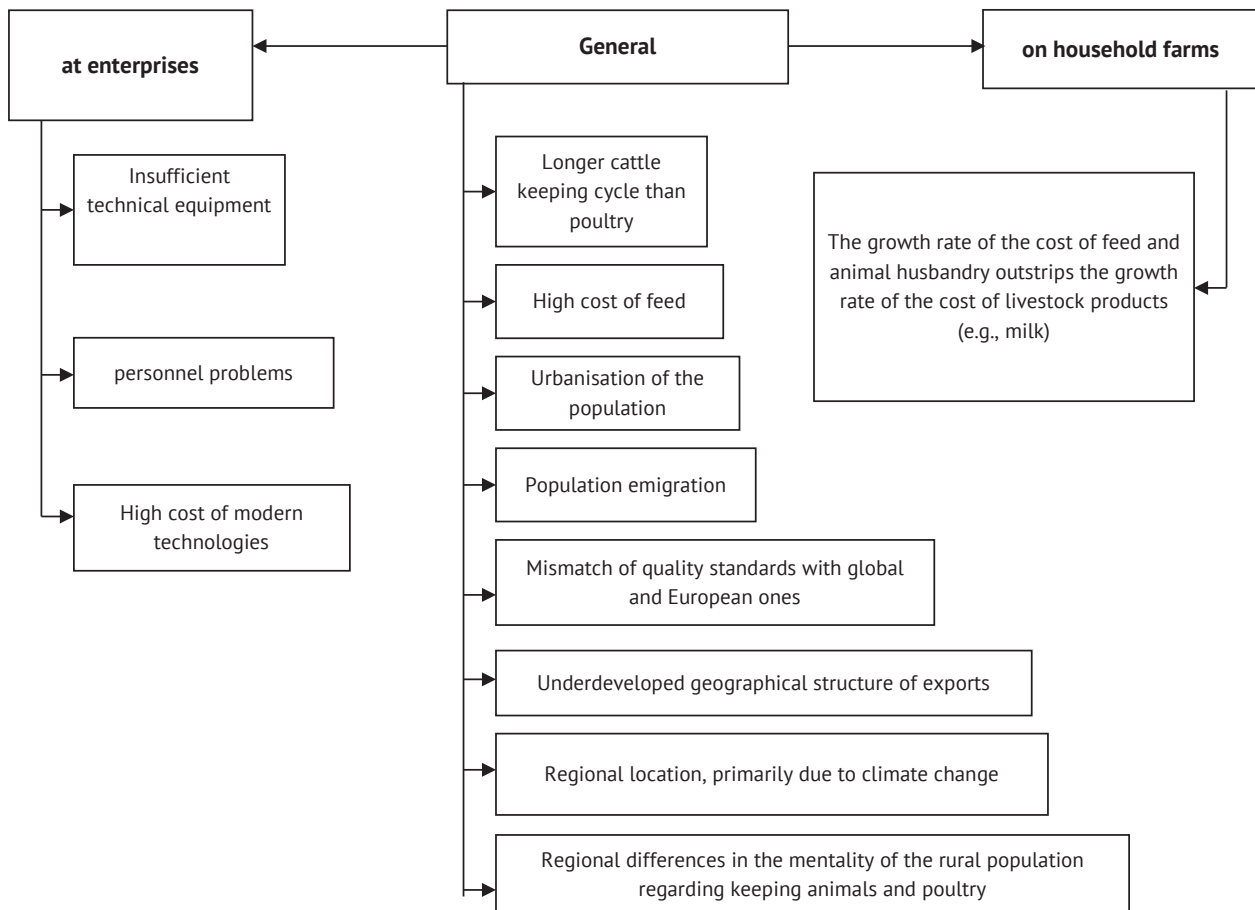


Figure 3. Reasons for the decline in cattle numbers

Source: developed and constructed by the authors of this study based on personal findings

Some of these issues can be addressed at the micro level, namely, through the involvement of highly skilled workers, while others require government intervention, primarily through the adoption of relevant regulations. Furthermore, the impact factor should also be considered, as the resolution of common causes affects businesses and household farms. For instance, lower feed costs should help to increase beef production in all categories of farms, while harmonisation of quality standards with European ones should increase beef exports, which will lead to a rise in production.

Considering the significance of exports for increasing production volumes, it is advisable to attach particular importance to raising quality standards, harmonising them with global and European standards, establishing joint ventures with business entities from countries that are significant exporters on the world market, and having trade agreements and free

trade zones in place between countries. Thus, Ukraine has trade agreements with dozens of countries, both individual countries and integration groups, including the EU, EFTA, Canada, the UK, Turkey, Israel, etc. While integration implies free trade and the removal or reduction of trade barriers, it also requires considering non-tariff barriers and country-specific features. Thus, while for EU countries it is primarily European quality standards, for the latter two it is the consumption of halal and kosher products. In an effort to expand its position in the markets of Eastern countries and given the growing consumption of kosher products in developed countries, it is advisable to stimulate the production of halal and kosher beef.

Considering the state and trends of beef and veal production in Ukraine and the world, the study outlined certain areas of promotion of its post-war development as follows: attracting foreign investment on favourable

terms; establishing joint ventures, including with the support of research institutions, advisory services for training, providing advice on efficient beef production, livestock maintenance, feeding optimisation, etc; state support, which includes direct payments (the amount of which will depend on the number of livestock, land availability, location, climate zone, and region) and indirect funds (through the mechanism of providing preferential loans, insurance system, and the procedure for providing high-yield cows for temporary use, whose calves will stay in the agricultural enterprise or household farm, and whose cows will be transferred to other farms).

Furthermore, it is proposed to introduce a procedure for granting benefits or tax holidays for enterprises/businesses that: raise new types of highly productive livestock or produce organic products; introduce modern technologies; provide the processing industry with quality raw materials. Therewith, it is necessary to stimulate the use of modern technologies, promote production efficiency, specifically feeding, etc. Moreover, it is worth promoting the introduction of innovative technologies, research results of Ukrainian scientific institutions, which may be cheaper and more suitable for local conditions. In addition, when increasing production, it is necessary to consider the specific features of the regions, namely, the zones and regions where production has historically been the most developed, where the most favourable natural and climatic conditions and feed resources are, as well as where production is noticeably declining, etc.

Consideration of the findings of this study and the recommendations provided will help to increase production volumes both in individual enterprises by eliminating the identified problems and in the industry as a whole by reducing existing threats and exploiting opportunities. The analysis suggests a rather negative trend of declining beef production in Ukraine, particularly in contrast to the global dynamics. To improve the efficiency of the development and functioning of the beef market in Ukraine, especially in the context of its convergence with the EU markets, V. Lyakhovets (2018) suggests the introduction of international experience. However, the authors of the present study believe that direct borrowing of the classical European model of the economic mechanism for regulating the beef market without factoring in the local organisational and economic conditions of the market environment is impossible, which is caused not only by the prohibitive cost of implementing its main principles against the background of the current crisis in the Ukrainian economy, but also by the need to find Ukraine's own and more efficient way of developing the beef market. The authors of this study believe that the amount of state support for the beef market depends on the real capabilities of the budget; tax, credit, price, investment, export-import, customs, monetary and credit policies, which affect the solvency of enterprises to provide production, material and technical resources.

A. Sakhno and I. Salkova (2021) agree with this, noting that in the production of meat sold on the internal market at prices higher than world prices, in the future, it is necessary to increase its own competitiveness not by increasing state support, but by reducing costs and improving the quality of meat products. The authors of the present study also came to the same conclusion. As noted above, and considering the findings of this study on the state, trends, features, and reasons for the decline in beef production, the issues of product competitiveness, increased profitability, consumer preferences, identification of regional reserves for production development, and improvement of product quality are still open. Yu. Sinyavina and T. Butenko (2021) note that quality improvement is an additional reserve for the economic efficiency of the industry. In Ukraine, this is one of the main prerequisites for both efficiency and growth in demand for products in the internal and external markets. Furthermore, the authors of the present study believe it is necessary to focus on quality as well, since it primarily affects public health and long-term demand.

The study proved the need for capital investment in the development of agricultural enterprises as a basis for the development of effective meat production activities. The authors of this study fully share the opinion of N.G. Kopitets and V.M. Voloshyn (2020) that meat production is provided by a range of agricultural and industrial sectors of the country, which requires a clear definition of priorities for the development of the meat market and mechanisms for state support for the livestock sector.

Therefore, considering the above, when developing the main aspects of the strategy for sustainable development of the country's agricultural sector in the future, the Ukrainian meat market should be given priority in the system of state regulation. M.O. Karpyak (2018) shares the same opinion, emphasising that today livestock production underlies sustainable development of crop production. In other words, the implementation of the state policy to support the development of livestock is a prerequisite for the sustainable development of the agricultural sector and has a considerable impact on improving food security and preserving the Ukrainian countryside. As noted above, consideration of the findings of this study on the state, trends, features, and reasons for the decline in beef production may help to increase it, but the issues of product competitiveness, increased profitability, consumer preferences, identification of regional reserves for production development, etc. are still open. These are promising areas for further research.

CONCLUSIONS

Thus, the study showed that global beef production is growing steadily, with its share not falling below 20.3% of total meat production in 1961-2022. Its main producers are primarily the countries of North and South

America, Asia, and Australia, and among European countries – Germany and France. Beef production in the EU declined, although there was an increase in exports and a decrease in imports, primarily from Brazil and the UK. Ukraine exports beef mainly to the East. Its largest consumers include Argentina, the US, and Brazil. Ukraine's share of global production is declining. The world's largest beef producers do not always produce the most beef per capita, as observed in China and India.

In terms of food self-sufficiency in Ukraine, consumption of beef and veal in 2016-2022 was consistently lower than production. It is found that the share of agricultural enterprises in the structure of beef production has noticeably decreased, but there is a direct correlation between changes in the number of enterprises and production volumes. Notably, enterprises have greater opportunities to apply modern technologies, attract highly qualified personnel, enter foreign markets, etc.

Regionally, most beef and veal are produced in the Forest-Steppe zone, with over 43% since 2019, and the other two zones alternating between them. The regions that stand out are Lviv, Ivano-Frankivsk, Kyiv, Vinnytsia, and Kharkiv. The decline in production was primarily caused by a significant reduction in the number of livestock (by 6.7 times compared to 1961) and a negative level of profitability of cattle meat production. The increase in average consumer prices is not helping to boost demand for beef. The primary reasons for the decrease in the number of cattle are proposed to be divided into general, individual enterprises and households, which should help to accelerate their solution, including through the influence of the state.

Therefore, considering the growth of beef production in the world, its consumption by the majority of the population, favourable natural and climatic conditions, and one of the world's largest grain production volumes in Ukraine, as well as the production of other products and raw materials for processing enterprises, it is advisable to promote cattle breeding and beef production, specifically through the adoption of the necessary regulations, improving product quality, introducing modern technologies, preferential insurance, lending, and a range of other measures by the state and individual enterprises.

The study found a decrease in the supply of live animals to processing enterprises in the post-war period, with a significant decrease in the purchase of animals from the population due to military operations. However, positive developments in production give grounds for the resumption of cattle breeding in the postwar period. Still, there is a decrease in meat stocks, particularly beef, in Ukraine. An analysis of beef production before and after the hostilities, including growth rates, suggests that its recovery may depend heavily on cooperation with foreign partners. In the future, it is advisable to expand the research on the development of beef production, considering the climate crisis and the increase in the share of Ukraine's exports in the world market.

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CONFLICT OF INTEREST

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Стан і перспективи розвитку виробництва яловичини та телятини в Україні в контексті міжнародної економічної інтеграції

Олександр Миколайович Нечипоренко

Доктор економічних наук, доцент
Національний науковий центр «Інститут аграрної економіки»
03127, вул. Героїв Оборони, 10, м. Київ, Україна
<https://orcid.org/0000-0002-1080-5633>

Надія Василівна Кривенко

Кандидат економічних наук, старший науковий співробітник
Національний науковий центр «Інститут аграрної економіки»
03127, вул. Героїв Оборони, 10, м. Київ, Україна
<https://orcid.org/0000-0003-3439-432X>

Дмитро Віталійович Людвенко

Доктор економічних наук, старший науковий співробітник
Національний науковий центр «Інститут аграрної економіки»
03127, вул. Героїв Оборони, 10, м. Київ, Україна
<https://orcid.org/0000-0002-7908-5718>

Вікторія Петрівна Рудь

Кандидат економічних наук, старший науковий співробітник
Інститут овочівництва і баштанництва Національної академії аграрних наук України
62478, вул. Інститутська, 1, с. Селекційне, Україна
<https://orcid.org/0000-0002-2464-0200>

Юрій Михайлович Носенко

Кандидат сільськогосподарських наук
Президія Національної академії аграрних наук України
01010, вул. Михайла Омеляновича-Павленка, 9, м. Київ, Україна
<https://orcid.org/0000-0002-6648-3418>

Анотація. Виробництво яловичини зумовлене необхідністю забезпечення продовольчої безпеки країни, задоволення потреби переробної промисловості в сировині, необхідністю збільшення надходжень до державного бюджету від експорту. Мета статті полягає у висвітленні стану, тенденцій виробництва у світі та Україні, виявленні проблем та визначенні напрямків розвитку з врахуванням міжнародної економічної інтеграції. Використано методи аналізу, синтезу, узагальнення, конкретизації, математичні та графічні. В результаті досліджень визначено тенденцію до зростання виробництва яловичини та телятини у світі, основні країни продуценти та переважну сталість їх частки у світових обсягах протягом десятиліть. Виявлено, що більше виробництво яловичини не завжди дозволяє забезпечити вищі обсяги на душу населення (наприклад в Китаї та Індії). Спостерігається зменшення продукування Україною та її частки у світових обсягах, хоча виробництво перевищує фонд споживання. Визначено, що у структурі суб'єктів господарювання зменшується частка сільськогосподарських підприємств та існує пряма залежність їх кількості та обсягів виробництва, яке переважає у зоні Лісостепу. Виділено області найбільші продуценти (Київську, Вінницьку, Харківську, Львівську), де передбачається першочерговий розвиток у післявоєнний період. Визначено основні причини зменшення виробництва, зокрема зниження поголів'я худоби, від'ємний рівень рентабельності, зростання середніх споживчих цін. За результатами SWOT-аналізу визначено перспективи розвитку та розроблено відповідні пропозиції. Запропоновано причини зменшення поголів'я худоби, яловичини виокремлювати як загальні, підприємств, господарств населення; наголошено на їх взаємозалежності. Наголошено на необхідності державної підтримки виробників через надання продовольчої допомоги населенню безкоштовно та через купівлю продукції за нижчими цінами залежно від їх матеріального рівня. Виокремлено міжнародну економічну інтеграцію як один з вагомих чинників збільшення виробництва яловичини. Підкреслено необхідність врахування споживчих переваг населення країн з якими укладено регіональні торговельні угоди, зокрема щодо виробництва кошерної та халяльної продукції. Результати досліджень та розроблені пропозиції можуть використовуватись при розробці державних заходів стосовно нарощування виробництва яловичини та підвищення її частки у світовому експорті

Ключові слова: обсяги виробництва; яловичина; державна політика; регіональні торговельні угоди; рентабельність; продовольча допомога

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Тел. (0412) 22-04-17
E-mail: info@sciencehorizon.com.ua
<https://sciencehorizon.com.ua>

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